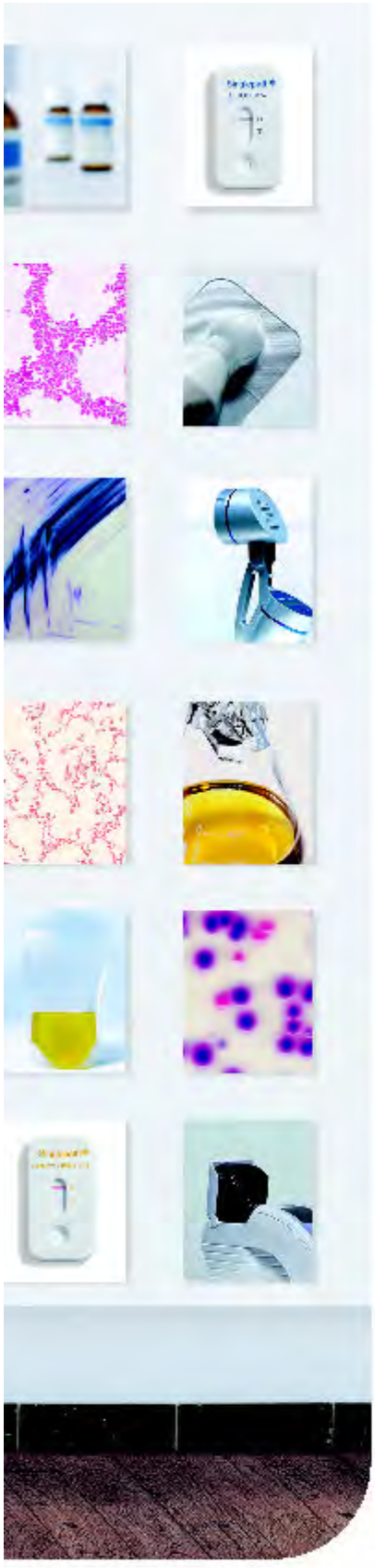
Microbiology Manual

12th Edition

MERCK

Contents


Welcome
Willkommen
Bienvenue
Bienvenido...
MERCK
MERCK



MERCK Microbiology

About this Manual

In 1885, more than 100 years ago, Merck started its activities in Microbiology. The history of the Merck's Microbiology Manual is much shorter than the tradition of Merck as manufacturer of dehydrated culture media. Before 1950, Merck microbiology products were listed in the Merck laboratory products catalog. In 1953 the first edition of Merck's Microbiology Manual was printed. It included 85 dehydrated culture media.

The new manual

The new Manual is the the 12th edition of the Merck Microbiology Manual and replaces all former editions. This Manual is available as hard copy and in digital form on CD-ROM.

The 12th edition greatly differs from the 11th edition of 2000. The layout has changed and the section with technical- and product information as well as the fields of application (in the new edition termed Review of Special Fields of Application) is updated and extended. New chapters were added, s.a. "About Merck and EMD" and "About Merck microbiology".

Dehydrated culture media and supplement product listing

The product listing for the dehydrated culture media and their supplements has changed. Chromocult® (media containing chromogenics) and Fluorocult® (media containing fluorogenics), ReadyCult®, egg yolk and egg yolk tellurite emulsion are listed in alphabetical order in the dehydrated culture media product monograph section of the 12th edition. The supplements monographs directly follow the appropriate dehydrated culture media monograph.

Dehydrated culture media naming

The name of some dehydrated culture media has been modified to match international descriptions. For example, Buffered Peptone Water is now listed under "B". Formerly it was to be found under "P" as Peptone Water, Buffered. Cereus Agar is listed under "M" because of MYP, the international description. Salmonella Enrichment Broth acc. to Rappaport-Vassiliadis (RVS) is now found under "R" from Rappaport-Vassiliadis Broth.

Compliance with reference methods

Each product monograph indicates when a product is in compliance with or recommended in a reference method protocol. The following abbreviations were used:

USP	United States Pharmacopoeia
EP	European Pharmacopoeia
DAB10	Deutsches Arzneimittelbuch
ISO	International Standardisation Organisation
AOAC	Official Methods of Analysis of AOAC International
BAM	US Food and Drug Administration Bacteriology Analytical Manual (**FDA**)
USDA	US Department of Agriculture; Microbiology Laboratory Guidebook (**USDA-FSIS**)
SMD	Standard Methods for the Examination of Dairy Products (**APHA**)
SMWW	Standard Methods for the Examination of Water and Wastewater (**APHA**)
EPA	Environmental Protection Agency (**USEPA**) **§35 LMBG** Lebensmittelgesetzbuch (German Food Law)
DIN	Deutsches Institut für Normung (German Institute for Standardisation)
CE	European certification of In Vitro Diagnostic Medical Devices (Directive 98/79/EC, October 27, 1998); effective date: December 7, 2003.

Review of Special Fields of Application

The tables contain the necessary information to be able to select the test parameters and the culture media recommended for the testing. Given the type of sample it lists the test parameters, Merck product and the reference method.

Other support services

In addition Merck offers a wide range of technical information, educational publications and product support services on the Merck website www.chemdat.de. These include:

- Brochures
- Certificates of Analysis (CoA)
- Certificates of Origin (CoO)
- Safety data sheets (SDS) on a ChemDAT CD-ROM
- Seminars and symposia worldwide
- Training courses (e.g Preparation of culture media)
- Locally prepared Merck or VWR micro-biology news letters or magazines

How to contact Merck?

On page 664 you find information how to contact your local Merck representation office worldwide.

About Merck and EMD

Brief profile - Introducing a modern company

The Merck Group is a global pharmaceutical and chemical company focusing on the development, manufacture, and marketing of innovative drugs, chemicals and laboratory products.

Merck's pharmaceutical business encompasses both prescription and patented drugs (e.g. for the treatment of cardiovascular diseases and diabetes), generic drugs, and self-medication products.

Our chemicals business sector concentrates on high-quality specialty chemicals: liquid crystals for displays - effect pigments for use in industry and cosmetics - electronic chemicals for chip manufacture - analytical reagents (incl. microbiology products) and test kits for the chemical, food and beverage, water, pharmaceutical and cosmetic industry and for environmental analysis- products and services for the entire process chain of the pharmaceutical industry.

Merck regards its talented, entrepreneurial employees, its application-oriented research and development, its consistent customer focus, and its responsible handling of natural resources as the keys to its success. Today, around 34,500 committed Merck employees are helping to continue the company's over **300-year-old tradition**

The beginning of Merck in 18th century:
The Engel Pharmacy in Darmstadt

Innovation - Courage and pioneering spirit for the development of new products

Research at Merck focuses on serving the well-being of many people, resolving social concerns, and on our corporate success. In order to expand our existing product range, we depend on employees who regard innovation as an opportunity for growth and as the key to new products for the world's most important markets. We have continually expanded our research capacities through company acquisitions, as well as partnerships and strategic alliances.

History - Innovative by tradition

Merck is the oldest chemical-pharmaceutical companies in the world. Its roots reach back to 1668, when Friedrich Jacob Merck purchased the "Engel-Apotheke" ('Angel pharmacy') in Darmstadt near Frankfurt. In 1827, Heinrich Emanuel Merck began the large-scale production of alkaloids, a then recently discovered class of highly effective plant constituents[1]. The pharmacy's laboratory developed into a chemical and pharmaceutical factory and in 1860 already manufactured more than 800 and by 1900 around 10,000 different basic pharmaceutical substances and chemicals of special purity. In 1889, Georg Merck, a grandson of Heinrich Emanuel Merck, took over the branch in New York and founded Merck and Co. in the United States. After the First World War, Merck lost many of its foreign branches, including its American subsidiary, which became an independent, self-standing company called Merck & Co.[2] in 1919.

Global teams ensure our success

To succeed in global competition we need employees with entrepreneurial spirit and intercultural skills. As a research-based company, people who look for innovative solutions are particularly important to us. Our success is attributable to our 34,500 employees, who are willing to keep learning, respect each other, and work in teams beyond national borders.

Responsible and committed for generations

Companies are increasingly including corporate responsibility as a mainstay of their corporate goals, actively taking on responsibility for employees, society, and the environment. The company's mission statement and code of conduct express the general legal and cultural terms and conditions of responsible business operations for the entire Merck Group. For us, the focal points of ethical business operations include the obligation of the company and its employees to respect established laws. Merck supports fair competition.

1. The highly effective plant constituents offered by Emanuel Merck around 1850 included the analgesic morphine.
2. *The two companies agreed for the name "Merck" to be used exclusively by Merck & Co. in the United States and Canada, while being used exclusively by us in Europe and the rest of the world. Accordingly, Merck & Co. operates outside North America under the name Merck Sharp & Dohme (in Germany under MSD Sharp & Dohme GmbH), while our companies in North America operate under EMD Pharmaceuticals, Inc. and EMD Chemicals, Inc.*

Guaranteeing work, plant, and product safety is as much a part of the rules as handling natural resources with care. In research and development respecting ethical borders, for example when using gene technology, is a high priority for us. As a research-based company, Merck uses patents to protect its inventions in order to be able to generate an adequate return on its innovations; in doing so, however, the company respects the intellectual property of others.

Merck end of 19th century:
The chemical and pharmaceutical industry in Darmstadt, Germany

High level of environmental protection and safety

We have undertaken to implement key aspects of sustainable development as described in Agenda 21 and to abide by the chemical industry's principles of Responsible Care®. Every employee of the Merck Group is required to be environmentally aware and safety-conscious at the workplace. We perform environmental audits at all our production sites. As part of sustainable development, we evaluate the results of these audits and take preventive action, and set ourselves new goals. Several more plants were certified according to the environmental management standard ISO 14001 in 2002, meaning that more than 80% of our global production capacities now operate in accordance with this environmental standard.

Expenses for environmental protection and safety equipment, e.g. for water protection, air pollution control, waste disposal, and work safety equipment, amounted to EURO 95 million in 2002. In order to ensure process efficiency, quality management (QM) systems are combined with environmental management systems and audited regularly.

Merck KGaA, Darmstadt, Germany

Fascination in dialog with the public

We regard communicating clearly in a way suitable for our target groups as an important exercise to clarify the reasons for and effects of our business activities. Continuous dialog with the public and a transparent information policy ensure our place in society as a research-based pharmaceutical and chemical company.

The Merck KGaA website at www.merck.de provides up-to-date information on the company and new products - often in four languages. Most of our publications can be downloaded. Internet portals relating to special health and chemicals issues and the web presences of our foreign companies enable direct contact with Merck. In 2000, we commenced a global project to strengthen our corporate brands, Merck and EMD (www.emd-chemicals.com). Our logos and new corporate design reflect our common identity and the values we believe in.

MERCK EMD

In Europe and rest of world we are known as Merck. In North America Merck operates under the name EMD.

As a listed company, shareholders, analysts, banks, and journalists are important to us. We provide up-to-date financial reports on our business development. These reports include in particular our Annual Report and quarterly reports, but also press releases and regular telephone conferences. We broadcast major press and analyst conferences live on the Internet. In terms of publishing its Annual Report for 2002, Merck KGaA was the second-fastest of the 100 largest publicly listed companies in Germany.

Our employees worldwide also use state-of-the-art communication media and information systems. Our "Corporate MerckNet" enables all companies of the Merck Group to simultaneously access current corporate information. In addition, our international employee magazine "pro" - which also has its own regional section in many places - is available to Merck employees at all our sites.

Merck Worldwide
Service around the globe

The Merck Group is a global company, represented by many subsidiaries operating on all six continents. Around 34,500 committed employees work in 58 countries in areas ranging from research and development through production to sales, offering comprehensive services, to turn our vision of today into the reality of tomorrow. We regard ourselves as part of society at our 82 research and production plants in 29 countries, where we act as active "fellow citizens". Our customers and business partners can expect to be met everywhere with the same friendly and competent service and the proverbial Merck quality - irrespective of whether they are dealing with a large subsidiary or a small representative office.

Further information ...

We will be glad to provide you with further information. Call us at +49(0)6151 720 or send an e-mail to service@merck.de.

Our Web site at www.merck.de provides important, up-to-date information.

The beginning

An important stimulus towards cultural techniques came with attempts to discredit the alleged miracle of red, blood-like stains on the bread and wafers used in the Catholic mass which were believed to be the blood of Christ. Bartholomeo Bizio (1817) looked at the red spots under a microscope and saw what he described as a "fungus" (bacteria). The "fungus" he named as Serratia marcescens multiplied through contact of the red polenta with fresh polenta. At growth that requires moisture and warmth. Bizio also showed that the red colouration can be passed to fresh bread by handling. In 1848 Christian Gottfried Ehrenberg (1795-1876) inoculated red spots found on "bloody bread" on to potatoes, bread and Swiss cheese kept in metal vessels, the atmosphere of which was kept moist with damp paper. In doing so he probably became the first person to cultivate bacteria. Ehrenberg introduced the term **bacteria** (meaning little rods). Hermann Hoffman (1813-1878) was the first who tried to cultivate the non chromogenic (colour-forming) microorganisms. He experimented with pieces of bread and potato; he boiled them, decanted the water, and then inoculated the surface of the bread or potatoes with yeast. Joseph Schroeter (1837-1894) found that chromogenic bacteria would grow on solid substrates such as potato, starch paste, flour paste, bread, egg albumen, and meat. Similar to Bizio's earlier findings Schroeter found that the colonies were capable of forming new colonies of the same colour, consisting of organisms of the same type.

Pure culturing: The start

Louis Pasteur introduced the concept that a disease organism might be cultured outside the body.

In 1873 Edwin Klebs (1834-1913) was one of the first bacteriologists to obtain separate cultures using what he called his fractional method (**Dilution method**). This method was basically sub-culturing small quantities from a liquid medium to another medium. The fractional procedure had the disadvantage that only the predominant organisms could be isolated from a mixture and that is was very labour and material intensive. Joseph Lister (1878) was the first to isolate a pure culture. He isolated a bacterium which he named *Bacterium lactis* from Pasteur's lactic ferment that caused milk souring. Lister employed the fractional dilution principle. A loopful of spoiled milk was transferred to sterile boiled water. Lister determined the required dilution using the microscope. He used a syringe to inoculate a glass slide with various dilutions. He determined microscopically the dilution which showed only one bacterium. He then diluted the milk sample with the required volume of boiled water and cultured the inoculated solution. Considering the lack of pure culturing techniques it is not surprising that microbiologists in the 19th century claimed that microorganisms varied in their morphological form and their physiological function (**pleomorphism**).

At the end of the 19th century the number of laboratories conducting microbiological testings increased rapidly. Particularly small laboratories, however, had problems with the preparation of their formulated culture media. There were high (heating) costs, batch to batch variations, the variation in growth performance of one lab compared to another and the lack of qualified staff to prepare the culture media. The shelf life of the culture media was very limited and culture media had to be disposed due to spoilage.

In 1908 Prof. R. Doerr suggested the preparation of culture media in a solid form for mobile laboratories. Doerr's solid medium was prepared by pouring Nutrient Agar on a sterile glass plate which was then set to dry. The dried material was grinded and could be reconstituted to a "ready to use" medium. A similar process was communicated by W.D Frost in 1910 at a meeting of the American Society of Bacteriologists.

Pure culturing on solid media

The German botanist Oscar Brefeld did most of the pioneering work on pure culturing on solid media. In 1872 he reported growing fungal colonies from single spores on gelatin surfaces. His solid medium was produced by adding gelatin to a liquid medium. Robert Koch realised the importance of a technique which would allow distinguishing one kind of bacteria from another and obtaining pure bacterial cultures in vitro. If the microbe could be isolated, then it might be possible to control the disease. Robert Koch struggled with Kleb's concept of using liquid media to get pure cultures. He experimented with cut potatoes which he placed into glass vessels. Because of the moist surface of the cut potato, motile bacteria can spread easily over the whole surface. The substrate is opaque making it difficult to see colonies. Also potato is not a good nutrient to grow many bacteria. Koch perceived that it would be better to solidify a liquid medium. The varying nutritional requirements could be met by modifying the liquid base.

In 1881 Koch obtained pure cultures of the anthrax bacillus using the aqueous humor of an ox's eye supplemented with 10% gelatin. The sterilised medium was poured onto sterile glass slides which were then stored under a bell jar to avoid external contamination. Using a sterile needle or platinum wire he subcultured a small amount by drawing several cross lines on the gelatin. Different colonies of the obtained isolates were transferred to test tubes containing sterile nutrient gelatin. In 1881 this pure culturing technique was reported together with a **"poured plate"** method. For the latter the bacterial inoculum was mixed with the melted gelatin and then poured onto a cold sterile plate.

Gelatin was not very attractive as solidifying agent. It solidifies at a temperature below 25°C making it difficult to be used during summer time as Walter Hesse, a collaborator of Koch found out. Also many organisms can digest gelatin. Gelatin plates could not be incubated at 37 °C, the temperature favoured by human and animal pathogens. While working as an assistant to Robert Koch, Friedrich Löeffler (1852-1915) reported using **nutrient broth** for the successful cultivation of the bacillus of mouse septicaemia. This medium is essentially the nutrient broth still in use to day. It consisted of meat water to which 2.0% peptone and 0.6% common salt was added, with the final product being made slightly alkaline with sodium phosphate. In 1880 Nägeli (1817-1891) proposed the use of **peptone** in the preparation of culture media.

Koch employed peptone water to isolate the cholera bacterium but they require more complex C sources like, for instance, sugars. The requirement for nitrogen may be satisfied in form of NH^{4+}, NO^{3-} or N_2, although many organisms need more complex organic nitrogenous compounds like inorganic acids and essential amino acids.

In 1882 Koch reported using heated serum to "stiffen" culture media for the cultivation of tubercle bacilli. The solution came in 1882, when Fanny Eilshemius, the wife and assistant of Walter Hesse suggested the use of agar-agar. Agar-agar remains solid at temperatures up to 100°C, it gels at 34-42°C, it is clear, and it resists digestion by bacterial enzymes.

The invention of the Petridish in 1887 is credited to another assistant of Robert Koch, R.J. Petri. It is likely that the Slavanian scientist Emanuel Klein (1885) and the English researcher Percy Frankland, (1886) suggested such dish earlier.

In 1902 Drigalski and Conradi, collaborators of Robert Koch, proposed the use of what is now known as the hockey stick or Drigalski spatula and the surface plating method. Drigalski and Conradi sterilised the hockey stick over a Bunsen burner and after letting it cool down dipped the short side of the stick in the sample and spread it in all direction over the agar plate and to a second, third and fourth plate.

The methods developed by Koch and his colleagues were so successful that by the early 20th century, the etiologic agent for nearly every important bacterial disease had been isolated and identified.

Non selective, general culture media

In 1855 Gimwade in England was the first to produce powdered milk.

The crucial question to culture microorganisms in the laboratory was: what must be added to water to make microorganisms grow?

Pasteur showed that yeasts consisted of carbohydrates, oxygen, hydrogen and a range of chemicals occurring in ashes of which potassium and phosphoric acid were the most important. Yeasts would grow when these elements were available as raw materials. Pasteur also found that yeasts use hydrogen and oxygen from water, nitrogen from ammonia and carbon, not sourced from CO_2 as plants do, but from sugar. In 1861 Pasteur formulated the first **liquid culture medium**. The medium was relatively simple consisting of 100 g distilled water, 10 g candy sugar, 1 part ammonium tartrate and 1 part ash from yeast. Burden and Sanderson demonstrated the growth of bacteria when water, blood or puss was inoculated in Pasteur`s medium. Cohn demonstrated that the sugar in Pasteur's medium favoured the growth of yeast and moulds at the cost of bacteria. The medium facilitated a good growth of bacteria when sugar was replaced with ammonium tatrate. Cohn improved the medium further after Adolf Mayer, in 1869, had identified the minerals in yeast ash. Cohn used Mayer's mineral salt solution to formulate his standard bacteria liquid medium: 0.1 g potassium phosphate, 0,1 magnesium sulphate, 0.2 g ammonium tartrate and 0,01 calcium hydrogen triphosphate in 20 g distilled water. He also used Wolf's or Knops' nutrient liquid consisting of potassium phosphate, magnesium sulphate and calcium nitrate or calcium chloride. Cohn (1872) established that bacateria, notably *Bacterium termo* could not grow in Mayers, Wolfs or Knops mineral nutrient solutions. However, the addition of ammonion tartrate resulted in growth as observed by turbidity. Cohn showed that bacteria could assimilate substitutes of ammonium tartrate, such as succinic acid, lactic acid, or acetic acid but also C- sources such as glucose, pyruvate, glycerin and cellulose. Ureum alone or as additive to mineral solution, could be assimilated also if a nitrogen-free C-source like potassium tartrate was present. The chemicals used by Cohn contained small amounts of ammonia. Therefore, it was not possible to conclude whether bacteria could assimilate potassium tartrate or cremor tartari.

The culturing of microrganisms is very complex and requires that the various essential substances are present in the right ratios. It became clear that growth requirements of microorganisms were also complex. A synthetic medium, a medium of defined C- and N-sources such as ammonium salts, asparagin, phosphate, sodium chloride, glycerin, magnesium sulphate, other amino acids, ureum and peptone was not growth promoting enough. Bacteriologists realised that the use of natural ingredients offered a better solution. A variety of aqueous extracts and decoctions of vegetable substances (hay, turnip, carrot etc.) along with milk and urine were also tried with differing results.

Selective culture media

The biodiversity and the different cultural demands of microorganisms became clearer. In 1883 Ulysse Gayon and Gabriel Dupetit isolated two strains of denitrifying bacteria in pure culture. They showed that individual organic compounds, such as sugars and alcohols, can replace complex organics and serve as reductants for nitrate as well as serving as carbon sources. The Dutch microbiologist Martinus W. Beijerinck (1851-1931) pioneered the principles of selective culture. Beijerinck was one of the first microbiologists to emphasise the ecological approach to microbiology. He realised that the use of non selective culture was not the way to discover the world of microorganisms. The detection of specific microorganisms from a natural sample requires the use of specific culture media and incubation conditions which favour the growth of one type of organism while constraining others. Different culture media support different microorganisms. Using his enrichment (selective culture) Beijerinck isolated the first pure cultures of many soil and aquatic microorganisms including aerobic sulfate reducing bacteria, nitrogen fixing root nodule bacteria and *Lactobacillus* spp.

Sergius Winogradsky (1856-1953) discovered bacteria capable of **autotrophic** ("self-feeding") growth using inorganic compounds such as H_2S as their only energy source, and CO_2 as their only C-source. This was the first discovery that organisms other than green plants or algae could exist without consuming organic matter. He also discovered groups of photosynthetic bacteria that do not produce oxygen as their waste. He developed a culture technique known as **Winogradsky column**. The glass column contained mud and water and was exposed to light. Over time, different microbial communities grew and inter-changed waste products and nutrients.

Limbourg (1889) is to be credited for proposing the use of bile salts. The sodium salt of cholic acid was added to a mixture of peptone and pancreas extract. Leubuscher (1890) used pure bile as growth medium for B. anthracis and B. typhosus. Coprrado (1891) grew organisms in pure bile and observed that it stimulates the growth of B. mallei, bactericidal to B. anthracis but showed no affect on B. typhosus and B. pneumoniae. Freankell and Krause reported that B. typhosus was not effected by an exposure of 24 hours to bile. Conradi (1906) strongly advocates the use of pure bile as a help towards the isolation of B. typhosus. Meyerstein ((1907) did growth studies with pure bile and bile diluted with nutrient medium. He found that B. pyocyaneus grew in a bile solution, B. coli grew in bile salt with nutrient medium but Staphylococus pyogenes aureus remained inhibited, although large amounts of nutrient medium were added to the bile solution. MacConkey experimented with a potato juice medium with commercial bile salts. In 1900 MacConkey proposed a bile salt lactose agar as growth medium for B. typhosus and B. coli communis. In 1901 MacConkey and Hill proposed a bile salt glucose broth as a simple test for faecal contamination. To include B. enteritidis, MacConkey proposed to substitute glucose with lactose. The composition of this MacConkey lactose agar was as follows: 0.5 % bile salts, 2.0% peptone, 1.0% lactose agar and 100ml tap water.

Around the 1900's scientists tried to find a method to isolate Bac. typhi (Salmonella typhi) and to solve the problem to differentiate Bac. coli (E.coli) from Bac. typhi. The approach explored was to add "antiseptica" to a nutrient medium. This inhibited, however, Bac. thyphi. Drigalski and Conradi (1902) proposed a different approach. They suggested using the differences in the metabolisms of sugars in the order to distinguish between the two bacteria. Wurtz (1892) proposed to use this difference of pure cultures and formulated a lithimus lactose agar. B. coli formed red colonies and B. typhi colonies remained blue in colour. Kashida (1898) added 1% ureum to the medium and observed that B. typhi colonies remained colourless whereas B. coli colonies turned red. This system coloured Coli-bacteria red after 18h and blue after 24 h at 37°C, whereas B. typhi remained colourless after 4-5 days of incubation. B. coli (+) and B. typhi (-) differ in the mono-, di- and polysaccharides. These were added to the standard nutrient agar (meat infusion, peptone, agar) and lithimus indicator. However, when testing faecal samples, the colour of the colony depends on the amount of lactose and the diffusion of the metabolites. This phenomenon was first reported by Beijerinck (1891). The elective approach worked well with pure cultures, but when faecal samples were tested, a large number of different types of cocci coloured the medium red due to acid formation, thus interfering with the detection of colourless typhi colonies. Drigalski and Conradi evaluated a range of dye "antiseptica" (malachite green, brilliant green, methyl blue, methyl violet and crystal violet) with different concentrations. They found that a concentration of 1/ 100.000 crystal violet inhibited the interfering cocci without affecting the colony formation of B. typhi. The preparation of the culture medium illustrates the state of the art of that time. The nutrient agar was prepared from 3 pounds of cut lean beef with 2 liter of water and allowed to stand until the next day. The meat water was decanted and the cut meat pressed. The meat water was boiled for 1 hour and filtered afterwards. 20 g peptone and 10g sodium chloride was added followed by 1 hour boiling and filtration. To the solution 60g of fine threaded agar was added followed by boiling for 3 hours. After a weak alkalinisation (using lithimus paper as indicator) it was filtered and boiled again for 1 hour. A lithimus lactose solution was added to the hot sterile nutrient agar and thoroughly mixed. 10% water free sodium hydroxide and 20 ml of freshly prepared crystal violet (0.1 g in 100ml distilled water) was added. The result is a nutrient agar with 13 % lithimus solution and 0.01% crystal violet. Plates were poured and the rest of the prepared medium was portioned into 200ml Erlenmeyer flasks. It is warned that if one overheats, the lactose is affected making the medium acidic. The loss of lactose affects the differential system. The red colour of B. coli colonies starts too early. Also it is warned that overheating of agar-agar should be avoided because this leads to precipitation.

Endo commented on Drigalski and Conradi's Lithimus lactose crystal violet agar that it was difficult to detect weakly red or bluish coloured typhi colonies against the blue coloured medium. His further studies finally resulted in the development of Endo agar.

In 1908, Eijkman demonstrated that coliform bacteria isolated from intestines of warm-blooded animals produce gas in glucose broth at a temperature of 46°C. Over time, the test has been modified to a fermentation in MacConkey broth at 44°C in an accurate, thermostatically controlled water bath.

In 1916 Holt-Harris & Teague developed Eosin methylenblue lactose saccharose (EMB) agar for the isolation and identification of pathogenic Entero-bacteriaceae.

In 1918 Gassner published his investigations using metachrom yellow as inhibitor of cocci and spore-formers.

Paul Ehrlich (late 1800's) discovered that certain dyes could specifically stain bacteria but not other cells. Scientists at his institute discovered arseno-phenylglycine, which was effective against African sleeping sickness and Salvarsan which was used to treat syphilis for many years. Alexander Fleming discovered lysozyme in 1922 and then, based on the work of others, developed penicillin in 1928.

Waksman, fueled by the discoveries of the 30s, surveyed many soil samples and participated in the discovery of a number of chemotherapeutic agents. He termed the agents *antibiotics*.

About Merck Microbiology

A tradition of more than 100 years of quality, innovation and safety

The beginning: peptones, gelatin and agar-agar

Merck is a pharmaceutical company with more than 100 years of experience in the manufacturing of base materials and culture media. Already in 1878 Merck manufactured peptones, initially as food supplements which were sold by pharmacies. Suggested by Naegeli, a coworker of Robert Koch these peptones became ingredients of culture media from 1881 onwards.

The use of agar-agar as gelling agent was suggested in 1882 by Fanny Eilshemius, the assistant and wife of Walter Hesse, another collaborator of Robert Koch. The Merck product catalogue of 1884 lists, beside peptones, gelatin, also sterile culture media and agar-agar.

The medicinal peptones were produced on a laboratory scale. In April 1885 Merck acquired a patent (see Figure) for the manufacturing casein (milk) peptone on industrial scale. The product information for casein (milk) peptone of 1886 recommends its use for culturing microorganisms.

Figure: Merck Quality Control Lab. in 1935

In 1892, Merck began the industrial production of casein peptone (Tryptone) as a microbiological ingredient. In the Merck report from 1892, under the header "Peptone and peptone preparations according to Dr. Adamkiewitz it says:

Peptone powder is particularly suitable for scientific purposes for physiological and bacteriological investigations (preparation of culture media, such as culture Gelatin Medium, Culture Agar (i.e. Nutrient Agar) and Culture Broth (i.e. Nutrient Broth)." Nutrient Broth and Nutrient Agar were developed by Loeffler. These media were widely used and served as base for other formulations, e.g. Endo agar.

In the beginning peptones, particularly casein and albumin peptones, did not dissolve completely resulting in turbid peptone solutions. Before use in culture media, the turbid peptone solution had to be filtered. As a result, the nutritive composition varied from lot to lot. In 1912 Merck had developed a procedure to manufacture serum albumin, and in 1913 a casein peptone which dissolved completely into a clear solution.

Merck - The "oldest" dehydrated culture media manufacturer in the world

In 1910, Merck started to manufacture and commercialise dehydrated culture media. The annual report of 1910 says: "I am launching some new products under the registered trade name RAGIT which I am manufacturing at the request of Prof. E. L Marx. They should make the preparation of culture media easier and cheaper, as is necessary in bacteriological laboratories and when traveling ...".

The first powdered dehydrated culture media from Merck were Ragit Broth (i.e. Nutrient Broth) and Ragit Agar (Nutrient Agar). Merck also produced Hart's Endo selective supplement (1909) i.e. a Ragit Endo tablet which was added to Ragit Agar to make Endo Agar.

In 1909, Marx pioneered the preparation of dehydrated powdered Nutrient Broth, Nutrient Agar and Endo Agar formulations on laboratory scale. For the preparation of these dehydrated media Marx used Merck's Ragit peptone, Ragit agar-agar powder, and Maggi's granulated meat extract powder. Hart (1909) had also suggested the use of commercially available meat extract (e.g. Von Liebig's- or Maggi's meat extract) to replace the tedious preparation of meat broth. This core ingredient of culture media was prepared from fresh meat chops at that time. Marx preferred Maggi's granulated meat extract, because it did not contain heat resistant spores. He prepared Endo agar by adding Endo powder mix, containing lactose, sodium sulfite, sodium hydroxide and fuchsin, to Nutrient Agar.

In 1915, the Merck price list contained, apart from Ragit Agar, Ragit Broth, Ragit Endo tablet, Ragit serum albumin, also various peptones and Ragit gelatin.

In the years to come other firms followed the path taken by Merck. In 1916 Difco (Digestive Ferments Company) started the manufacturing of dehydrated Nutrient Agar and Nutrient Broth. In 1935, the Baltimore Biological company (BBL) was established, which was acquired in 1955 by Becton & Dickinson Inc. The latter acquired Difco in 1997. Oxoid, the medical division of LEMCO (Liebig's Extract Meat Company) began manufacturing dehydrated culture media in the 1950's. Beginning of 2004, Oxoid was acquired by Fisher Scientific.

Identifying the hazards when working with powdered culture media containing toxic substances, Merck developed a granulation process in 1950. In these early days it was a wet granulation process. Further improvements of the process followed and since 1978 Merck's dehydrated culture media are primarily produced in a dry granulation process. In this unique process, a powdered dehydrated culture medium is compressed to granules without the use of any additional chemicals or additives and avoiding heating to give granulated dehydrated culture media. Not all culture media can be granulated because some components cannot be granulated (e.g. vitamin assay media). If the performance of a dehydrated culture medium in granulated

form is negatively affected, the medium remains in powdered form to the benefit of our customers.

In 1953 the Merck microbiology product portfolio had grown to 85 powdered or granulated dehydrated culture media.

To date Merck is one of the leading manufacturers of dehydrated culture media in the world with a product portfolio of more than 400 dehydrated culture media formulations and constantly expanding the product offerings.

Tradition of quality

Merck KGaA is a pharmaceutical company and this is unique in culture media manufacturing. Merck has always taken great care to ensure that its products are of the highest quality. In 1851 Heinrich Emanuel Merck wrote: "I guarantee the constant purity of my products and take full responsibility for any disadvantage that may be caused due to any of these products being contaminated." This tradition has been maintained.

Since 1885, the beginning of microbiology at Merck, the principles of quality control (QC) and quality assurance (QA) implemented at Merck's pharmaceutical division were applied to microbiology. Merck microbiology products are thoroughly tested in the central quality control laboratory before being marketed worldwide.

Merck microbiology is in an excellent position to set up and maintain a high quality. In addition to the central quality control laboratory and its specialized staff, Merck microbiology has access to the services of a corporate Regulatory Affairs (RA) Department with specialists in microbiology affairs, a corporate Quality Management (QM) Department with internal specialised auditors, a corporate purchasing department, and numerous specialised laboratories in fields of pharmaceutical, chemical, analytical and diagnostic research (R&D).

Figure: Merck Microbiology facilities at Halle around 1903

Merck's certificates of analysis (CofA's) for dehydrated culture media are a benchmark.

Merck specifies a recovery rate of >70% for non selective media and <30% for selective media. The international committee, working on ISO 11133 "Guidelines on preparation and production of culture media; Part 2 Practical guidelines on performance testing", recognised Merck's benchmark quality criteria. They implemented Merck's benchmark performance criteria into ISO11133 part 2. A lot specific certificate of analysis can be downloaded and/or printed from the internet at www.merck.de.

Tradition of innovation

For more than 100 years Merck contributed to the innovation of microbiology and in particular to the culture of microorganisms.

The quality of culture media was not high in the beginning days compared to today's standards. Ingredients and composed culture media had to be filtered, or additives had to be added to clear the solution. Due to the numerous filtration steps it was impossible to standardise the performance. And more so since there was a lack of qualified staff to prepare culture media.

Merck's innovative approach to culturing microorganisms by manufacturing dehydrated culture media formulations (1910) with both, serum albumin (1912) and casein (milk) peptone (1913), resulting in clear solutions. The importance of these novel and standardised products is obvious when reading the 1923 annual report: "because in hospitals and governmental laboratories increasingly culture media were prepared in dedicated culture media "kitchens" where an increasing number of staff worked, the microbiologist missed the hands on experience on the problems of raw materials and preparation. The bacteriologist had to rely on the work of "competent staff" who did not fully understand what was required, because they did not know what work was done in the laboratory with the products they prepared".

In 1923 Merck replaced the Ragit Broth (Nutrient Broth) and Ragit Agar (Nutrient Agar) by Standard I- and Standard II Culture Broth and Agar. This improvement followed a suggestion by Kuczynski and Ferner. These biochemists showed the importance of free amino acids for the growth of microorganisms and emphasized the need for more "defined" culture media.

In 1926 Merck introduced dehydrated culture media in a **glass tube**. In the annual report of 1926 it can be read: "it (Merck) is a modern culture media manufacturer with an enormous innovative capacity ... to make culture media for bacteria in a way that makes it possible to store them ready for use and to sell them as a normal commercial commodity. I manufacture most required culture media in this form, such as Nutrient Broth, Nutrient Agar for slant cultures and plates, as well as various culture media for differentiating Salmonella typhi from Escherichia coli. The media are delivered in hermetically sealed glass vessels and are therefore protected from mould and drying out and can be stored indefinitely ...".

In the beginning of the 1950's, Merck pioneered the granulation of powdered dehydrated culture media. In 1953 the microbiology portfolio consisted of 85 different granulated and powdered dehydrated culture media. In the late 1970's, the culture media portfolio included 220 mostly granulated dehydrated culture media, 21 different culture medium base materials (peptones, yeast extracts etc.) sundry preparation (a.o. anaerobiosis rings, Kovac's indole reagents etc.) and 90 related products (staining agents, sugars, bile salts etc.).

Figure: Sterikon® plus bioindicator

In 1970, the Sterikon® bioindicator, an ampoule containing Nutrient Broth, sugar, a pH indicator and a spore suspension of non-pathogenic Geobacillus stearothermophilus was launched, at a time where paper test strips were the standard product. It enabled to check the adequacy of the steam sterilization process of an autoclave (15 minutes at 121°C) without opening the ampoule. So the contents always remain integer.

Sterikon® bioindicator was further improved and in 1990 replaced by **Sterikon® plus bioindicator**. Sterikon® became the gold standard for validating the sterilisation of culture media by use of an autoclave.

In the mid 1970's, Merck had further improved the process of manufacturing granulated culture media.

Figure: Merck's granulated dehydrated media (free flowing less dust, no clumping, dissolve easily and long shelf life)

The wet granulation, developed in the 1950's, was replaced by a dry granulation process.

In 1982, the **slow release** principle used in drugs was applied to the selective culturing of Salmonella. The Salmosyst® tablet allowed the slow release of the selective components of tetrathionate broth into the non-selective Salmosyst® Enrichment broth. The non-selective broth became selective over the incubation time allowing pre-injured Salmonella to survive and grow also.

In 1986 Fluorocult® media were introduced to shorten the time to result and to reduce the costs per test. The principle of these Fluorocult® media is that an enzyme characterising the target organism or group of organisms splits a fluorogenic substrate, like 4-methyllumbelliferyl-ß-D-glucuronide (MUG), into a sugar or an amino acid part and a carbohydrate rest, the methylumbelliferone. This methylumbelliferone becomes visible under UV light. Fluorogenic culture media are read in the dark, exposed to UV light at 366 nm. A typical blue fluorescence shows a positive result.

MUG is added into traditional media for the detection of Escherichia coli (e.g. Brilliant Green 2%-Bile Broth, Lauryl Sulphate Broth, EC Broth, MacConkey Agar, ECD Agar) or E.coli O157 (e.g. E. coli O157:H7 Agar). For the rapid detection of Clostridium perfringens the substrate methylumbelliferyl-phosphate (MUP) was introduced. The Acid phosphatase, characterising Clostridum perfringens, cleaves MUP producing the fluorogen methyllumbelliferone.

Figure: Fluorocult® LMX broth (left and right)
Tube 1 and 2 S. enteritidis
Tube 3 E.coli
Tube 4 C.freundii
Tube 5 Ent. cloacae

Merck also pioneered the manufacturing of **chromogenic culture media**. In these type of media, the traditional differential system of sugar and pH indicator is replaced by a chromogenic substrate. This is commonly an indolyl derivative, e.g. 5-bromo-4-chloro-3-indolyl (X), 5-bromo-6-chloro-3-indolyl (magenta) or 6-chloro-3-indolyl (salmon).

The principle of a chromogenic medium is that a chromogenic substrate is cleaved by an enzyme characterising a target organism or target group of organisms and split into a sugar part and a chromogen. In the presence of oxygen the chromogen forms a dimer that colours a broth or a typical colony (not like a pH indicator colouring the medium around a colony). The colour is not affected by pH. The use of a combination of different

fluorogenic and/or chromogenic substrates, e.g. used in **Fluorocult® LMX Broth** or in **Chromocult® Coliform Agar** - both media were EPA approved in 2002 - allows the simultaneous detection of coliform bacteria and E.coli within 24 hours without further confirmation. Chromocult® Enterococci Broth and Agar have been developed for the detection and enumeration of faecal streptococci and *Enterococcus* spp.

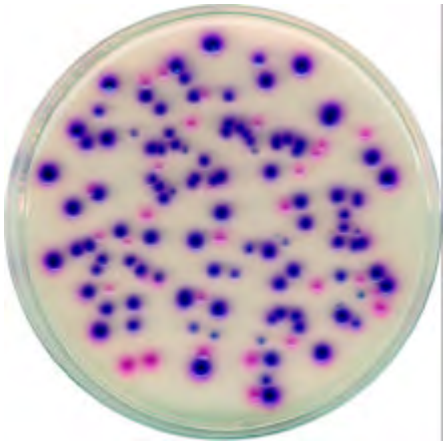


Figure: Chromocult® coliform agar (Dark violet: E.coli)

In 1995, Merck introduced **Readycult®** Coliforms and **Readycult®** Enterococci. A convenient snap pack containing sterile preweighed Fluorocult® LMX Broth or Chromocult® Enterococci Broth. The use of Readycult® together with Merck's Mini-incubator allows microbial water analysis in the field.

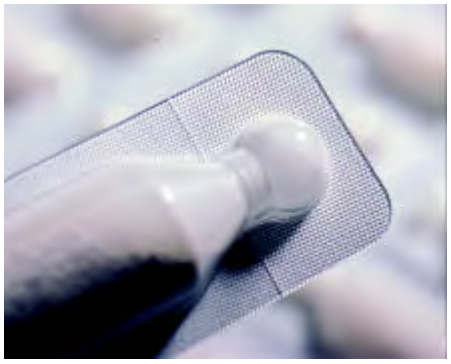


Figure: Readycult® convenient snap pack format

The first formulations of chromogenic culture media were designed for water analysis. These media could also be used for the examination of processed food, but the selectivity was not sufficient for testing wastewater and fresh foods.

In 2002 Merck introduced **Chromocult® Agar ES**, which can be used for the simultaneous detection of coliform bacteria and E.coli in drinking water, waste-water, processed food, raw food and environmental samples. In the same year Merck also launched the **E.coli/coliform supplement** to increase the seletivity for E.coli/coliforms when added to various enrichment broths and plating agars (Flourocult® LMX Broth, Lauryl Sulphate Broth, EC broth, MacConkey Agar and Chromocult® Coliform Agar) without impairing the productivity.

Figure: Coliform/ E.coli Supplement enhances selectivity of Enterobacteriaceae, coliform and E.coli media

In 2000 Merck introduced **gamma-irradiated, triple sealed Tryptic Soy Broth (TSB)**. Standard dehydrated culture media are not sterile carrying a bioburden of spores and/or small bacteria (e.g. *H. pseudoflava*), or mycoplsma's (e.g. *A. laidlawii*) which are capable of passing through a 0,1µ and 0.22µ filter.

The sterility of Merck's new irradiated TSB is assured and the pre-sterilisation process in the laboratory can be completely eliminated. The preparation of TSB for sterility testing is now more convenient and easy. The transfer of the granulated medium to sterile water produces no spread of dust. The granulated medium dissolves quickly and completely within minutes.

In 2001 **TSB non animal** was introduced. This medium is free of any animal derived material and gives pharmaceutical companies concerned about **transmissible spongiform encephalitis (TSE)** the opportunity to conduct sterility controls and media-fills safely.

In 2003, Merck launched five rapid immuoassays, **Singlepath®** and **Duopath®**, for the detection of major food pathogens together with the dedicated enhanced enrichment media. Duopath® Verotoxins is a unique product which allows the detection of verotoxin 1 and 2 in one test.

Tradition of safety

Working with powdered dehydrated culture media is a health risk. During the preparation of powdered culture media dust spreads around, is inhaled and settles on exposed body surface. When bile salt containing media are weighted, an immediate coughing response signals the inhalation of airborne dehydrated powdered culture medium. To minimise the risk of powdered culture media, Merck decided to introduce **granulated** dehydrated culture media in the 1950's.

Figure: Granulated culture media

As a pharmaceutical company Merck is aware of the potential risk of **transmissible spongiform encephalitis (TSE)** via dehydrated culture media and other raw materials e.g. peptones. Since the end of year 2000 Merck only manufactures dehydrated culture media which are in compliance with the European Directorate for Quality of Medicines (EDQM) and the European Pharmacopoeia (EP) recommendations as outlined in the Resolution of the Public Health Committee (Partial Agreement, Resolution AP-CSP (99) 4) and EP Section 5.2.8 Minimising the risk of transmissible spongiform encephalitis in medicinal preparations. Furthermore it is a Merck policy to prefer using ingredients of non animal origin. When there is no no alternative, only ingredients are used, which are recommended by the EDQM for the preparation of medicines, belonging to TSE Category B (Lower-infectivity tissues) or Category C (Tissues with no detected infectivity).

Custom Made Media Formulations and Production Support

Merck KGaA offers a wide range of base materials for cell culture and fermentation applications as well as for use in dehydrated culture media.

For cell culture and fermentation applications Merck also runs a custom made Peptone-Extract, a custom made media programme, and an optimisation support program.

Since 1892 Merck manufactures high quality base materials. Todays base materials are mostly granulated.

The base material portfolio includes

- agar-agar
- meat peptones
- casein peptones
- custom made peptones and extracts
- extracts (malt and yeast)
- non-animal peptones
- bile salts
- gelatin

Merck offers a comprehensive line of chemicals and reagents, which are listed in the General Chemical catalogue and additionally antibiotics through CNBI, an affiliated company of EMD Chemicals Inc.

Media for quality control, sterility control and molecular genetics

Merck also offers a broad range of granulated dehydrated culture media for molecular genetics, pharmaceutical testing, quality control, antibiotic, and vitamin testing. These are listed in the section Dehydrated culture media. For an overview of Merck products per application see the Review of Special Fields of Application.

Custom made media and formulations

Merck shares it's over 100 years of experience in peptone and culture media manufacturing with the customers. A dedicated team of responsive media specialists, microbiologists, chemists, regulatory affair specialists at Merck are available for the customer.

Merck manufactures specific culture media made to customers specific requirements. The quality control and testing protocols are agreed upon by both sides and a certificate of analysis is set up. Regulatory Affairs Department helps it's customers with regard to Regulatory Documentation needs.

All information the customer shares with Merck is proprietary and confidential. An appropriate confidentiality agreement is signed by both sides. A pre-sample is prepared which at Merck is quality controlled against the agreed certificate of analysis.

When the pre-sample passes the quality control, the sample is shipped to the customer. If the customer's validation testing approves the sample, Merck can start producing this special medium.

The shipment to the customer is accompanied with the necessary documentation papers, e.g. a certificate of analysis, certificate of origin, safety data sheets and other technical documentation.

Media optimisation program

The media optimisation program allows the biopharmaceutical customers to benefit from Merck's experience in selecting the right base materials, yield enhancement and media formulation scale-up.

About ... A Tradition of Quality and Service

"Quality and Service is Our Commitment"

Merck KGaA has always taken great care to ensure that its products are of a high quality. As early as 1850, Heinrich Emmanuel Merck offered guarantees on his products. He wrote in a letter to a customer that he would always vouch for the purity of his products and was prepared to cover his customers against any disadvantage arising from an impure product. In 1850 Merck already had a central quality assurance laboratory conducting quality controls on raw materials and final products to ensure a consistent high quality.

In the mid 1800's Merck quality policy was unique. When in 1885 the new department of Microbiology was founded, Merck's quality policy was also applicable to its microbiology products. Since then, all components used for any microbiology product were tested at the central quality assurance laboratory.

At Merck, quality control and quality assurance is a basic pre-condition.

Merck microbiology is in an excellent position to set up and maintain a total quality management system. As a multinational pharmaceutical company, Merck microbiology benefits from the know how of a corporate quality department, numerous specialised laboratories in the fields of pharmaceutical, chemical, analytical and diagnostic research.

The following areas are carefully controlled:

- The quality and the safety of raw materials
- The standardisation of raw materials, manufacturing, products, quality control and quality assurance
- The production process
- The safety of finished products
- The quality of finished product via testing each batch at the central quality assurance laboratory
- The regulatory documentation
- The labelling
- The dispatch of products via inspection on appearance, sealing, labelling etc.

Merck not only pays attention to the quality of its products, but also does its utmost to meet and fulfil all its customers' requirements. Today quality at Merck also means prompt delivery, excellent technical support, immediate information, a flexible custom made dehydrated culture media manufacturing programme, safety, user-friendly handling, convenience products, cost saving products, environmental conservation and much more.

Merck's Central quality assurance laboratory

Every Merck employee is trained to practice quality for the benefit of customers. Everything we do is done according to the maxim:

"Merck creates quality. Quality is our commitment"

Service

Merck supports its microbiology customers world wide with technical information, educational publications and product support services. These include:

- Custom made peptones and culture media programme
- Fermentation and cell culture optimisation programme
- Brochures (www.chemdat.info)
- Meaningful batch specific certificates of analysis that can be downloaded from the internet (www.chemdat.info)
- Safety data sheets on a ChemDAT CD-ROM or via the internet (www.chemdat.info)
- Organising seminars and symposia
- Organising training courses
- (Local Merck or VWR) microbiology news letters, or magazines
- Local hot lines for technical support
- A microbiology Regulatory Affairs unit
- Microbiology sales specialists
- Innovative products

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EUROPEAN PHARMACOPOEIA 2002 Fourth edition Section 5.2.8 Minimising the risk of transmitting animal spongiform encephalopathy agents via medicinal products.

INTERNATIONAL STANDARDISATION STANDARD ISO 11133 part 2 2002 Microbiology of food and animal feeding stuffs. Guidelines on preparation and production of culture media - Part2: Practical guidelines on performance testing of culture media.

NATIONAL COMMITTEE FOR CLINICAL LABORATORY STANDARDS 1996. Approved standard M22-A2. Quality assurance for commercially prepared microbiological culture media, 2nd ed. NCCLS, e, Pa.

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About Regulatory Affairs and Documentation

Regulatory Affairs Department

The approval processes in the biopharmaceutical industry are becoming more and more stringent and comprehensive. This is also true for dehydrated culture media. The government agencies' regulations are becoming increasingly strict as is illustrated by the increasing demands for documentation and certification on the safety of products used in Microbiology laboratories.

Merck KGaA is an international operating pharmaceutical and chemical company with a worldwide network of subsidiaries and well prepared to meet all of the customer's regulatory requirements. Merck's Regulatory Affairs Department operates in a matrix structure with generalists and dedicated specialists for each area of expertise, as for example, for microbiology. This matrix structure leverages existing long standing pharmaceutical regulatory know-how to specific microbiology regulatory issues and promotes synergies.

The Regulatory Affairs Department plays a key leadership role in internal - and external (supplier) auditing, purchasing, product development, manufacturing, dispensing, quality control and quality assurance. Our customers can be provided with competent support in a continually evolving and complex manufacturing, quality control and quality assurance. The microbiology Regulatory Affair specialists interact with customers, scientists and experts within Merck, as well as in academia and at renowned research centres, and is involved in industry associations, for example, the International Pharmaceutical Excipients Council.

Merck microbiology offers product-specific answers to numerous time-sensitive inquiries, for example, about the topic of Transmissible Spongiform Encephalopathy (TSE), the use of genetically modified base materials, kosher and halal certificates, safety issues etc.

Merck customers can download certificates of analysis and safety data information sheets from the Merck website at www.chemdat.info.

Merck's key accounts, those from the pharmaceutical sector, among others, source products not only from one, but from two or more divisions at Merck. Merck has harmonised and standardised its regulatory affairs processes. Customers no longer have to differentiate between the divisions where they buy their products **- they just buy them "at Merck"**.

Transmissible spongiform encephalopathy (TSE)

Culture media contain animal-based raw materials and these are at risk of transmitting agents of animal spongiform encephalopathy. Merck prides itself that all culture media are in compliance with the guidelines recommended by European Department for Quality of Medicins (EDQM) on the manufacturing of drugs: Resolution of the Public Health Committee Partial Agreement, Resolution AP-CSP (99-4) and the European Pharmacopoeia section 5.2.8 Minimising the risk of transmitting animal spongiform encephalopathy agents via medicinal products.

A **certificate of suitability** is obtained for all animal based raw materials at TSE risk employed in the culture media manufacturing. These certificates of suitability are only one part of the measures to minimise the risk of TSE via culture media. The animal based peptones Merck uses for the manufacturing of culture media are coming from non-BSE countries only: New Zealand, Australia and the United States of America.

Other animal based ingredients have been replaced by synthetic or non-animal based ones, where possible. Any change in ingredients has to be approved by Regulatory Affairs and documented.

Merck operates a trackable supply chain with information on the origin of the product, its quality and processing, and provides this information to our customers. Furthermore, Regulatory Affairs Department evaluates the origin and the production processes of our suppliers of raw materials. Regulatory Affairs performs regularly detailed internal and external audits. Merck has implemented the ISO 9001 quality assurance system assuring in particular traceability and batch consistency. Non commingling procedures are practised and TOC monitoring is put in place to validate strict adherence to hygiene.

When all of the evidence is conclusive, Merck issues a certificate documenting in detail what steps were taken to minimise the TSE risk.


MERCK

BSE/TSE-Certificate

Figure: A typical example of a TSE certificate

Certificate of suitability

The Resolution of the Public Health Committee (Partial Agreement, Resolution AP-CSP (99) 4) states that suppliers of raw materials with TSE risk used in the production or preparation of medicinal products can apply for a certificate of suitability concerning the evaluation of the reduction of TSE risk. All animal based raw materials with risk of transmitting TSE which are to be used in the manufacturing of culture media at Merck have obtained this certification.

A certificate of suitability is only granted by the Certification Secretariat of the European Directorate for the Quality of Medicines (EDQM). A manufacturer of an animal based raw material must proof to EDQM that the quality of the substance is suitably controlled by the relevant monographs of the European Pharmacopoeia. The manufacturer provides EDQM with a full dossier describing in detail the source of animals and tissues used, manufacturing method, traceability, Quality System in force and a declaration of its willingness to be inspected upon request by a relevant authority. If independent experts whose impartiality is guaranteed by their status have assessed and approved the application, EDQM will grant a certificate of suitability.

The certificate of suitability certifies that a raw material is suitable for use in medicinal products. The certificate ensures that all possible impurities and contamination from the specified and documented route of manufacture (including source materials) can be fully controlled.

The certificate of suitability allows a manufacturer of culture media to communicate the compliance with Directives 2001/83/EC and 2001/82/EC in their marketing and promotional materials.

Table: Overview of Certificates of suitability (CEP) for meat peptones

	Certificate of suitability EDQM Reference number CEP
Peptone from Meat pancreatic digested	2000-238-Rev
Peptone from Meat peptic digested	2000-253-Rev
Meat extract	2000-12-Rev

Change of notification program

Merck offers an automated notification programme to customers who requested notification of changes in manufacturing, quality control, quality assurance, and documentation. As a pharmaceutical company, Merck understands the importance of changes and and well aware to communicate such changes to its customers. The change of notification program is also part of its ISO certified quality systems.

Compliance with pharmacopeia and standards

Merck manufactures all dehydrated culture media in compliance with the formulations as specified in the European Pharmacopeia (EP), United States Pharmacopeia (USP), Deutsches Arzneimittelbuch (DAB10), and reference protocols as outlined in other international standards like FDA-BAM, AOAC, APHA, USEPA, Standard Methods for the Detection of Water and Wastewater (SMWW) and Standard Methods for the Detection of Dairy Products (SMDP), just to name a few, and/or to publications of the developer(s).

All dehydrated culture media manufacturers, sometimes have to "adjust formulations" due to the nature of ingredients, in particular the chemically undefined "natural" ingredients like peptones to guarantee a consistent lot to lot performance. The formulations are therefore typical compositions similar to ISO.

ISO 11133 part 2, 2003 states that manufacturers of dehydrated culture media may modulate the composition of a formulation to meet performance criteria.

In the product monographs of this Microbiology Manual peptones are listed by their traditional names as documented in reference protocols, or, if applicable, in the article published by culture media developer(s). Unlike on the product label, the chemical ingredients in the product monographs are listed without the water content. Furthermore the amount of an infusion has been converted to the equivalent dry weight, which is consistent with its use in dehydrated culture media.

For checking the composition against a reference formulation it is advised to use the composition on the label.

Label

The label of a dehydrated culture medium or supplement gives information for the name of the product, the typical composition, required supplements, preparation instructions, pH value, storage information, expiration date and catalogue number, lot number, health & safety information, CE certification, usage in vitro information, and pack size.

Batch or lot

The contents of a pot of dehydrated culture medium is traceable to a defined amount of bulk, semi-finished product or end product. A lot or batch number is given to the medium which has been produced within one defined production run.

The batch or lot number is printed on the label (Figure). A batch or lot is consistent in type and quality A product with a lot number reaches the end user when the medium passed in-process control (the requirements of production) and the final quality assurance testing at the central quality control laboratory at Merck that operates independent of production, sales and marketing.

Technical Data Sheet

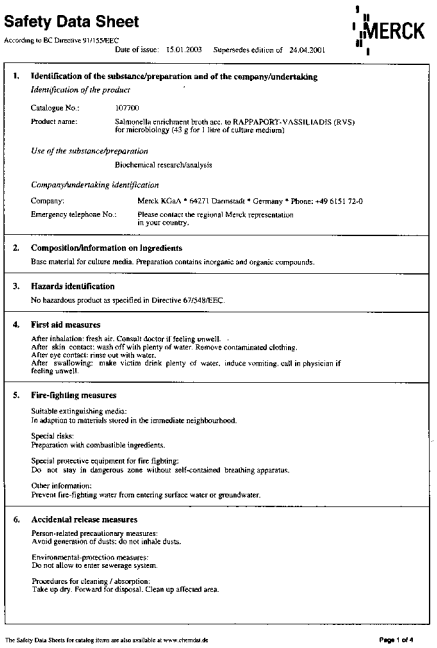
A technical data sheet is given in the ChemDAT database. The information is available on CD-ROM or can be downloaded from internet www.chemdat.info.

A technical data sheet gives:

- the catalogue number
- complete product name
- general product information (Bulk density, health and safety code, storage temperature, R-phrase, S-phrase)
- chemical and physical data (Odour, form, colour, pH value, solubility in water)
- toxicological data
- (Package information, catalogue number, package, and packaging)
- important notes/links providing additional information

Safety Data Sheet (SDS)

Dehydrated culture media are For Laboratory Use. Some dehydrated culture media contain hazardous and possibly toxic components. These media are labeled accordingly. Hazardous and/or toxic substances in culture media formulations include a.o. sodium selenite, sodium azide, cycloheximide, dichloran, brilliant green, malachite green, bile salts, fuchsin, tetrathionate, thiocyanate, rose bengal, chloramphenicol, kanamycin, lithium chloride and tergitol. In particular, selenite and sodium azide are very toxic and dangerous when inhaled.



Safety Data Sheet

According to EC Directive 91/155/EEC

Date of issue: 15.01.2003 Supersedes edition of 24.04.2001

1. Identification of the substance/preparation and of the company/undertaking

Identification of the product

Catalogue No.: 107700

Product name: Salmonella enrichment broth acc. to RAPPAPORT-VASSILIADIS (RVS) for microbiology (45 g for 1 liter of culture medium)

Use of the substance/preparation

Biochemical research/analysis

Company/undertaking identification

Company: Merck KGaA * 64271 Darmstadt * Germany * Phone: +49 6151 72-0

Emergency telephone No.: Please contact the regional Merck representation in your country.

2. Composition/information on ingredients

Base material for culture media. Preparation contains inorganic and organic compounds.

3. Hazards identification

No hazardous product as specified in Directive 67/548/EEC.

4. First aid measures

After inhalation: fresh air. Consult doctor if feeling unwell.
After skin contact: wash off with plenty of water. Remove contaminated clothing.
After eye contact: rinse out with water.
After swallowing: make victim drink plenty of water, induce vomiting, call in physician if feeling unwell.

5. Fire-fighting measures

Suitable extinguishing media:
In adaption to materials stored in the immediate neighbourhood.

Special risks:
Preparation with combustible ingredients.

Special protective equipment for fire fighting:
Do not stay in dangerous zone without self-contained breathing apparatus.

Other information:
Prevent fire-fighting water from entering surface water or groundwater.

6. Accidental release measures

Person-related precautionary measures:
Avoid generation of dusts; do not inhale dusts.

Environmental-protection measures:
Do not allow to enter sewerage system.

Procedures for cleaning / absorption:
Take up dry. Forward for disposal. Clean up affected area.

The Safety Data Sheets for catalog items are also available at www.chemdat.de

Page 1 of 4

Figure: A typical example of a safety data sheet

Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. When disposing, flush with a large volume of water to prevent creation of such azides.

Safety information on ingredients and culture media are summarised in ChemDAT manual. The ChemDAT manual is available on CD-ROM or can be down loaded from internet www.chemdat.info.

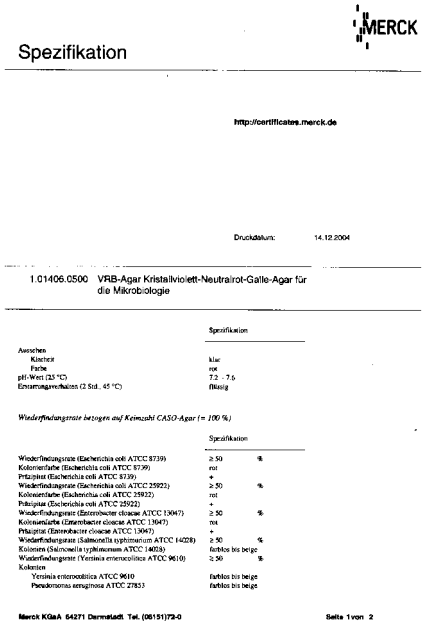
A safety data sheet gives in a total of 16 sections information about the name of the substance or product, information on the preparation, information on the composition, the identification of hazardous ingredients, first aid measures, fire fighting measures, accident release measures, handling and storage, exposure controls and personal protection, physical and chemical properties, toxicological information, ecological information, disposal considerations, stability and reactivity, information on transport, regulatory information and in section 16 other safety information that are country specific.

Certificate of Analysis and ISO 11133

Merck provides a batch or lot specific certificate of analysis (CofA). The necessary tests on the finished product are done at Merck's central quality assurance laboratory which operates independent from production, sales and marketing. The procedures for testing of dehydrated culture media and its supplements comply with ISO 11133 part 2 and the requirements of the European (EP) and the United States Pharmacopoeia (USP) and Deutsche Arzneimittelbuch (DAB 10).

A typical certificate of analysis contains basic information, s.a. the catalogue number, the product name and the batch number followed by technical information about appearance i.e. colour, clearness, solidification point (for agar media), pH, stability test

i.e. colour and haemolysis. Then the batch specific microbiological test results follow: defined collection test strains used (e.g. ATCC) are listed and the test results s.a. growth performance which includes recovery rate, selectivity ratio, incubation conditions. As an electronic copy it also contains the name of QC supervisor who is responsible to release a product lot to be sold worldwide.



MERCK

Spezifikation

http://certificates.merck.de

Druckdatum: 14.12.2004

1.01406.0500 VRB-Agar Kristallviolett-Neutralrot-Galle-Agar für die Mikrobiologie

	Spezifikation
Aussehen	
Klarheit	klar
Farbe	rot
pH-Wert (25 °C)	7.2 - 7.6
Einsatzungsverhalten (2 Std., 45 °C)	flüssig

Wiederfindungsrate bezogen auf Keimzahl CASO-Agar (= 100 %)

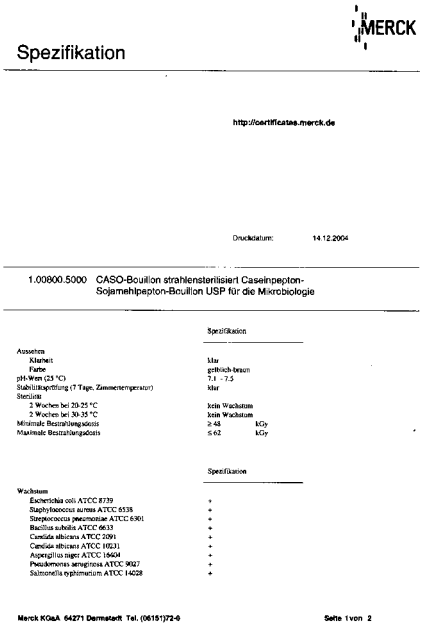
	Spezifikation	
Wiederfindungsrate (Escherichia coli ATCC 8739)	≥ 50	%
Kolonienfarbe (Escherichia coli ATCC 8739)	rot	
Prüfspezifität (Escherichia coli ATCC 8739)	+	
Wiederfindungsrate (Escherichia coli ATCC 25922)	≥ 50	%
Kolonienfarbe (Escherichia coli ATCC 25922)	rot	
Prüfspezifität (Escherichia coli ATCC 25922)	+	
Wiederfindungsrate (Enterobacter cloacae ATCC 13047)	≥ 50	%
Kolonienfarbe (Enterobacter cloacae ATCC 13047)	rot	
Prüfspezifität (Enterobacter cloacae ATCC 13047)	+	
Wiederfindungsrate (Salmonella typhimurium ATCC 14028)	≥ 50	%
Kolonien (Salmonella typhimurium ATCC 14028)	farblos bis beige	
Wiederfindungsrate (Yersinia enterocolitica ATCC 9610)	≥ 50	%
Kolonien		
Yersinia enterocolitica ATCC 9610	farblos bis beige	
Pseudomonas aeruginosa ATCC 27853	farblos bis beige	

Merck KGaA 64271 Darmstadt Tel. (06151)72-0 Seite 1 von 2

Figure: A certificate of analysis for a plating agar

The recovery rate and selectivity ratio of a plating agar is expressed in %. For a specific test strain it is the ratio of the count on the newest batch compared to a non selective reference medium (e.g. blood agar) times one hundred (ISO 11133 part 2: the ratio as such is given as e.g 0.7 and is identical to the >70% Merck specifies on the certificate of analysis). But this is not enough: on top of this the performance of a gold standard medium (medium batch with best performance) is run in parallel. If the new batch performs better than the previous gold standard, it becomes the new gold standard. Media from other manufacturers are also tested at the same time, so that Merck can ensure that its culture media have a consistent high quality.

Also for a liquid media (broth) the certificate of analysis specifies, similar to a CofA for plating agars, quantitative performance information (see Figure).



MERCK

Spezifikation

http://certificates.merck.de

Druckdatum: 14.12.2004

1.00800.5000 CASO-Bouillon strahlensterilisiert Caseinpepton-Sojamehlpepton-Bouillon USP für die Mikrobiologie

	Spezifikation	
Aussehen		
Klarheit	klar	
Farbe	gelblich-braun	
pH-Wert (25 °C)	7.1 - 7.5	
Stabilitätsprüfung (7 Tage, Zimmertemperatur)	klar	
Sterilität		
2 Wochen bei 20-25 °C	kein Wachstum	
2 Wochen bei 30-35 °C	kein Wachstum	
Minimale Bestrahlungsdosis	≥ 48	kGy
Maximale Bestrahlungsdosis	≤ 62	kGy

	Spezifikation
Wachstum	
Escherichia coli ATCC 8739	+
Staphylococcus aureus ATCC 6538	+
Streptococcus pneumoniae ATCC 6301	+
Bacillus subtilis ATCC 6633	+
Candida albicans ATCC 2091	+
Candida albicans ATCC 10231	+
Aspergillus niger ATCC 16404	+
Pseudomonas aeruginosa ATCC 9027	+
Salmonella typhimurium ATCC 14028	+

Merck KGaA 64271 Darmstadt Tel. (06151)72-0 Seite 1 von 2

Figure: A certificate of analysis for a liquid medium

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An Overview of Merck Microbiology Products and Services

Why choosing Merck microbiology products?

As one of the global market leaders in analytical reagents, Merck is a company whose products are found in virtually the entire world's scientific and quality control laboratories. Merck's innovative strength stems not only from a thorough knowledge of the market and product applications but also through its close co-operations with customers to guarantee of having special user-target features for own new and custom-made products. Merck undertakes everything itself: research and development, manufacturing and distribution.

All over the world Merck and EMD (Merck's name in North America) supply a complete product range for laboratory and production applications for microbiology, microscopy, for food, beverage, disinfectants residue, water and wine analyses.

Merck has always taken great care to ensure that its products are of the highest quality. As early as about 1850, Heinrich Emanuel Merck already offered guarantees on his products. This tradition has been maintained and will in the future.

Since the beginning of microbiology at Merck in 1885, the principles of quality control and quality assurance, implemented at Merck's pharmaceutical division, were applied to microbiology. It is a tradition since 1892 that Merck microbiology products are tested in the central quality assurance laboratory.

In addition to the central quality assurance laboratory, Merck microbiology benefits from a corporate Regulatory Affairs Department with a specialisation in microbiology, a corporate Quality Assurance Department, a corporate purchasing department, corporate dedicated auditors and numerous specialised laboratories in fields of pharmaceutical, chemical, analytical and diagnostic research.

Merck is a pharmaceutical company and this is unique in culture media manufacturing. Merck has more than 100 years experience in the manufacturing of agar-agar, peptones and dehydrated culture media. Merck manufactures granulated agar-agar, peptones and dehydrated culture media. Granulated culture media combine safety and optimal performance with clear advantages over powdered material from other suppliers.

Choosing Merck microbiology products is a choice to benefit from the more than 100 years of experience, a tradition of quality, innovation and service combined with technical support from specialists, a pharmaceutical supplier with audit and quality assurance programs, TSE low risk (Category B or C) products, clear and meaningful certificates of analysis, compliance with standards, innovative products, convenience products and global representation.

The product portfolio described in this manual is limited to microbiology products. Information on Merck products for non microbial analysis of drinking water, wastewater, food, beverages, wine, disinfectant residues can be found in other specific manuals, brochures and on the Merck website at www.microbiology.merck.de or www.emdchemicals.com.

Agars and peptones

Working with powdered agar-agar and peptones is unhealthy and risky. Powdered base materials spread dust into the environment and this dust is inhaled by people and also contaminates the production area. Merck manufacturers granulated agar-agar and peptones which combine safety and optimal performance and offer clear advantages.

Working with granulated base materials produces far less environmental contaminations. Granulated base materials have optimal flow properties and dissolve quickly.

Safe

Animal-based raw materials bare the risk of transmitting animal spongiform encephalopathy (TSE). As a pharmaceutical company, Merck is well equipped to ensure that its animal base materials comply with the guidelines of the European Department for Quality of Medicine (EDQM), that is, Resolution of the Public Health Committee (Partial Agreement, Resolution AP-CSP (99) and European Pharmacopoeia Section 5.2.8 Minimising the risk of transmitting animal spongiform encephalopathy agents via medicinal products. Ingredients at TSE risk belonging to safety category B or C and sourced from New Zealand or Australia.

Contact us if you need a base material that you do not find in this manual. Our technical support and research group is available to work with you to prepare custom-made base material or offer the support in optimising your yield or help you with media formulation scale up.

A choice for Merck base materials is a choice for:

Experience	More than 100 years
Unique products	Granulated base materials
Convenience	Granules do not form lumps
Cost savings	Granules dissolve quickly Batch size up to 4.5 tons
Documentation	Certificates of Suitability (CofS) Certificates of Origin (CofO) Certificates of Analysis (CofA)
Flexibility	Custom-made base materials Custom-made Certificates of Analysis
High yields	High quality base materials Optimisation support
Low TSE risk	Base materials at TSE risk sourced from New Zealand and Australia and Category B or C

Non animal peptone mixes

Safety	50% less dust
Service	Custom-made program Optimisation program

Custom-made peptones and media formulations

Merck runs a custom-made media programme. In this programme, Merck shares with customers its over 100 years of experience in peptone and culture media manufacturing. A dedicated team of responsive media specialists, microbiologists, chemists and regulatory affairs specialists at Merck are available for the customer.

Merck is your **partner** if you have specialised requirements for:

- a formulation not listed in this manual
- a formulation listed in this manual
- a specific fermentation medium
- a proprietary formulation
- granulated mixes
- quality control/quality assurance
- certificates
- container configurations

In the custom-made media programme Merck manufactures the culture medium that satisfies the customer's specific requirements. In a **partnership** with the customer, a certificate of analysis is set up and agreed. If the information the customer shares with Merck is proprietary, a confidentiality agreement is signed in the beginning to protect the customer's information. Merck prepares a pre-sample which is quality controlled against the agreed certificate of analysis. When the pre-sample passes quality control, it is shipped to the customer. If the customer's validation testing approves the pre-sample, Merck will produce the special medium with the raw materials which were reserved at the time when the pre-sample was prepared and approved. Merck then conducts a final quality assurance testing and provides the documentation a.o. the certificate of analysis, certificate of origin and technical documentation.

For cost reasons, a custom made culture medium should have a minimum batch size of 200 kg per order. Also these custom-made culture media have a shelf-life of 3-5 years (depends on ingredients and if powder or granulated) Batches below 200 kg may be possible (contact your local Microbiology specialist).

At Merck your special media requirements count!

Media optimisation programme

The media optimisation program allows the biopharmaceutical customers to benefit from Merck's experience in yield enhancement and media formulation scale up.

Upon customer's request, Merck media experts can study your production engineering, your organisms and your environment. If the information the customer shares with Merck is proprietary a signed confidentiality agreement will protect your information.

Dehydrated culture media

Granulation

Merck's dehydrated culture media are, apart from a few exceptions, produced in granulated form. Granulated culture media offer several advantages when compared to equivalent powder culture media:

- Considerably less dust is formed when handling the media: the danger of allergic reactions and inhalation of toxic substances are largely eliminated.
- Better flow properties: the media do not stick to the walls of vessels or apparatus and therefore easier to weigh out.
- Better coating of the granules with water: reduces the time required for suspending and dissolving the media. Formation of clumps, which are hard to dissolve, is thus prevented.
- Homogeneous distribution of the package contents is ensured even after prolonged storage. Because of the granulation the components do not separate out.
- Longer shelf life (3-5 years) due to
  - low water content
  - homogeneous distribution of the contents

No need for additonal supplements

Supplements are expensive. Whenever possible, Merck's granulated culture media already contain the selective components in the base medium. There is not always the need to purchase expensive supplements.

Overview of the media in which selective ingredients are incorporated in the base

Parameter	Selective ingredients included in base	Cat. no.
Cl. perfringens	SPS Agar	1.10235.
Dermatophytes	DTM Agar	1.10896.
E.coli O157	m-EC Broth	1.14582.
	m-TSB Broth	1.09205.
Enterococci	KAA Agar	1.05222.
	KF Agar	1.10707.
	Kranep Agar	1.05395.
Listeria	LEB Broth	1.10549.
	UVM Broth	1.10824
Salmonella	Selenite media	1.07709.
		1.07717.
Yeast and moulds	DG18 Agar	1.00465.
	DRBC Agar	1.00466.
	RBC Agar	1.00467.
	YGC Agar	1.16000.
Y.enterocolitica	YSE Broth	1.16701.

Transmissible Spongiform Encephalitis (TSE)

All Merck dehydrated culture media comply with guidelines recommended by European Department on Quality of Medicine (EDQM) i.e. Resolution of the Public Health Committee (Partial Agreement, Resolution AP-CSP (99) and European Pharmacopoeia Section 5.2.8 Minimising the risk of transmitting animal spongiform encephalopathy agents via medicinal products. The ingredients at TSE risk belong to safety category B or C. Animal based materials at TSE risk originate from New Zealand. Merck prefers the use of ingredients of non-animal origin.

TSB non-animal gives pharmaceutical companies concerned about transmissible spongiform encephalopathy (TSE) the opportunity to conduct sterility controls with a medium of non-animal origin.

Compliance with standards

All Merck's dehydrated culture media are formulated and tested using International Standard Organisation, United States- , European-, Culture media pharmacopoeia, Deutsches Arzneimittelbuch, FDA-BAM, USDA-FSIS, APHA, AOAC, §35 as benchmarks.

Certificates of analysis

Merck provides for its dehydrated culture media meaningful certificates of analysis, technical data sheets, product safety data sheets.

Typical composition

Some of the basic constituents of the culture media are natural products and their properties may therefore vary from batch to batch. In order to obtain reproducible results, when cultivating microorganisms, these variations must be corrected in certain cases by adjusting the amounts of substances used in the manufacture of the dehydrated culture media. Therefore, in compliance with, for example, ISO 11133 part 2 "typical" compositions of the dehydrated culture media are specified.

As with all our products for microbiological applications, dehydrated culture media undergo stringent quality controls from the raw materials to the finished product. With these controls we want to ensure, that despite of the variations, which always occur with natural substances, we can provide high consistent quality dehydrated culture media to our customers. In the descriptions of the individual culture media the strains, which our laboratories use for testing in quality control and their reactions are listed. These define the microbiological properties of the individual culture medium concerned.

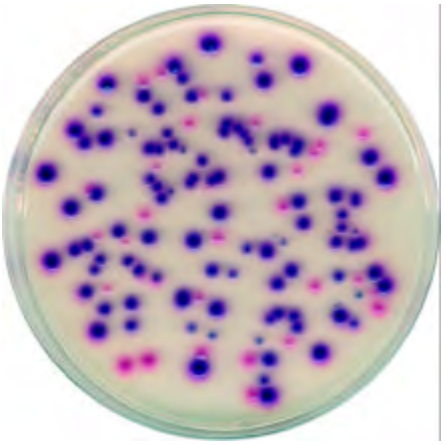


Figure: Chromocult® Coliform Agar
Salmon to red colonies: coliform bacteria
Dark violet colonies: E.coli

Fluorocult® and Chromocult® media

Detection of characteristic bacterial enzymes provides a fast way of detecting and identifying bacteria. With Fluorocult® media, fluorescence from a specific enzyme reaction provides the evidence when the sample contains E.coli or *Cl.perfringens*.

Chromocult® is Merck's colourful way of achieving savings in preparation, incubation, material and labour costs. The chromogenic substrates used in Chromocult® media give a clear distinguishable colour to each separate colony type allowing clear differentiation and identification.

Typical composition

The opening of glass and metal-capped supplements is not easy. Why do it the difficult way if there is an easy way. Merck's supplements have a user-friendly screw cap, which is easily turned to open the vial without running the risk of cutting, creating aerosols and possibly inhaling supplement contents.

Supplements are expensive. There is not always the need to purchase such expensive supplements. Save on your culture media expenditure. Merck offers granulated dehydrated culture media in which selective agents are incorporated in the base medium.

Ready to use culture media

The range of ready-to-use agar media include plating agars, single or triple wrapped contact plates, Envirocheck® contact slides and ReadyBag, pre-weighted bags of sterile granular enrichment media.

Agar plates

Since 1970 Merck uses a fully automated manufacturing process of preparing plating agars in a class 100 cleanroom environment.

The process starts with the manufacturing of the Petridishes using prepared polystyrene beads. Afterwards each Petridish is filled with culture medium. The culture medium is pumped via a pipe system directly from the autoclave to the filling station. The freshly poured plates are cooled on a conveyor belt. At last a robot arm puts a lid on each plate. The plates are automatically stacked to a set of 5 plates and a foil shrink-wrapped around each stack. The foiled stack of plates are then transported from the class 100 environment to a class 10.000 production area and packaged into boxes.

Like all other Merck products, each batch of ready-to-use culture media is quality controlled in the central quality control laboratory. This represents a second quality control, because the used media for the prepared plates were already quality controlled before and passed.

Envirocheck® contact plates

Envirocheck® Contact plates are used for hygiene monitoring. Merck's contact plates for surface testing are 56mm in diameter, triple wrapped and gamma irradiated for use in isolator/cleanroom environments. The programme includes contact plates for total viable counts (with and without neutralisers), and yeast and moulds.

Contact plates are also available in a blister packaging. This type of snap pack format originates from pharmaceutical, enabling the use of a single plate while the other plates in the pack remain sterile. The double packaging and sterilisation by gamma-irradiation allows usage in sterile environments.

Economical	Use of a single plate
Convenient	Storage at room temperature
Multifunctional	Double or triple wrapped for use cleanroom environments

Envirocheck® contact slides

Envirocheck® contact slides with a flexible paddle are widely used in the cleaning and disinfection control of surfaces and liquids. They can also be used for testing other samples. Here a 0.02 ml sample is spread over the surface of the slide.

Merck's Envirocheck® contact slides programme includes products for total colony counts, coliform bacteria, E.coli, Enterobacteriaceae, and yeast and moulds.

Liquid media - ReadyBag system

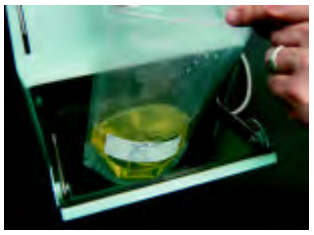
In 2004 Merck launched its new ReadyBag system, special plastic bags of different sizes with pre-weighted granular media and pre-sterilised by gamma-irradiation. The 3 step sample preparation system is easy to handle, requires minimal storage space, saves cost and reduces waste disposal.

Just add sterile water !

The granules dissolve within minutes.

Figure: **Pre-weighed sterile granular media:**
1. add sterile water
2. add sample
3. place in blender/homogeniser



Anaerobic and microaerophilic culturing

The different culturing methods require different environments. For an anaerobic or microaerophilic environment, the Merck programme includes a 2.5liter anaerobic jar, plate basket and the necessary anaerobic and microaerophilic gas kits called Anaerocult® A and Anaerocult® C. Plates can also be incubated separately in the appropriate pouch sytem, called Anaerocult® P, Anaerocult® A-mini and Anaerocult® C-mini. For microtiter plates Anaerocult® IS is used. All **Anaerocult®** products are **catalyst-free** for a safe environment.

Fixing and staining

Dyes are used in microscopy whenever microorganisms, cell and tissue components in animal and plant material have to be visualised. In order to make microorganisms suitable for microscopic staining, they have to be fixed and stained with suitable dyes. Gram-staining, spore staining and the detection of mycobacteria are of particular importance. In the investigation of foodborne outbreaks, fluorescence staining using acridine orange staining is employed.

The products for fixing and staining of micro-organmisms summarised in this manual are just a small part of the microscopy product line of Merck, which includes universal reagents and dyes, products for electron microscopy, haematology, histology and parasitology.

Identification

For identification of bacteria Merck has developed the Bactident® product range. This producy line includes key identification tests s.a. aminopeptidase (Gram-positive or negative test), oxidase, catalase, indole and coagulase test. Bactident® E.coli allows the identification of E.coli and Bactident® Staph plus that of coagulase positive Staphylococci (*Staph. aureus*).

Apart from these tests, the programme includes basal media such as DNase Test Agar, Kligler Agar, MR-VP Broth, Nutrient Gelatin Agar, OF-Basal Medium, Phenol Red Broth, SIM Medium, Simmons Citrate Agar, Triple Sugar Iron Agar, Tryptone Water and Urea Agar. Moreover, Kovac's indole reagent and a range of sugars and chemicals (e.g. potassium tellurite) are summarised in the Merck Chemical Product Manual.

MAS - Air sampler

There are strict rules governing the quality standards and the regular monitoring of air in areas such as cleanrooms in the pharmaceutical industry, surgery theatres in hospitals and other high risk areas in the food industry, e.g. in the filling area of beverage companies.

The measurement of a possible air contamination in cleanrooms requires a highly precise and reliable air sampler. The MAS-100 series is a family of air samplers developed by a team of users from the international pharmaceutical industry together with metrology engineers, technicians and designers. The MAS-100 series combine high-tech expertise and swiss made reliability with a unique design. The MAS-100 series include: MAS-100, MAS-100Ex (exlosion proof version), MAS-100Eco (version for the food industry), MAS-100ISO (unit for isolator and clean-room) and MAS-100CG (for compressed gases, e.g. nitrogen).

The MAS-100 Air Sampler series is based on the Anderson principle, the most accepted concept of microbial impactation samplers. The air samplers comply with ISO 14698 part 1, in which the air flow is specified with 100 liters per minute and a horizontal air velocity of 0.45 m per second, which is isokinetic. MAS-100 air samplers have a built-in compensation feature, which guarantees a constant 100 liter measurement with every sample taken.

MAS-100 air samplers use standard 90-100 mm Petridishes. This means, the same plates that have been validated for use in the laboratory can be used for air monitoring as well. No additional validation is needed, which saves a lot of money. There is no need to purchase expensive special plates or strips, which have to be validated additionally, adding more costs to each test. Apart from 7 pre-selected volumes any volume between 1-2000 liters can be chosen.

The MAS-100Eco was developed for less demanding fields which are not (yet) regulated by special standards. The MAS-100Eco does not have a built-in airflow compensator.

Figure: **MAS-100 Air Sampler (top), perforated lid (middle) and MAS-100 ISO (bottom)**

Hygiene monitoring

Hygiene monitoring has become an extremely important component of a safe production and hospital environment. Its aim is to bring the monitoring of all safety and quality-relevant factors forward within a production or hospital environment and minimising risks right from the beginning.

The hygiene monitoring programme includes traditional culture media, i.e. contact plates and dip-slides as well as the HY-LiTE® instrument based ATP-bioluminescence system and additionally HY-RiSE®, a simple rapid testing test strip based on NAD technology measurements.

The HY-LiTE®2 system enables objective assessment of cleanliness within 2 minutes. It is based upon the rapid, quantitative detection of ATP in food residues and other sources of contamination. The HY-LiTE®2 system is a portable and lightweight luminometer with a large and easy to read display and a built-in printer. The luminometer can be operated as a 'Test Only Device' with direct printout of test results, as a 'Test and Store Device' storing up to 2000 results for later printing or for downloading to a computer or as a part of an HACCP plan using the integrated TREND®2 software, a powerful analysis and data presentation package compatible to Windows 95, NT and XT. The system also includes a sampling pen, a pre-prepared, one shot device available in two formats - one for surface testing - the other one for liquid testing, i.e. rinse waters.

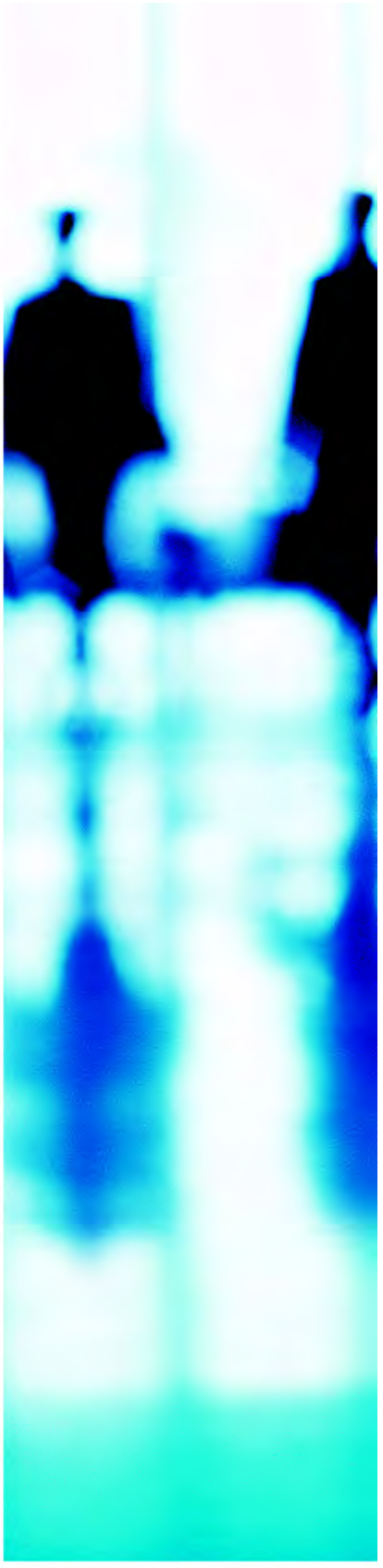
HY-RiSE® is a non instrumental test strip for checking the cleanliness of surfaces, liquids and also used for hand-testing. It is a simple three-drop test that produces a test result on cleanliness in a colour reaction. It is based upon the rapid qualitative (clean/unclean) detection of NAD/NADH in food residues and other sources of contamination.

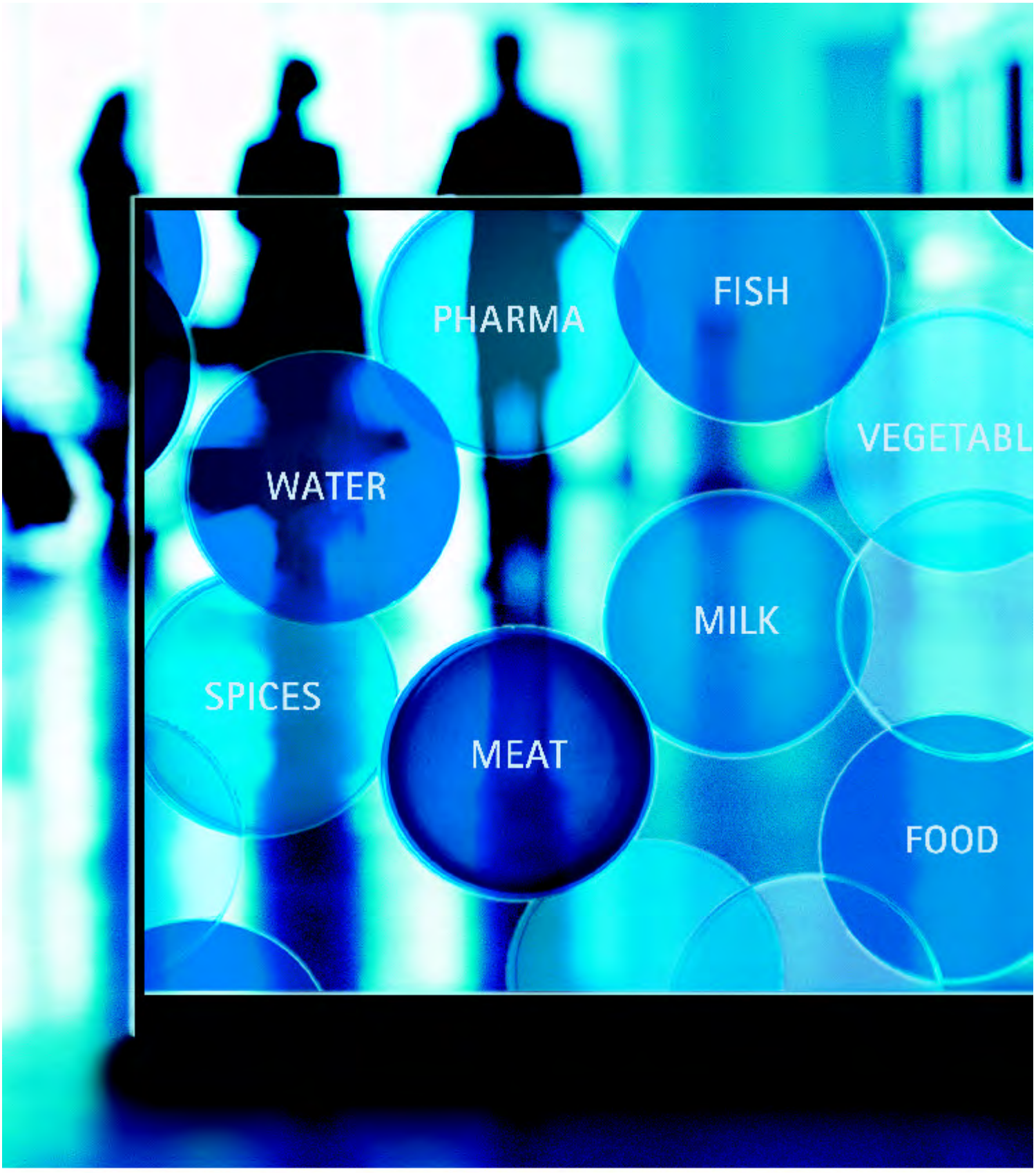
Sterility testing

The sterility testing programme includes dedicated dehydrated culture media, spore suspensions and Sterikon®plus, an ampoule used to check the efficiency of a steam sterilisation process of an autoclave. For sterility control and media fill test the pharmacopoeia recommend Tryptic Soy Broth (TSB), Fluid Thioglycollate Medium (FTM) and Thioglycollate Broth. These Merck products are marked by a high clearness combined with excellent filtration properties. The granulated form of these media ensures less dust, no sticking and quick dissolution. To ensure product safety for a vaccine manufacturer, Merck introduced TSB non-animal origin, triple wrapped and gamma-irradiated. This medium is guaranteed free of Mycoplasma, HEPA cells and spores.

Figure: **HY-LiTE®2 bioluminescence hygiene monitoring**

Review of Special Fields of Application




PHARMA
FISH
VEGETABL
WATER
MILK
SPICES
MEAT
FOOD

CANNED FOOD

Detection of Pathogens

	Merck Cat. No.	Reference Method	Bacillus cereus	Pathogenic bacilli	Cl. perfringens	Listeria	Salmonella	Staph. aureus
Pre-Enrichment								
Buffered Peptone Water	1.07228.	BAM, APHA					✓	
Lactose Broth	1.07661.	APHA, SMWW, EPA, EP, USP, AOAC					✓	
Enrichment								
Buffered Listeria Enrichment Broth + FDA-BAM 1995 Supplement	1.09628. + 1.11781.	BAM, APHA				✓		
DIASALM + Novobiocin Supplement	1.09803. + 1.09874.						✓	
Differential Reinforced Clostridial Medium (DRCM)	1.11699.	DIN			✓			
Fraser Broth Base + Fraser Supplement	1.10398. + 1.10399.	ISO				✓		
Giolitti-Cantoni Broth	1.10675.	DIN, IDF, ISO, APHA						✓
Listeria Enrichment Broth (LEB)	1.10549.	BAM, APHA				✓		
Listeria Enrichment Broth Base + FDA BAM 1992 Supplement	1.11951. + 1.11883.	IDF, BAM				✓		
Listeria Enrichment Broth Base + FDA-BAM 1995 Supplement	1.11951. + 1.11781.	IDF, BAM				✓		
L-PALCAM Broth	1.10823.					✓		
M Broth	1.10658.	SMWW, AOAC, APHA					✓	
MKTTn Broth	1.05878.	ISO					✓	
MSRV Medium Base Modified + Supplement	1.09878. + 1.09874.	AOAC					✓	

CANNED FOOD

Detection of Pathogens

	Merck Cat. No.	Reference Method	Bacillus cereus	Pathogenic bacilli	Cl. perfringens	Listeria	Salmonella	Staph. aureus
Rappaport Vassiliadis (RVS) Broth	1.07700.	ISO					✓	
Salmosyst Broth + Salmosyst Supplement	1.10153. + 1.10141.						✓	
Selenite Cystine Broth	1.07709.	APHA, DIN, USP, ISO, AOAC, BAM					✓	
Staphylococcus Broth (Baird)	1.07899.							✓
Tryptic Soy Broth + Polymyxin Supplement	1.05459. + 1.09875.		✓	✓				
UVM Broth	1.10824.	AOAC, APHA				✓		
UVM II Broth	1.10824. + 1.04039.	USDA				✓		

CANNED FOOD

Detection of Pathogens

	Merck Cat. No.	Reference Method	Bacillus cereus	Pathogenic bacilli	Cl. perfringens	Listeria	Salmonella	Staph. aureus
Isolation & Enumeration								
Baird Parker Agar Base + Egg-Yolk Tellurite Emulsion	1.05406. + 1.03785.	AOAC, BAM, ISO, EP, APHA, SMWW, USDA, USP						✓
Bismuth Sulfite Agar	1.05418.	AOAC, APHA, BAM, SMWW, USP					✓	
BPL Agar	1.07236.						✓	
BPLS Agar	1.07237.						✓	
Brilliant Green Agar Modified	1.10747.	ISO					✓	
Chapman Agar	1.05469.							✓
Deoxycholate Lactose Agar	1.02894.	APHA					✓	
Hektoen Enteric Agar	1.11681.	AOAC, BAM, ISO, APHA					✓	
MYP Agar + Polymyxin Supplement	1.05267. + 1.09875.	AOAC, BAM, ISO, APHA, USDA	✓	✓				
Oxford Agar Base + Oxford Supplement	1.07004. + 1.07006.	AOAC, BAM, ISO, APHA, USDA				✓		
PALCAM Listeria Agar Base + PALCAM Supplement	1.11755. + 1.12122.	BAM, APHA, ISO				✓		
Rambach Agar	1.07500.	FDA					✓	
Reinforced Clostridial Agar (RCA)	1.05410.				✓			
SPS Agar	1.10235.						✓	
SS Agar	1.07667.	APHA					✓	
TSC Agar	1.11972.	APHA, DIN, ISO			✓			
Vogel Johnson Agar	1.05405.	USP, EP, BAM			✓			
XLD Agar	1.05287.	APHA, EP, SMWW, AOAC, BAM, USP					✓	

CANNED FOOD

Marker Organisms Testing

	Merck Cat. No.	Reference Method	Aerobic Plate Count	Anaerobic Plate Count	Bacillaceae	Sulfite reducing Clostridia
Enrichment						
Differential Reinforced Clostridial Medium (DRCM)	1.11699.	DIN				✓
Reinforced Clostridial Medium (RCM)	1.05411.	EP				✓
Tryptic Soy Broth + Polymyxin Supplement	1.05459. + 1.09875.					✓
Isolation & Enumeration						
MYP Agar + Polymyxin Supplement	1.05267. + 1.09875.	AOAC, BAM, ISO, APHA, USDA			✓	
Plate Count Agar	1.05463.	APHA, ISO, USDA, EPA, SMDP, SMWW, AOAC, BAM	✓			
Differential Clostridial Agar (DCA) acc. to Weenk	1.10259.					✓
Reinforced Clostridial Agar (RCA)	1.05410.					✓
SPS Agar	1.10235.					✓
Standard I Nutrient Agar	1.07881.		✓			
Tryptic Soy Agar	1.05458.	EP, USP, AOAC, BAM, APHA, ISO, SMWW, USDA	✓			
TSC Agar	1.11972.	APHA, DIN, ISO		✓		
TSC Agar + Polymyxin Supplement	1.11972. + 1.09875.					✓

CANNED FOOD

Spoilage Organisms

	Merck Cat. No.	Reference Method	Aerobic Plate Count	Anaerobic Plate Count	Bacillaceae	Butyric anaerobes	Clostridia	Lactic acid bacteria (fastidious)	Lipolytic microorganisms	Spoilage	(Heat resistant) Fungi
Enrichment											
Bryant & Burkey Broth	1.01617.					✓	✓			✓	
Differential Reinforced Clostridial (DRCM) Broth	1.11699.	DIN					✓				
Reinforced Clostridial Medium (RCM)	1.05411.	EP					✓				
Isolation & Enumeration											
APT Agar	1.10453.	APHA, USDA	✓					✓			
Bryant & Burkey Agar (use broth and add Agar)	1.01617. + 1.01614.					✓	✓				
Dextrose Tryptone Agar	1.10860.	APHA, NCA			✓					✓	
Orange Serum Agar	1.10673.	APHA			✓						
Plate Count Agar	1.05463.	APHA, ISO, USDA, EPA, SMDP, SMWW, AOAC, BAM	✓								
Potato Dextrose Agar	1.10130.	APHA, USP, AOAC, BAM									✓
Reinforced Clostridial Medium (RCM)	1.05411.	EP					✓				
Sabouraud Dextrose Agar	1.05438.	EP, USP, BAM, APHA									✓
SPS Agar	1.10235.						✓				
Standard I Nutrient Agar	1.07881.		✓								
TSC Agar	1.11972.	APHA, DIN, ISO					✓				
TSC Agar + Polymyxin Supplement	1.11972. + 1.09875.						✓				
Yeast Extract Agar	1.03750.										✓
Yeast Extract Glucose Chloramphenicol Agar (YGC)	1.16000.	IDF, ISO, DIN									✓

NCA = National Canners Association;

CEREALS

Detection of Pathogens

	Merck Cat. No.	Reference Method	Bacillus cereus	Pathogenic bacilli	Cl. perfringens	L. monocytogenes	Salmonella	Staph. aureus
Pre-Enrichment								
Buffered Peptone Water	1.07228.	BAM, APHA, ISO, DIN					✓	
Lactose Broth	1.07661.	SMWW, EPA, APHA, USP, EP, AOAC					✓	
Enrichment								
Buffered Listeria Enrichment Broth + FDA-BAM 1995 supplement	1.09628. + 1.11781.	IDF, BAM				✓		
DIASALM + Novobiocin supplement	1.09803. + 1.09874.						✓	
Differential Reinforced Clostridial Medium (DRCM)	1.11699.	DIN		✓				
Fraser Broth Base + Fraser supplement	1.10398. + 1.10399.	ISO, AFNOR				✓		
Giolitti-Cantoni Broth	1.10675.	DIN, IDF, ISO, APHA					✓	
Listeria Enrichment Broth (LEB)	1.10549.	BAM, APHA				✓		
Listeria Enrichment Broth Base + FDA BAM 1992 supplement	1.11951. + 1.11883.	IDF, BAM				✓		
Listeria Enrichment Broth Base + FDA-BAM 1995 supplement	1.11951. + 1.11781.	IDF, BAM				✓		
L-PALCAM Broth	1.10823.					✓		
M Broth	1.10658.	SMWW, AOAC, APHA					✓	
MKTTn Broth	1.05878.	ISO					✓	
MSRV Medium Base Modified + supplement	1.09878. + 1.09874.	AOAC					✓	
Rappaport Broth (Salmonella Enrichment Broth)	1.10236.						✓	
Rappaport Vassiliadis (RVS) Broth	1.07700.	ISO					✓	

CEREALS

Detection of Pathogens

	Merck Cat. No.	Reference Method	Bacillus cereus	Pathogenic bacilli	Cl. perfringens	L. monocytogenes	Salmonella	Staph. aureus
Reinforced Clostridial Medium (RCM)	1.05411.	EP			✓			
Salmosyst Broth + Salmosyst supplement	1.10153. + 1.10141.						✓	
Selenite Cystine Broth	1.07709.	APHA, DIN, USP, ISO, AOAC, BAM					✓	
Staphylococcus Broth (Baird) + Egg-Yolk Tellurite Emulsion	1.07899. + 1.03785.							✓
Tetrathionate Broth Muller-Kauffmann	1.10863.	ISO					✓	
Tryptic Soy Broth + polymyxin supplement	1.05459. + 1.09875.		✓	✓				
UVM Broth	1.10824.	AOAC, APHA, USDA				✓		
UVM II Broth	1.10824. + 1.04039.	USDA				✓		

CEREALS

Detection of Pathogens

	Merck Cat. No.	Reference Method	Bacillus cereus	Cl. perfringens	L. monocytogenes	Moulds	Salmonella	Staph. aureus
Isolation & Enumeration								
Baird Parker Agar Base + Egg-Yolk Tellurite Emulsion	1.05406. + 1.03785.	AOAC, BAM, APHA, EP, ISO, SMWW, USDA, USP						✓
Bismuth Sulfite Agar	1.05418.	AOAC, APHA, BAM, SMWW, USP					✓	
Chapman Agar	1.05469.							✓
Deoxycholate Lactose Agar	1.02894.	APHA					✓	
Differential Clostridial Agar (DCA) acc. to Weenk	1.10259.						✓	
DRBC	1.00466.	BAM, APHA				✓		
Hektoen Enteric Agar	1.11681.	AOAC, BAM, APHA, ISO					✓	
Mannitol Salt Agar	1.05404.	USP, BAM						✓
MYP Agar	1.05267.	AOAC, BAM, APHA, ISO, USDA	✓					
OGYE Agar + OGY supplement	1.05978. + 1.09877.	APHA, ISO				✓		
Potato Dextrose Agar	1.10130.	APHA, USP, AOAC, BAM				✓		
Rambach Agar	1.07500.	FDA					✓	
Rose Bengal Chloramphenicol Agar (RBC)	1.00467.	SMWW				✓		
SPS Agar	1.10235.			✓				
SS Agar	1.07667.	APHA					✓	
TSC Agar	1.11972.	DIN, APHA, ISO		✓				
TSN Agar	1.05264.			✓				
Vogel Johnson Agar Base	1.05405.	EP, USP, BAM						✓
XLD Agar	1.05287.	APHA, EP, USP, AOAC, SMWW, BAM					✓	

CEREALS

Marker Organisms Testing

	Merck Cat. No.	Reference Method	Coliforms	E. coli	Enterobacteriaceae	Enterococcus	Staphylococci	Sulfite reducing Clostridia
Enrichment								
Brilliant Green 2%-Bile Broth	1.05454.	SMWW, EPA, AOAC, BAM, APHA, ISO		✓				
Brilliant Green 2%-Bile Broth with MUG, Fluorocult®	1.12587.		✓	✓				
Differential Reinforced Clostridial Medium (DRCM)	1.11699.	DIN						✓
EC Broth	1.10765.	APHA, SMWW, EPA, AOAC, BAM, ISO, USDA	✓	✓				
EE Broth Mossel	1.05394.	EP, APHA			✓			
Enterococci Broth, Chromocult®	1.10294.					✓		
Giolitti-Cantoni Broth	1.10675.	DIN, IDF, ISO, APHA					✓	
Lauryl Sulfate Broth	1.10266.	SMWW, ISO, EPA, AOAC, BAM, APHA, USDA	✓	✓				
Lauryl Sulfate Broth with MUG, Fluorocult®	1.12588.	AOAC, BAM, APHA, SMWW	✓	✓				
LMX Broth with MUG, Fluorocult® + E.coli supplement	1.10620. + 1.00898.	EPA	✓	✓				
Reinforced Clostridial Medium (RCM)	1.05411.	EP						✓
Staphylococcus Broth (Baird)	1.07899.						✓	

CEREALS

Marker Organisms Testing

Isolation & Enumeration	Merck Cat. No.	Reference Method	Aerobic Plate Count	Coliforms	E. coli	Enterobacteriaceae	Enterococcus	Staphylococci + S. aureus	Sulfite reducing Clostridia
Baird Parker Agar Base + Egg-Yolk Tellurite Emulsion	1.05406. + 1.03785.	AOAC, BAM, APHA, EP, ISO, SMWW, USDA, USP						✓	
Bile Esculine Azide Agar	1.00072.	ISO					✓		
Chapman Agar	1.05469.							✓	
China-Blue Lactose Agar	1.02348.		✓	✓		✓			
Coliform Agar ES, Chromocult®	1.00850.			✓	✓				
Coliform Agar, Chromocult®	1.10426.	EPA		✓	✓			✓	
Count Agar Sugar-Free	1.10878.								
Differential Clostridial Agar (DCA) acc. to Weenk	1.10259.								✓
E.coli Direct Agar with MUG, Fluorocult®	1.04038.			✓	✓				
Enterococcus Agar, Chromocult®	1.00950.						✓		
Kanamycin Esculin Azide Agar	1.05222.			✓	✓		✓		
KF Streptococcus Agar	1.10707.	APHA, SMWW, EPA					✓		
MacConkey Agar	1.05465.	EP, USP, EPA, SMWW, AOAC, BAM, APHA		✓	✓	✓			
Mannitol Salt Agar	1.05404.	USP, BAM						✓	
Plate Count Agar	1.05463.	APHA, ISO, USDA, SMDP, SMWW, EPA, AOAC, BAM							
Reinforced Clostridial (RCA) Agar	1.05410.								✓
SPS Agar	1.10235.								✓
Standard I Nutrient Agar	1.07881.								
Tryptone Glucose Extract (TGE) Agar	1.10128.	SMWW, APHA, API, AOAC							

CEREALS

Marker Organisms Testing

	Merck Cat. No.	Reference Method	Aerobic Plate Count	Coliforms	E. coli	Enterobacteriaceae	Enterococcus	Staphylococci + S. aureus	Sulfite reducing Clostridia
TSC Agar + polymyxin supplement	1.11972. + 1.09875.								✓
TSN Agar	1.05264.								✓
Vogel Johnson Agar	1.05405.	EP, USP, BAM						✓	
VRB Agar	1.01406.	APHA, SMDP, IDF, BAM, ISO		✓	✓				
VRB Agar with MUG, Fluorocult®	1.04030.	APHA, BAM		✓	✓				
VRBD Agar	1.10275.	EP, ISO, APHA				✓			

CEREALS

Spoilage Organisms

	Merck Cat. No.	Reference Method	Aerobic Plate Count	Bacillaceae	Yeast and Moulds
Enumeration					
Count Agar Sugar-Free	1.10878.		✓		
Dextrose Tryptone Agar	1.10860.	APHA, NCA		✓	
DG 18 Agar	1.00465.				✓
DRBC Agar	1.00466.	BAM, APHA			✓
MYP Agar	1.05267.	AOAC, BAM, APHA, ISO, USDA		✓	
OGYE Agar Base + OGY supplement	1.05978. + 1.09877.	APHA, ISO			✓
Potato Dextrose Agar	1.10130.	APHA, USP, AOAC, BAM			✓
Rose Bengal Chloramphenicol Agar (RBC)	1.00467.	SMWW			✓
Yeast Extract Agar	1.03750.				✓
Yeast Extract Chloramphenicol Agar (YGC)	1.16000.	IDF, ISO, DIN			✓

	Merck Cat. No.	Reference Method	Bacillus cereus	Campylobacter	Cl. perfringens	E. coli 0157:H7 (VTEC)	Listeria	Salmonella	Staph. aureus	Y. enterocolitica
Pre Enrichment										
Buffered Peptone Water	1.07228.	BAM, APHA, ISO, DIN						✓		
Lactose Broth	1.07661.	SMWW, EPA, APHA, USP, EP, AOAC						✓		
Enrichment										
Bolton Broth + Bolton Supplement	1.00068. + 1.00069.	BAM		✓						
Buffered Listeria Enrichment Broth + FDA-BAM 1995 Supplement	1.09628. + 1.11781.	IDF, BAM					✓			
DIASALM + Novobiocin Supplement	1.09803. + 1.09874.							✓		
Differential Reinforced Clostridial (DRCM) Broth	1.11699.	DIN			✓					
EC Broth Modified (w/ novobiocin)	1.14582.	USDA, APHA				✓				
Fraser Broth Base + Fraser Supplement	1.10398. + 1.10399.	ISO, AFNOR					✓			
Giolitti-Cantoni Broth	1.10675.	DIN, IDF, ISO, APHA							✓	
GN Broth Hajna	1.10756.	APHA, USDA						✓		
Lactose Broth	1.07661.	SMWW, EPA, APHA, USP, EP, AOAC						✓		
Listeria Enrichment Broth (LEB)	1.10549.	BAM, APHA					✓			
Listeria Enrichment Broth Base + FDA BAM 1992 Supplement	1.11951. + 1.11883.	IDF, BAM					✓			
Listeria Enrichment Broth Base + FDA-BAM 1995 Supplement	1.11951. + 1.11781.	IDF, BAM					✓			
L-PALCAM Broth	1.10823.						✓			
M Broth	1.10658.	SMWW, AOAC, APHA						✓		
MKTTn Broth	1.05878.	ISO						✓		

	Merck Cat. No.	Reference Method	Bacillus cereus	Campylobacter	Cl. perfringens	E. coli 0157:H7 (VTEC)	Listeria	Salmonella	Staph. aureus	Y. enterocolitica
MSRV Medium Base Modified + Supplement	**1.09878. + 1.09874.**	**AOAC**						✓		
Rappaport Broth (Salmonella Enrichment Broth)	**1.10236.**							✓		
Rappaport Vassiliadis (RVS) Broth	**1.07700.**	**ISO**						✓		
Reinforced Clostridial Medium (RCM)	**1.05411.**	**EP**			✓					
Salmosyst Broth + Salmosyst® Supplement	**1.10153. + 1.10141.**							✓		
Selenite Cystine Broth	**1.07709.**	**APHA, DIN, USP, ISO, AOAC, BAM**						✓		
Staphylococcus Broth (Baird)	**1.07899.**	**DIN**							✓	
Tetrathionate Broth, Modified	**1.10863.**	**ISO**						✓		
Tryptic Soy Broth + Polymyxin Supplement	**1.05459. + 1.09875.**	**ISO**	✓							
Tryptic Soy Broth, Modified (w/ novobiocin)	**1.09205.**	**DIN, BAM**				✓				
UVM Broth	**1.10824.**	**AOAC, APHA, USDA**					✓			
UVM II Broth	**1.10824. + 1.04039.**	**USDA**					✓			
Yersinia Selective Enrichment Broth	**1.16701.**									✓

DAIRY

Detection of Pathogens

	Merck Cat. No.	Reference Method	Bac. cereus	Brucella	Campylobacter	Cl. perfringens	Listeria	Salmonella/Shigella	Staph. aureus	Y. enterocolitica	VTEC/E. coli 0157:H7
Isolation & Enumeration											
Baird Parker Agar Base + Egg-Yolk Tellurite Emulsion	1.05406. + 1.03785.	AOAC, BAM, APHA, EP, ISO, SMWW, USDA, USP							✓		
Bismuth Sulfite Agar	1.05418.	AOAC, APHA, BAM, SMWW, USP						✓			
BPL Agar	1.07236.							✓			
BPLS Agar	1.07237.							✓			
Brilliant Green Agar Modified	1.10747.	ISO						✓			
Brucella Agar	1.10490.	EP, ISO, DIN, USP, USDA		✓							
Campylobacter Agar Base + Skirrow Supplement	1.02248. + 1.02249.	APHA, ISO, SMWW			✓						
Campylobacter Blood-Free Agar Base (modified CCDA) + CCDA Supplement	1.00070. + 1.00071.	ISO			✓						
Chapman Agar	1.05469.								✓		
Deoxycholate Lactose Agar	1.02894.	APHA						✓			
Hektoen Enteric Agar	1.11681.	AOAC, BAM, APHA, ISO						✓			
Mannitol Salt Agar	1.05404.	USP, BAM							✓		
MYP Agar + Polymixin Supplement	1.05267. + 1.09875	AOAC, BAM, APHA, ISO, USDA	✓								
Rambach Agar®	1.07500.	FDA(510K)						✓			
Sorbitol MacConkey Agar (SMAC)	1.09207.	DIN, BAM, APHA, ISO, SMWW, USDA									✓
Sorbitol MacConkey Agar (SMAC) + CT Supplement	1.09207. + 1.09202.	DIN, BAM, APHA, ISO, SMWW, USDA									✓

DAIRY

Detection of Pathogens

	Merck Cat. No.	Reference Method	Bac. cereus	Brucella	Campylobacter	Cl. perfringens	Listeria	Salmonella/Shigella	Staph. aureus	Y. enterocolitica	VTEC/E. coli 0157:H7
SPS Agar	**1.10235.**					✓					
SS Agar	**1.07667.**	**APHA**						✓			
TSC Agar	**1.11972.**	**DIN, APHA, ISO**				✓					
Vogel Johnson Agar	**1.05405.**	**EP, USP, BAM**							✓		
XLD Agar	**1.05287.**	**APHA, EP, USP, AOAC, SMWW, BAM**						✓			
Yersinia Agar (CIN) + CIN Supplement	**1.16434. + 1.16466.**	**APHA, BAM, ISO**								✓	

DAIRY

Marker Organisms Testing

	Merck Cat. No.	Reference Method	Coliforms	E. coli	Listeria	Staphylococci	Sulfite reducing Clostridia
Enrichment							
Brilliant Green 2%-Bile Broth	1.05454.	SMWW, EPA, AOAC, BAM, APHA, ISO	✓	✓			
Brilliant Green 2%-Bile Broth with MUG, Fluorocult®	1.12587.		✓	✓			
Differential Reinforced Clostridial Medium (DRCM)	1.11699.	DIN					✓
EC Broth	1.10765.	APHA, SMWW, EPA, AOAC, BAM, ISO, USDA	✓	✓			
Fraser Broth Base + Fraser Supplement	1.10398. + 1.10399.	ISO, AFNOR			✓		
Giolitti-Cantoni Broth	1.10675.	DIN, IDF, ISO, APHA				✓	
Lauryl Sulfate Broth	1.10266.	SMWW, ISO, EPA, AOAC, BAM, APHA, USDA	✓	✓			
Lauryl Sulfate Broth with MUG, Fluorocult®	1.12588.	AOAC, BAM, APHA, SMWW, ISO	✓	✓			
Listeria Enrichment Broth (LEB)	1.10549.	BAM, APHA			✓		
Listeria Enrichment Broth Base + FDA BAM 1992 Supplement	1.11951. + 1.11883.	IDF, BAM			✓		
Listeria Enrichment Broth Base + FDA-BAM 1995 Supplement	1.11951. + 1.11781.	IDF, BAM			✓		
LMX Broth, Fluorocult® + E.coli Supplement	1.10620. + 1.00989	EPA	✓	✓			

DAIRY

Marker Organisms Testing

	Merck Cat. No.	Reference Method	Coliforms	E. coli	Listeria	Staphylococci	Sulfite reducing Clostridia
L-PALCAM Broth	**1.10823.**				✓		
Reinforced Clostridial Medium (RCM)	**1.05411.**	**EP**					✓
Staphylococcus Broth (Baird)	**1.07899.**	**DIN**				✓	
UVM Broth	**1.10824.**	**AOAC, APHA, USDA**			✓		
UVM Broth + UVM II Supplement	**1.10824. + 1.04039.**	**USDA**			✓		

DAIRY

Marker Organisms Testing

	Merck Cat. No.	Reference Method	Aerobic Plate Count	Anaerobic Plate Count	Coliforms	E. coli	Enterobacteriaceae	Enterococcus	Listeria	Staphylococci	Sulfite reducing Clostridia
Isolation & Enumeration											
APT Agar	1.10453.	APHA, USDA	✓								
Baird Parker Agar Base + Egg-Yolk Tellurite Emulsion	1.05406. + 1.03785.	AOAC, BAM, APHA, EP, ISO, SMWW, USDA, USP								✓	
Bile Esculine Azide Agar	1.00072.	ISO						✓			
Chapman Agar	1.05469.									✓	
China-Blue Lactose Agar	1.02348.		✓		✓		✓				
Coliform Agar ES, Chromocult®	1.00850.				✓	✓					
Coliform Agar, Chromocult®	1.10426.	EPA			✓	✓					
Count Agar Sugar-Free	1.10878.		✓								
E.coli Direct Agar with MUG, Fluorocult®	1.04038.					✓					
Enterococcus Agar, Chromocult®	1.00950.							✓			
Kanamycin Esculin Azide Agar	1.05222.							✓			
KF Streptococcus Agar	1.10707.	APHA, SMWW, EPA						✓			
MacConkey Agar	1.05465.	EP, USP, EPA, SMWW, AOAC, BAM, APHA			✓	✓	✓				
Mannitol Salt Agar	1.05404.	USP, BAM								✓	
Oxford Agar Base + Oxford Supplement	1.07004. + 1.07006.	AOAC, BAM, APHA, ISO, USDA							✓		
PALCAM Listeria Agar Base + PALCAM Supplement	1.11755. + 1.12122.	BAM, APHA, ISO							✓		
Plate Count Agar	1.05463.	APHA, ISO, USDA, SMDP, SMWW, EPA, AOAC, BAM	✓								
Plate Count Skim Milk Agar	1.15338.	DIN, IDF	✓								

DAIRY

Marker Organisms Testing

	Merck Cat. No.	Reference Method	Aerobic Plate Count	Anaerobic Plate Count	Coliforms	E. coli	Enterobacteriaceae	Enterococcus	Listeria	Staphylococci	Sulfite reducing Clostridia
Reinforced Clostridial Agar (RCA)	**1.05410.**			✓							✓
SPS Agar	**1.10235.**			✓							✓
Standard I Nutrient Agar	**1.07881.**		✓								
Tryptone Glucose Extract (TGE) Agar	**1.10128.**	**SMWW, APHA, API, AOAC**	✓								
TSC Agar	**1.11972.**	**DIN, APHA, ISO**									✓
Vogel Johnson Agar	**1.05405.**	**EP, USP, BAM**								✓	
VRB Agar	**1.01406.**	**APHA, SMDP, IDF, BAM, ISO**			✓	✓					
VRB Agar with MUG, Fluorocult®	**1.04030.**	**APHA, BAM**			✓	✓					
VRBD Agar	**1.10275.**	**EP, ISO, APHA**					✓				

DAIRY

Spoilage Organisms

	Merck Cat. No.	Reference Method	Antibiotic recidues	Bacillaceae ("flat sour")	Clostridia	Coliforms	Proteolytic organisms	Lipolytic microorganisms	Lactic acid bacterial Lactobacillaceae	Streptococci	Yeast & Moulds
Enrichment											
Brilliant Green 2%-Bile Broth	1.05454.	SMWW, EPA, AOAC, BAM, APHA, ISO				✓					
Bryant & Burkey Broth	1.01617.							✓			
Differential Reinforced Clostridial (DRCM) Broth	1.11699.	DIN			✓						
LMX Broth, Fluorocult® + E.coli Supplement	1.10620. + 1.00898					✓					
M-17 Broth	1.15029.									✓	
MRS Broth	1.10661.	DIN, APHA							✓	✓	
Reinforced Clostridial Medium (RCM)	1.05411.	EPA			✓						
Tryptic Soy Broth + Polymyxin Supplement	1.05459. + 1.09875.			✓							
Isolation & Enumeration											
Antibiotic Agar No. 1	1.05272.	EP, USP, AOAC	✓								
APT Agar	1.10453.	APHA, USDA	✓						✓		
Bryant & Burkey Agar (agar added to broth) Agar-agar	1.01617. + 1.01614.							✓			
Calcium Caseinate Agar	1.05409.						✓				
Coliform Agar ES, Chromocult®	1.00850.					✓					
Coliform Agar, Chromocult®	1.10426.	EPA				✓					
Dextrose Tryptone Agar	1.10860.	APHA, NCA		✓							
DG 18 Agar	1.00465.										✓
DRBC Agar	1.00466.	BAM, APHA									✓
M-17 Agar	1.15108.	APHA								✓	
MacConkey Agar	1.05465.	EP, USP, EPA, SMWW, AOAC, BAM, APHA				✓					
MacConkey Agar with MUG, Fluorocult®	1.04029.					✓					

DAIRY

Spoilage Organisms

	Merck Cat. No.	Reference Method	Antibiotic recidues	Bacillaceae ("flat sour")	Clostridia	Coliforms	Proteolytic organisms	Lipolyti microorganisms	Lactic acid bacterial/Lactobacillaceae	Streptococci	Yeast & Moulds
MRS Agar	**1.10660.**	**DIN, APHA**							✓	✓	
MYP Agar + Polymixin Supplement	**1.05267. + 1.09877.**	**AOAC, BAM, APHA, ISO, USDA**	✓								
OGYE Agar Base + OGYE Supplement	**1.05978. + 1.09877.**	**APHA, ISO**									✓
Potato Dextrose Agar	**1.10130.**	**APHA, USP, AOAC, BAM**									✓
Reinforced Clostridial Agar	**1.05410.**				✓						
Reinforced Clostridial Medium (RCM)	**1.05411.**	**EP**			✓						
Rogosa Agar	**1.05413.**	**APHA**							✓		
Rose Bengal Chloramphenicol Agar (RBC)	**1.00467.**	**SMWW**									✓
SPS Agar	**1.10235.**				✓						
Trybuturin Agar Base	**1.01957.**							✓			
TSC Agar	**1.11972.**	**DIN, APHA, ISO**			✓						
VRB Agar	**1.01406.**	**APHA, SMDP, IDF, BAM, ISO**				✓					
VRB Agar with MUG, Fluorocult®	**1.04030.**	**APHA, BAM**				✓					
Yeast Extract Agar	**1.03750.**										✓
Yeast Extract Chloramphenicol Agar (YGC)	**1.16000.**	**IDF, ISO, DIN**									✓

EGG PRODUCTS

Detection of Pathogens

	Merck Cat. No.	Reference Method	Bacillus cereus	Pathogenic Bacilli	Campylobacter	Listeria	Salmonella	Staph. aureus	Yersinia enterocolitica
Pre-Enrichment									
Buffered Peptone Water	1.07228.	BAM, APHA, ISO, DIN					✓		
Lactose Broth	1.07661.	SMWW, EPA, APHA, USP, EP, AOAC					✓		
Enrichment									
Bolton Broth + Bolton Supplement	1.00068. + 1.00069.	BAM, ISO			✓				
Buffered Listeria Enrichment Broth + FDA-BAM 1995 Supplement	1.09628. + 1.11781.	IDF, BAM				✓			
DIASALM + Novobiocin Supplement	1.09803. + 1.09874.						✓		
Fraser Broth Base + Fraser Supplement	1.10398. + 1.10399.	ISO, AFNOR				✓			
Giolitti-Cantoni Broth	1.10675.	DIN, IDF, ISO, APHA						✓	
Listeria Enrichment Broth (LEB)	1.10549.	BAM, APHA				✓			
Listeria Enrichment Broth Base + FDA BAM 1992 Supplement	1.11951. + 1.11883.	IDF, BAM				✓			
Listeria Enrichment Broth Base + FDA-BAM 1995 Supplement	1.11951. + 1.11781.	IDF, BAM				✓			
L-PALCAM Broth	1.10823.					✓			
M Broth	1.10658.	SMWW, AOAC, APHA					✓		
MKTTn Broth	1.05878.	ISO					✓		
MSRV Medium Base Modified + Supplement	1.09878. + 1.09874.	AOAC					✓		
Rappaport Broth (Salmonella Enrichment Broth)	1.10236.						✓		
Rappaport Vassiliadis (RVS) Broth	1.07700.	ISO					✓		

EGG PRODUCTS

Detection of Pathogens

	Merck Cat. No.	Reference Method	Bacillus cereus	Pathogenic Bacilli	Campylobacter	Listeria	Salmonella	Staph. aureus	Yersinia enterocolitica
Salmosyst® Broth + Salmosyst® Supplement	1.10153. + 1.10141.						✓		
Selenite Cystine Broth	1.07709.	APHA, DIN, USP, ISO, AOAC, BAM					✓		
Staphylococcus Broth (Baird)	1.07899.	DIN						✓	
Tryptic Soy Broth + Polymyxin Supplement	1.05459. + 1.09875.	DIN, ISO	✓	✓					
UVM Broth	1.10824.	AOAC, APHA, USDA				✓			
UVM Broth + UVM II Supplement	1.10824. + 1.04039.	USDA				✓			
Yersinia Selective Enrichment Broth	1.16701.								✓

EGG PRODUCTS

Detection of Pathogens

	Merck Cat. No.	Reference Method	Bacillus cereus	Pathogenic Bacilli	Campylobacter	Listeria	Salmonella	Staph. aureus	Yersinia enterocolitica
Isolation & Enumeration									
Baird Parker Agar Base + Egg-Yolk Tellurite Emulsion	1.05406. + 1.03785.	AOAC, BAM, APHA, EP, ISO, SMWW, USDA, USP						✓	
Bismuth Sulfite Agar	1.05418.	AOAC, APHA, BAM, SMWW, USP					✓		
BPL Agar	1.07236.						✓		
BPLS Agar	1.07237.						✓		
Brilliant Green Agar Modified	1.10747.	ISO					✓		
Campylobacter Agar Base + Skirrow Supplement	1.02248. + 1.02249.	APHA, ISO, SMWW			✓				
Campylobacter Blood-Free Agar Base (modified CCDA) + CCDA Supplement	1.00070. + 1.00071.	ISO			✓				
Chapman Agar	1.05469.							✓	
Deoxycholate Lactose Agar	1.02894.	APHA					✓		
Hektoen Enteric Agar	1.11681.	AOAC, BAM, APHA, ISO					✓		
Mannitol Salt Agar	1.05404.	USP, BAM						✓	
MYP Agar + Polymixin Supplement	1.05267. + 1.09877	AOAC, BAM, APHA, ISO, USDA	✓	✓					
Oxford Agar Base + Oxford Supplement	1.07004. + 1.07006.	AOAC, BAM, APHA, ISO, USDA				✓			
PALCAM Listeria Agar Base + PALCAM Supplement	1.11755. + 1.12122.	BAM, APHA, ISO				✓			

EGG PRODUCTS

Detection of Pathogens

	Merck Cat. No.	Reference Method	Bacillus cereus	Pathogenic Bacilli	Campylobacter	Listeria	Salmonella	Staph. aureus	Yersinia enterocolitica
Rambach® Agar	1.07500.	FDA(510K)					✓		
SS Agar	1.07667.	APHA					✓		
TSC Agar	1.11972.	DIN, APHA, ISO	✓						
Vogel Johnson Agar	1.05405.	EP, USP, BAM						✓	
XLD Agar	1.05287.	APHA, EP, USP, AOAC, SMWW, BAM					✓		
Yersinia Agar (CIN) + CIN Supplement	1.16434. + 1.16466.	APHA, BAM, ISO							✓

EGG PRODUCTS

Marker Organisms Testing

	Merck Cat. No.	Reference Method	E. coli	Coliforms	Enterobacteriaceae	Enterococcus	Listeria	Staphylococci	Sulfite reducing Clostridia
Enrichment									
Brilliant Green 2%-Bile Broth	1.05454.	SMWW, EPA, AOAC, BAM, APHA, ISO	✓	✓					
Brilliant Green 2%-Bile Broth with MUG, Fluorocult®	1.12587.		✓	✓					
Differential Reinforced Clostridial (DRCM) Broth	1.11699.	DIN						✓	
EC Broth	1.10765.	APHA, SMWW, EPA, AOAC, BAM, ISO, USDA	✓	✓					
EE Broth Mossel	1.05394.	EP, APHA			✓				
Enterococci Broth, Chromocult®	1.10294.					✓			
Fraser Broth Base + Fraser Supplement	1.10398. + 1.10399.	ISO, AFNOR					✓		
Giolitti-Cantoni Broth	1.10675.	DIN, IDF, ISO, APHA						✓	
Lauryl Sulfate Broth	1.10266.	SMWW, ISO, EPA, AOAC, BAM, APHA, USDA	✓	✓					
Lauryl Sulfate Broth with MUG, Fluorocult®	1.12588.	AOAC, BAM, APHA, SMWW,ISO	✓	✓					
Listeria Enrichment Broth (LEB)	1.10549.	BAM, APHA					✓		
Listeria Enrichment Broth Base + FDA BAM 1992 Supplement	1.11951. + 1.11883.	IDF, BAM					✓		
Listeria Enrichment Broth Base + FDA-BAM 1995 Supplement	1.11951. + 1.11781.	IDF, BAM					✓		
LMX Broth, Fluorocult® + E.coli Supplement	1.10620. + 1.00898	EPA	✓	✓					

EGG PRODUCTS

Marker Organisms Testing

	Merck Cat. No.	Reference Method	E. coli	Coliforms	Enterobacteriaceae	Enterococcus	Listeria	Staphylococci	Sulfite reducing Clostridia
L-PALCAM Broth	**1.10823.**						✓		
Reinforced Clostridial Medium (RCM)	**1.05411.**	**EP**							✓
Staphylococcus Broth (Baird)	**1.07899.**	**DIN**							✓
UVM Broth	**1.10824.**	**BAM, APHA**					✓		
UVM Broth + UVM II Supplement	**1.10824. + 1.04039.**	**USDA**					✓		

EGG PRODUCTS

Marker Organisms Testing

	Merck Cat. No.	Reference Method	Aerobic Plate Count	Bacillaceae	Coliforms	E. coli	Enterobacteriaceae	Enterococcus	Listeria	Staphylococci & S. aureus	Sulfite reducing Clostridia
Isolation & Enumeration											
Baird Parker Agar Base + Egg-Yolk Tellurite Emulsion	1.05406. + 1.03785.	AOAC, BAM, APHA, EP, ISO, SMWW, USDA, USP								✓	
Bile Esculine Azide Agar	1.00072.	ISO						✓			
Chapman Agar	1.05469.									✓	
China-Blue Lactose Agar	1.02348.		✓		✓		✓				
Coliform Agar, Chromocult®	1.10426.	EPA			✓	✓					
Coliform Agar ES, Chromocult®	1.00850.				✓	✓					
E.coli Direct Agar with MUG, Fluorocult®	1.04038.					✓					
Enterococcus Agar, Chromocult®	1.00950.							✓			
Kanamycin Esculin Azide Agar	1.05222.							✓			
KF Streptococcus Agar	1.10707.	APHA, SMWW, EPA						✓			
MacConkey Agar	1.05465.	EP, USP, EPA, SMWW, AOAC, BAM, APHA			✓	✓	✓				
Mannitol Salt Agar	1.05404.	USP, BAM								✓	
Oxford Agar Base + Oxford Supplement	1.07004. + 1.07006.	AOAC, BAM, APHA, ISO, USDA							✓		
PALCAM Listeria Agar Base + PALCAM Supplement	1.11755. + 1.12122.	BAM, APHA, ISO							✓		
Plate Count Agar	1.05463.	APHA, ISO, USDA, SMDP, SMWW, EPA, AOAC, BAM	✓								
Standard I Nutrient Agar	1.07881.		✓								

EGG PRODUCTS

Marker Organisms Testing

	Merck Cat. No.	Reference Method	Aerobic Plate Count	Bacillaceae	Coliforms	E. coli	Enterobacteriaceae	Enterococcus	Listeria	Staphylococci & S. aureus	Sulfite reducing Clostridia
Tryptone Glucose Extract (TGE) Agar	**1.10128.**	**SMWW, APHA, API, AOAC**	✓								
TSC Agar + Polymyxin Supplement	**1.11972. + 1.09875.**										✓
Vogel Johnson Agar	**1.05405.**	**EP, USP, BAM**								✓	
VRB Agar	**1.01406.**	**APHA, SMDP, IDF, BAM, ISO**			✓	✓					
VRB Agar with MUG, Fluorocult®	**1.04030.**	**APHA, BAM**			✓	✓					
VRBD Agar	**1.10275.**	**EP, ISO, APHA**					✓				

EGG PRODUCTS

Spoilage Organisms

	Merck Cat. No.	Reference Method	Aerobic Plate Count	Bacillaceae	Coliforms	Enterobacteriaceae	Lactic acid bacteria	Pseudomonadaceae	Staphylococci	Yeast and Moulds
Isolation & Enumeration										
Baird Parker Agar Base + Egg-Yolk Tellurite Emulsion	1.05406. + 1.03785.	AOAC, BAM, APHA, EP, ISO, SMWW, USDA, USP							✓	
Calcium Caseinate Agar	1.05409.							✓		
Chapman Agar	1.05469.								✓	
China-Blue Lactose Agar	1.02348.				✓	✓				
Coliform Agar ES, Chromocult®	1.00850.				✓					
Coliform Agar, Chromocult®	1.10426.	EPA			✓					
Dextrose Tryptone Agar	1.10860.	APHA, NCA		✓						
DG 18 Agar	1.00465.									✓
DRBC Agar	1.00466.	BAM, APHA								✓
MacConkey Agar	1.05465.	EP, USP, EPA, SMWW, AOAC, BAM, APHA			✓	✓				
Mannitol Salt Agar	1.05404.	USP, BAM							✓	
OGYE Agar Base + OGY Supplement	1.05978. + 1.09877.	APHA, ISO								✓
Plate Count Agar	1.05463.	APHA, ISO, USDA, SMDP, SMWW, EPA, AOAC, BAM	✓							
Potato Dextrose Agar	1.10130.	APHA, USP, AOAC, BAM								✓
Rose Bengal Chloramphenicol Agar (RBC)	1.00467.	SMWW								✓
Standard I Nutrient Agar	1.07881.		✓							

EGG PRODUCTS

Spoilage Organisms

	Merck Cat. No.	Reference Method	Aerobic Plate Count	Bacillaceae	Coliforms	Enterobacteriaceae	Lactic acid bacteria	Pseudomonadaceae	Staphylococci	Yeast and Moulds
Tryptone Glucose Extract (TGE) Agar	1.10128.	SMWW, APHA, API, AOAC	✓							
Vogel Johnson Agar	1.05405.	EP, USP, BAM							✓	
VRB Agar	1.01406.	APHA, SMDP, IDF, BAM, ISO			✓					
VRB Agar with MUG, Fluorocult®	1.04030.	APHA, BAM			✓					
VRBD Agar	1.10275.	EP, ISO, APHA				✓				
Yeast Extract Agar	1.03750.									✓
Yeast Extract Chloramphenicol Agar (YGC)	1.16000.	IDF, ISO, DIN								✓

	Merck Cat. No.	Reference Method	Aeromonas hydrophila	Bacillus cereus	Cl. perfringens	Listeria	Pl shigelloides	Salmonella	Staph. aureus	Y. enterocolitica	Vibro spp.
Pre-Enrichment											
Alkaline Peptone Water	1.01800.	BAM, APHA, ISO									✓
Buffered Peptone Water	1.07228.	BAM, APHA, ISO, DIN						✓			
Enrichment											
Alkaline Peptone Water	1.01800.	ISO									✓
Buffered Listeria Enrichment Broth + FDA-BAM 1995 Supplement	1.09628. + 1.11781.	IDF, BAM				✓					
DIASALM + Novobiocin Supplement	1.09803. + 1.09874.							✓			
Differential Reinforced Clostridial Medium (DRCM)	1.11699.	DIN			✓						
Fraser Broth Base + Fraser Supplement	1.10398. + 1.10399.	ISO, AFNOR				✓					
Giolitti-Cantoni Broth	1.10675.	DIN, IDF, ISO, APHA							✓		
GN Broth Hajna	1.10756.	APHA, USDA					✓				
Lactose Broth	1.07661.	SMWW, EPA, APHA, USP, EP, AOAC						✓			
Listeria Enrichment Broth (LEB)	1.10549.	BAM, APHA				✓					
Listeria Enrichment Broth Base + FDA BAM 1992 Supplement	1.11951. + 1.11883.	IDF, BAM				✓					
Listeria Enrichment Broth Base + FDA-BAM 1995 Supplement	1.11951. + 1.11781.	IDF, BAM				✓					
L-PALCAM Broth	1.10823.					✓					
M Broth	1.10658.	SMWW, AOAC, APHA						✓			
MKTTn Broth	1.05878.	ISO						✓			
MSRV Medium Base Modified + Supplement	1.09878. + 1.09874.	AOAC						✓			

	Merck Cat. No.	Reference Method	Aeromonas hydrophila	Bacillus cereus	Cl. perfringens	Listeria	Pl shigelloides	Salmonella	Staph. aureus	Y. enterocolitica	Vibro spp.
Rappaport Broth (Salmonella Enrichment Broth)	1.10236.							✓			
Rappaport Vassiliadis (RVS) Broth	1.07700.	ISO						✓			
Reinforced Clostridial Medium (RCM)	1.05411.	EP			✓						
Salmosyst® Broth + Salmosyst® Supplement	1.10153. + 1.10141.							✓			
Selenite Cystine Broth	1.07709.	APHA, DIN, USP, ISO, AOAC, BAM						✓			
Staphylococcus Broth (Baird)	1.07899.	DIN							✓		
Tetrathionate Broth Muller-Kauffmann	1.10863.	ISO						✓			
Tryptic Soy Broth + Polymyxin Supplement	1.05459. + 1.09875.	ISO		✓							
UVM Broth	1.10824.	AOAC, APHA, USDA				✓					
UVM Broth + UVM II Supplment	1.10824. + 1.04039.	USDA				✓					
Yersinia Selective Enrichment Broth	1.16701.									✓	

	Merck Cat. No.	Reference Method	Aeromonas hydrophila	Bacillus cereus	Cl. perfringens	Listeria	Pl shigelloides	Salmonella	Shigella	S. aureus	Y. enterocolitica	Vibrio spp.
Isolation & Enumeration												
Baird Parker Agar Base + Egg-Yolk Tellurite Emulsion	1.05406. + 1.03785.	AOAC, BAM, APHA, EP, ISO, SMWW, USDA, USP								✓		
Bismuth Sulfite Agar	1.05418.	AOAC, APHA, BAM, SMWW, USP						✓				
Brilliant Green Agar Modified	1.10747.	ISO						✓				
Chapman Agar	1.05469.									✓		
Deoxycholate Lactose Agar	1.02894.	APHA						✓				
GSP agar	1.10230.		✓				✓					
Hektoen Enteric Agar	1.11681.	AOAC, BAM, APHA, ISO						✓				
MacConkey Agar	1.05465.	EP, USP, EPA, SMWW, AOAC, BAM, APHA					✓	✓				
Mannitol Salt Agar	1.05404.	USP, BAM								✓		
MYP Agar + Polymixin Supplement	1.05267. + 1.09875.	AOAC, BAM, APHA, ISO, USDA		✓								
Oxford Agar Base + Oxford Supplement	1.07004. + 1.07006.	AOAC, BAM, APHA, ISO, USDA				✓						
PALCAM Listeria Agar Base + PALCAM Supplement	1.11755. + 1.12122.	BAM, APHA, ISO				✓						
Rambach® Agar	1.07500.	FDA						✓				
SPS Agar	1.10235.				✓							
SS Agar	1.07667.	APHA					✓	✓	✓			
TCBS agar	1.10263.	APHA, WHO, AOAC, BAM, ISO, SMWW										✓

	Merck Cat. No.	Reference Method	Aeromonas hydrophila	Bacillus cereus	Cl. perfringens	Listeria	Pl shigelloides	Salmonella	Shigella	S. aureus	Y. enterocolitica	Vibrio spp.
Triple Sugar Iron Agar (TSI)	**1.03915.**	**DIN, EP, USP, ISO, AOAC, BAM, APHA, USDA**						✓				
TSC Agar + TSC Supplement	**1.11972. + 1.00888.**				✓							
TSN Agar	**1.05264.**				✓							
Vogel Johnson Agar	**1.05405.**	**EP, USP, BAM**								✓		
XLD Agar	**1.05287.**	**APHA, EP, USP, AOAC, SMWW, BAM**					✓	✓				
Yersinia Agar (CIN) + CIN Supplement	**1.16434. + 1.16466.**	**APHA, BAM, ISO**									✓	

FISH

Marker Organisms

	Merck Cat. No.	Reference Method	Coliform bacteria	E. coli	Enterobacteriaceae	Enterococcus	Listeria	Staphylococci	Sulfite reducing Clostridia
Enrichment									
Brilliant Green 2%-Bile Broth	1.05454.	SMWW, EPA, AOAC, BAM, APHA, ISO	✓	✓					
Brilliant Green 2%-Bile Broth with MUG, Fluorocult®	1.12587.		✓	✓					
Differential Reinforced Clostridial (DRCM) Broth	1.11699.	DIN							✓
EC Broth	1.10765.	APHA, SMWW, EPA, AOAC, BAM, ISO, USDA	✓	✓					
EE Broth Mossel	1.05394.	EP, APHA			✓				
Enterococci Broth, Chromocult®	1.10294.					✓			
Giolitti-Cantoni Broth	1.10675.	DIN, IDF, ISO, APHA						✓	
Lauryl Sulfate Broth	1.10266.	SMWW, ISO, EPA, AOAC, BAM, APHA, USDA	✓	✓					
Lauryl Sulfate Broth with MUG, Fluorocult®	1.12588.	AOAC, BAM, APHA, SMWW, ISO	✓	✓					
Listeria Enrichment Broth (LEB)	1.10549.	BAM, APHA					✓		
Listeria Enrichment Broth Base + FDA BAM 1992 Supplement	1.11951. + 1.11883.	IDF, BAM					✓		
Listeria Enrichment Broth Base + FDA-BAM 1995 Supplement	1.11951. + 1.11781.	IDF, BAM					✓		

FISH

Marker Organisms

	Merck Cat. No.	Reference Method	Coliform bacteria	E. coli	Enterobacteriaceae	Enterococcus	Listeria	Staphylococci	Sulfite reducing Clostridia
LMX Broth, Fluorocult® + E.coli Supplement	**1.10620. + 1.00898**	**EPA**	✓	✓					
L-PALCAM Broth	**1.10823.**						✓		
Reinforced Clostridial Medium (RCM)	**1.05411.**	**EP**							✓
Staphylococcus Broth (Baird)	**1.07899.**	**DIN**						✓	
UVM Broth	**1.10824.**	**AOAC, APHA**					✓		
UVM Broth + UVM II Supplement	**1.10824. + 1.04039.**	**USDA**					✓		

FISH

Marker Organisms

	Merck Cat. No.	Reference Method	Aerobic Plate Count	Anaerobic Plate Count	Coliforms	E. coli	Enterobacteriaceae	Enterococcus	Listeria	Staphylococci	Sulfite reducing Clostridia
Isolation & Enumeration											
Baird Parker Agar Base + Egg-Yolk Tellurite Emulsion	1.05406. + 1.03785.	AOAC, BAM, APHA, EP, ISO, SMWW, USDA, USP								✓	
Bile Esculine Azide Agar	1.00072.	ISO						✓			
Chapman Agar	1.05469.									✓	
China-Blue Lactose Agar	1.02348.		✓		✓		✓				
Coliform Agar ES, Chromocult®	1.00850.				✓	✓					
Coliform Agar, Chromocult®	1.10426.	EPA			✓	✓					
Count Agar Sugar-Free	1.10878.		✓								
E.coli Direct Agar with MUG, Fluorocult®	1.04038.					✓					
Enterococcus Agar, Chromocult®	1.00950.							✓			
Kanamycin Esculin Azide Agar	1.05222.							✓			
KF Streptococcus Agar	1.10707.	APHA, SMWW, EPA						✓			
MacConkey Agar	1.05465.	EP, USP, EPA, SMWW, AOAC, BAM, APHA			✓	✓	✓				
Mannitol Salt Agar	1.05404.	USP, BAM								✓	
Oxford Agar Base + Oxford Supplement	1.07004. + 1.07006.	AOAC, BAM, APHA, ISO, USDA							✓		
PALCAM Listeria Agar Base + PALCAM Supplement	1.11755. + 1.12122.	BAM, APHA, ISO							✓		
Plate Count Agar	1.05463.	APHA, ISO, USDA, SMDP, SMWW, EPA, AOAC, BAM	✓								
Reinforced Clostridial Agar (RCA)	1.05410.										✓
SPS Agar	1.10235.										✓

FISH

Marker Organisms

	Merck Cat. No.	Reference Method	Aerobic Plate Count	Anaerobic Plate Count	Coliforms	E. coli	Enterobacteriaceae	Enterococcus	Listeria	Staphylococci	Sulfite reducing Clostridia
Standard I Nutrient Agar	**1.07881.**		✓								
Tryptone Glucose Extract (TGE) Agar	**1.10128.**	**SMWW, APHA, API, AOAC**	✓								
TSC Agar	**1.11972.**	**APHA, DIN, ISO**		✓							
TSC agar with Polymixin Supplement	**1.11972. + 1.09875.**	**APHA, DIN, ISO**									✓
Vogel Johnson Agar	**1.05405.**	**EP, USP, BAM**								✓	
VRB Agar	**1.01406.**	**APHA, SMDP, IDF, BAM, ISO**			✓	✓					
VRB Agar with MUG, Fluorocult®	**1.04030.**	**APHA, BAM**			✓	✓					
VRBD Agar	**1.10275.**	**EP, ISO, APHA**					✓				

FISH

Spoilage Organisms

	Merck Cat. No.	Reference Method	Bacillaceae ("flat sour")	Clostridia	Lipolytic organisms	Proteolytic organisms	Pseudomonadaceae	Lactic acid bacteria	Yeast & Moulds
Enrichment									
Differential Reinforced Clostridial (DRCM) Broth	1.11699.	DIN		✓					
MRS Broth	1.10661.	DIN, APHA						✓	
Reinforced Clostridial Medium (RCM)	1.05411.	EP		✓					
Tryptic Soy Broth + Polymyxin Supplement	1.05459. + 1.09875.	ISO	✓						
Isolation & Enumeration									
APT Agar	1.10453.	APHA, USDA						✓	
Bryant & Burkey Agar (agar added to broth) Agar agar	1.01617. + 1.01614.				✓				
Calcium Caseinate Agar	1.05409.					✓			
Dextrose Tryptone Agar	1.10860.	APHA, NCA	✓						
DG 18 Agar	1.00465.								✓
DRBC Agar	1.00466.	BAM, APHA							✓
GSP Agar	1.10230.						✓		
MRS Agar	1.10660.	DIN, APHA						✓	
MYP Agar + Polymixin Supplement	1.05267.	AOAC, BAM, APHA, ISO, USDA	✓						
OGYE Agar Base + OGYE Supplement	1.05978. + 1.09877.	APHA, ISO							✓
Potato Dextrose Agar	1.10130.	APHA, USP, AOAC, BAM							✓
Pseudomonas Agar Base + Pseudomonas CFC Selctive Supplement	1.07620. + 1.07627.	ISO					✓		
Reinforced Clostridial Agar (RCA)	1.05410.			✓					
Rogosa Agar	1.05413.	APHA						✓	
Rose Bengal Chloramphenicol Agar (RBC)	1.00467.	SMWW							✓
SPS Agar	1.10235.			✓					
Trybuturin Agar Base	1.01957.				✓				
TSC Agar	1.11972.	DIN, APHA, ISO		✓					
Yeast Extract Agar	1.03750.								✓
Yeast Extract Chloramphenicol Agar (YGC)	1.16000.	IDF, ISO, DIN							✓

	Merck Cat. No.	Reference Method	General Enrichment	Bacillus	Campylobacter	Clostridia	Coliforms	Coliform (fecal)	E. coli	E. coli 0157	Enterobacteriaceae	Enterococcus	Lactobacilli	Listeria	Salmonella / Shigella	Staphylococci	Sulfite reducing Clostridia	Yeast & Moulds	Yersinia
Pre-Enrichment																			
Buffered Peptone Water	1.07228.	BAM, APHA, ISO, DIN													✓				
Lactose Broth	1.07661.	SMWW, EPA, APHA, USP, EP, AOAC													✓				
Enrichment																			
Azide Dextrose Broth	1.01590.	EPA, SMWW										✓							
Bolton Broth Base + Bolton Supplement	1.00068. + 1.00069.	BAM, ISO			✓														
Brilliant Green 2%-Bile Broth	1.05454.	SMWW, EPA, AOAC, BAM, APHA, ISO					✓		✓										
Brilliant Green 2%-Bile Broth with MUG, Fluorocult®	1.12587.						✓		✓										
Bryant & Burkey Broth	1.01617.					✓													
Buffered Listeria Enrichment Broth + FDA-BAM 1992 Supplement	1.09628. + 1.11883.	IDF, BAM												✓					
Buffered Listeria Enrichment Broth + FDA-BAM 1995 Supplement	1.09628. + 1.11781.	IDF, BAM												✓					
Campylobacter Blood-Free Agar Base (modified CCDA) + CCDA Supplement	1.00070. + 1.00071.	ISO			✓														
Campylobacter Selective Agar Base + Skirrow Supplement	1.02248. + 1.02249.	APHA, ISO, SMWW			✓														
CAYE Broth Base, Modified + CAYE Supplement	1.00060. + 1.00051.									✓									
DIASALM + Novobiocin Supplement	1.09803. + 1.09874.														✓				
Differential Reinforced Clostridial Medium (DRCM)	1.11699.	DIN				✓												✓	

	Merck Cat. No.	Reference Method	General Enrichment	Bacillus	Campylobacter	Clostridia	Coliforms	Coliform (fecal)	E. coli	E. coli 0157	Enterobacteriaceae	Enterococcus	Lactobacilli	Listeria	Salmonella / Shigella	Staphylococci	Sulfite reducing Clostridia	Yeast & Moulds	Yersinia
EC Broth	1.10765.	APHA, SMWW, EPA, AOAC, BAM, ISO, USDA					✓	✓	✓										
EC Broth Modified (w/ novobiocin)	1.14582.	USDA, APHA								✓									
EE Broth Mossel	1.05394.	EP, APHA									✓								
ENDO Broth MF	1.10750.	SMDP, SMWW, APHA, EPA					✓		✓										
Enterococcus Broth, Chromocult®	1.10294.											✓							
Fluid Thioglycollate Medium	1.08191.	EP, USP, AOAC, BAM, APHA, USDA				✓													
Fluid Thioglycollate Medium G	1.16761.					✓													
Fraser Broth Base + Fraser Supplement	1.10398. + 1.10399.	ISO, AFNOR												✓					
Giolitti-Cantoni Broth	1.10675.	DIN, IDF, ISO, APHA														✓			
GN Broth Hajna	1.10756.	APHA, USDA							✓						✓				
Lactose Broth	1.07661.	SMWW, EPA, APHA, USP, EP, AOAC	✓				✓		✓						✓				
Lauryl Sulfate Broth	1.10266.	SMWW, ISO, EPA, AOAC, BAM, APHA, USDA					✓	✓	✓										
Lauryl Sulfate Broth with MUG, Fluorocult®	1.12588.	AOAC, BAM, APHA, SMWW, ISO					✓		✓										
Listeria Enrichment Broth (LEB)	1.10549.	BAM, APHA												✓					
Listeria Enrichment Broth Base + FDA BAM 1992 Supplement	1.11951. + 1.11883.	IDF, BAM												✓					

	Merck Cat. No.	Reference Method	General Enrichment	Bacillus	Campylobacter	Clostridia	Coliforms	Coliform (fecal)	E. coli	E. coli 0157	Enterobacteriaceae	Enterococcus	Lactobacilli	Listeria	Salmonella / Shigella	Staphylococci	Sulfite reducing Clostridia	Yeast & Moulds	Yersinia
Listeria Enrichment Broth Base + FDA-BAM 1995 Supplement	1.11951. + 1.11781.	IDF, BAM												✓					
LMX Broth, Fluorocult® + E.coli Supplement	1.10620. + 1.00898.	EPA					✓		✓										
L-PALCAM Broth	1.10823.													✓					
M Broth	1.10658.	SMWW, AOAC, APHA													✓				
MacConkey Broth	1.05396.	EP					✓		✓										
MKTTn Broth	1.05878.	ISO													✓				
MRS Broth	1.10661.	DIN, APHA											✓						
Nutrient Broth	1.05443.	SMDP, APHA, USP (Fluid K), AOAC, BAM	✓																
Rappaport Broth (Salmonella Enrichment Broth)	1.10236.														✓				
Rappaport Vassiliadis (RVS) Broth	1.07700.	ISO													✓				
Reinforced Clostridial Medium (RCM)	1.05411.	EP				✓											✓		
Sabouraud 2%-Dextrose Broth	1.08339.	EP, USP, AOAC																✓	
Salmosyst® Broth + Salmosyst® Supplement	1.10153. + 1.10141.														✓				
Selenite Broth	1.07717.	APHA, SMWW													✓				
Selenite Cystine Broth	1.07709.	APHA, DIN, USP, ISO, AOAC, BAM													✓				
Standard I Nutrient Broth	1.07882.		✓																
Staphylococcus Broth (Baird)	1.07899.	DIN														✓			
TBG Broth, Modified (Tetrathionate Brilliant Green Bile Broth)	1.05178.	EP													✓				

	Merck Cat. No.	Reference Method	General Enrichment	Bacillus	Campylobacter	Clostridia	Coliforms	Coliform (fecal)	E. coli	E. coli 0157	Enterobacteriaceae	Enterococcus	Lactobacilli	Listeria	Salmonella / Shigella	Staphylococci	Sulfite reducing Clostridia	Yeast & Moulds	Yersinia
Tetrathionate Broth	**1.05285.**	**APHA, USP, DIN, AOAC, BAM, SMWW**													✓				
Tetrathionate Broth Muller-Kauffmann	**1.10863.**	**ISO**													✓				
Tetrathionate Crystal Violet Broth (Preuss)	**1.05173.**														✓				
Tryptic Soy Broth	**1.05459.**	**EP, USP, AOAC, BAM, APHA, USDA, ISO, SMWW**	✓																
Tryptic Soy Broth + Polymyxin Supplement	**1.05459. + 1.09875.**	**ISO**		✓															
Tryptic Soy Broth Modified (w/novobiocin)	**1.09205.**	**DIN, BAM**								✓									
Tryptone Water	**1.10859.**	**APHA, SMWW, ISO**							✓										
Tryptose Broth	**1.10676.**	**BAM, APHA**							✓					✓	✓	✓			
UVM Broth, Modified	**1.10824.**	**AOAC, APHA, USDA**												✓					
UVM Broth + UVM II Supplement	**1.10824. + 1.04039.**	**USDA**												✓					
Wort Broth	**1.05449.**																	✓	
Yersinia Selective Enrichment Broth	**1.16701.**																		✓

	Merck Cat. No.	Reference Method	General plating	Alicyclobacillus	Antibiotic Residue Test	Antimicrobial Inhibitor Test	Bacillus	Campylobacter	Clostridia	Coliforms	E. coli	E. coli 0157	Enterobacteriaceae	Enterococcus	Environmental Test	Lactobacilli	Lipase Test	Listeria	Salmonella / Shigella	Staphylococci	Sulfite reducing Clostridia	Total Plate Count	Vibrio	Yeast & Moulds	Yersina
Isolation & Enumeration																									
Antibiotic Agar No. 1	1.05272.	EP, USP, AOAC				✓																			
APT Agar	1.10453.	APHA, USDA														✓									
Baird Parker Agar Base + Egg-Yolk Tellurite Emulsion	1.05406. + 1.03785.	AOAC, BAM, APHA, EP, ISO, SMWW, USDA, USP																		✓					
BAT Medium	1.07994.	IFU		✓																					
Bile Esculin Azide Agar	1.00072.	ISO													✓										
Bismuth Sulfite Agar	1.05418.	AOAC, APHA, BAM, SMWW, USP																		✓					
Blood Agar Base (add defibrinated sheep blood)	1.10886.	BAM, APHA	✓				✓		✓												✓				
Blood Agar Base No.2 (add defibrinated sheep blood)	1.10328.	BAM, ISO	✓				✓		✓												✓				
BPL Agar	1.07236.																			✓					
BPLS Agar	1.07237.																			✓					
Brilliant Green Agar Modified	1.10747.	ISO																		✓					
BROLAC Agar	1.01639.									✓	✓														
Bromocresol Purple Azide Broth	1.03032.														✓										
Bryant & Burkey Agar (use broth and add Agar)	1.01617. + 1.01614.								✓																
Calcium Caseinate Agar	1.05409.						✓																		
Campylobacter Agar Base + Skirrow Supplement	1.02248. + 1.02249.	APHA, ISO, SMWW						✓																	
Campylobacter Blood-Free Agar Base (modified CCDA) + CCDA Supplement	1.00070. + 1.00071.	ISO						✓																	
CATC Agar	1.10279.	DIN													✓										
Cereus Selective Agar Base + Cereus Supplement	1.05267. + 1.09875.	AOAC, BAM, APHA, ISO, USDA					✓																		
Chapman Agar	1.05469.																				✓				

	Merck Cat. No.	Reference Method	General plating	Alicyclobacillus	Antibiotic Residue Test	Antimicrobial Inhibitor Test	Bacillus	Campylobacter	Clostridia	Coliforms	E. coli	E. coli 0157	Enterobacteriaceae	Enterococcus	Environmental Test	Lactobacilli	Lipase Test	Listeria	Salmonella / Shigella	Staphylococci	Sulfite reducing Clostridia	Total Plate Count	Vibrio	Yeast & Moulds	Yersina
China-Blue Lactose Agar	1.02348.									✓											✓				
Coliform Agar ES, Chromocult®	1.00850.									✓	✓														
Coliform Agar, Chromocult®	1.10426.	EPA								✓	✓														
Count Agar Sugar-Free	1.10878.		✓																						
DCLS Agar	1.10270.												✓											✓	
Desoxycholate Citrate Agar, Modified	1.02896.	EP																		✓					
Desoxycholate Lactose Agar	1.02894.	APHA								✓	✓									✓					
Dextrose Tryptone Agar	1.10860.	APHA, NCA					✓							✓											
DG 18 Agar	1.00465.																							✓	
DIASALM Medium Base + MSRV Supplement	1.09803. + 1.09874.																			✓					
Differential Clostridial Agar (DCA) acc. to Weenk	1.10259.																					✓			
DNase Test Agar	1.10449.	APHA																			✓				
DRBC Agar	1.00466.	BAM, APHA																						✓	
E.coli 0157:H7 Agar with MUG, Fluorocult®	1.04036.											✓													
E.coli Direct Agar with MUG (ECD), Fluorocult®	1.04038.									✓	✓														
EMB Agar	1.01347.	SMWW											✓							✓					
ENDO Agar	1.04044.	SMDP, APHA, SMWW								✓	✓														
Enterococcus Agar	1.05262.	SMWW													✓										
Enterococcus Agar Base	1.05289.	SMWW													✓										
Enterococcus Agar, Chromocult®	1.00950.														✓										
Hektoen Enteric Agar	1.11681.	AOAC, BAM, APHA, ISO								✓	✓									✓					
Kanamycin Esculin Azide Agar	1.05222.														✓										
KF Streptococcus Agar	1.10707.	APHA, SMWW, EPA													✓										

	Merck Cat. No.	Reference Method	General plating	Alicyclobacillus	Antibiotic Residue Test	Antimicrobial Inhibitor Test	Bacillus	Campylobacter	Clostridia	Coliforms	E. coli	E. coli 0157	Enterobacteriaceae	Enterococcus	Environmental Test	Lactobacilli	Lipase Test	Listeria	Salmonella / Shigella	Staphylococci	Sulfite reducing Clostridia	Total Plate Count	Vibrio	Yeast & Moulds	Yersina
Kligler Agar	1.03913.	BAM, APHA, ISO									✓	✓								✓					
Lactose TTC Agar with Tergitol® 7	1.07680.	ISO									✓	✓													
Letheen Agar, Modified	1.10404.	BAM, USP														✓									
Levine EMB Agar	1.01342.	SMWW									✓	✓													
Lysine Iron Agar	1.11640.	AOAC, BAM, SMWW, USDA									✓	✓								✓					
M Endo Agar LES	1.11277.	SMWW, EPA, APHA									✓	✓													
M-17 Agar	1.15108.	APHA													✓										
MacConkey Agar	1.05465.	EP, USP, EPA, SMWW, AOAC, BAM, APHA									✓	✓		✓						✓					
MacConkey Agar with MUG, Fluorocult®	1.04029.										✓	✓													
MacConkey Sorbitol Agar (SMAC)	1.09207.	DIN, BAM, APHA, ISO, SMWW, USDA											✓												
MacConkey Sorbitol Agar (SMAC) + CT Supplement	1.09207. + 1.09202.												✓												
Malt Extract Agar	1.05398.																							✓	
Mannitol Salt Agar	1.05404.	USP, BAM																			✓				
Meat Liver Agar	1.15045.								✓																
mFC Agar	1.11278.	SMWW, EPA, AOAC									✓	✓													
Microbial Content Test Agar	1.07324.	USP														✓									
MRS Agar	1.10660.	DIN, APHA															✓								
MYP Agar + Cereus Supplement	1.05267.+ 1.09875.	AOAC, BAM, APHA, ISO, USDA					✓																		

	Merck Cat. No.	Reference Method	General plating	Alicyclobacillus	Antibiotic Residue Test	Antimicrobial Inhibitor Test	Bacillus	Campylobacter	Clostridia	Coliforms	E. coli	E. coli 0157	Enterobacteriaceae	Enterococcus	Environmental Test	Lactobacilli	Lipase Test	Listeria	Salmonella / Shigella	Staphylococci	Sulfite reducing Clostridia	Total Plate Count	Vibrio	Yeast & Moulds	Yersina
Nutrient Agar	1.05450.	SMDP, APHA, SMWW, EPA, AOAC, BAM, ISO, USDA	✓																						
OGYE Agar Base + OGYE Supplement	1.05978. + 1.09877.	APHA, ISO																						✓	
Orange Serum Agar	1.10673.	APHA														✓									
Oxford Agar Base + Oxford Supplement	1.07004. + 1.07006.	AOAC, BAM, APHA, ISO, USDA																✓							
PALCAM Listeria Agar Base + PALCAM Supplement	1.11755. + 1.12122.	BAM, APHA, ISO																✓							
Plate Count Agar	1.05463.	APHA, ISO, USDA, SMDP, SMWW, EPA, AOAC, BAM																					✓		
Plate Count Skim Milk Agar	1.15338.	DIN, IDF																					✓		
Potato Dextrose Agar	1.10130.	APHA, USP, AOAC, BAM																						✓	
Rambach® Agar	1.07500.	FDA(510K)																		✓					
Reinforced Clostridial Agar (RCA)	1.05410.									✓															
Reinforced Clostridial Medium (RCM)	1.05411.	EP								✓												✓			
Rogosa Agar	1.05413.	APHA														✓									
Rose Bengal Chloramphenicol Agar (RBC)	1.00467.	SMWW																						✓	
Sabouraud 2%-Dextrose Agar	1.07315.	SMWW																						✓	
SIM Medium	1.05470.	APHA, BAM												✓											
Sorbitol MacConkey Agar (SMAC) + CT Supplement	1.09207. + 1.09202.	DIN, BAM, APHA, ISO, SMWW, USDA																							
SPS Agar	1.10235.									✓															
SS Agar	1.07667.	APHA																		✓					

	Merck Cat. No.	Reference Method	General plating	Alicyclobacillus	Antibiotic Residue Test	Antimicrobial Inhibitor Test	Bacillus	Campylobacter	Clostridia	Coliforms	E. coli	E. coli 0157	Enterobacteriaceae	Enterococcus	Environmental Test	Lactobacilli	Lipase Test	Listeria	Salmonella / Shigella	Staphylococci	Sulfite reducing Clostridia	Total Plate Count	Vibrio	Yeast & Moulds	Yersina
SSDC (Salmonella Shigella Agar w/sodium desoxy-cholate and calcium chloride)	1.16724.	ISO																							✓
Standard Count Agar	1.01621.		✓																				✓		
Standard I Nutrient Agar	1.07881.		✓																				✓		
Standard II Nutrient Agar	1.07883.		✓																				✓		
Staphylococcus Medium 110, Chapman	1.05469.																				✓				
Sulphite Iron Agar + Polymyxin Supplement	1.10864. + 1.09875.	ISO								✓															
TCBS Agar	1.10263.	APHA, WHO, AOAC, BAM, ISO, SMWW																						✓	
Test Agar pH 8.0	1.10664.	DIN					✓																		
Tributyrin Agar Base	1.01957.																	✓							
Triple Sugar Iron Agar (TSI)	1.03915.	DIN, EP, USP, ISO, AOAC, BAM, APHA, USDA												✓						✓					
Tryptic Soy Agar (TSA)	1.05458.	EP, USP, AOAC, BAM, APHA, ISO, SMWW, USDA	✓																						
Tryptic Soy Agar with Polysorbate® 80 & Lecithin	1.07324.	USP														✓									
Tryptone Glucose Extract (TGE) Agar	1.10128.	SMWW, APHA, API, AOAC	✓																				✓		
Tryptose Agar	1.10237.	BAM, APHA																✓							
TSC Agar	1.11972.	DIN, APHA, ISO								✓															
TSC Agar + Polymyxin Supplement	1.11972. + 1.09875.									✓															
TSC Agar + TSC Supplement	1.11972. + 1.00888.									✓															
TSN Agar	1.05264.									✓															

FOOD & BEVERAGE Testing

Isolation, Enumeration & Identification

	Merck Cat. No.	Reference Method	General plating	Alicyclobacillus	Antibiotic Residue Test	Antimicrobial Inhibitor Test	Bacillus	Campylobacter	Clostridia	Coliforms	E. coli	E. coli 0157	Enterobacteriaceae	Enterococcus	Environmental Test	Lactobacilli	Lipase Test	Listeria	Salmonella / Shigella	Staphylococci	Sulfite reducing Clostridia	Total Plate Count	Vibrio	Yeast & Moulds	Yersina
Universal Beer Agar	1.00445.																✓								✓
Vogel Johnson Agar	1.05405.	EP, USP, BAM																			✓				
VRB Agar	1.01406.	APHA, SMDP, IDF, BAM, ISO									✓	✓													
VRB Agar with MUG, Fluorocult®	1.04030.	APHA, BAM									✓	✓													
VRBD Agar	1.10275.	EP, ISO, APHA												✓											
WL Nutrient Agar	1.10866.																								✓
Wort Agar	1.05448.																								✓
XLD Agar	1.05287.	APHA, EP, USP, AOAC, SMWW, BAM												✓						✓					
Yeast Extract Agar	1.03750.																								✓
Yeast Extract Chloramphenicol Agar (YGC)	1.16000.	IDF, ISO, DIN																							✓
Yersinia Agar (CIN) + CIN Supplement	1.16434. + 1.16466.	APHA, BAM, ISO																							✓
Rapid Identification																									
Bactident® E.coli	1.13303.											✓													
Singlepath® Campylobacter	1.04143.	AOAC							✓																
Singlepath® E.coli 0157	1.04141.	AOAC											✓												
Duopath® Verotoxins	1.04144.	AOAC											✓												
Singlepath® Listeria	1.04142.	AOAC pending																✓							
Singlepath® Salmonella	1.04140.	AOAC																		✓					

HEAT PROCESSED FOOD

Enrichment

	Merck Cat. No.	Reference Method	Aerobic organisms	Anaerobic organisms	Bacillaceae	Bacillus cereus	Coliforms	E. coli	Enterobacteriaceae	Enterococcus	Lipolytic microorganisms	Proteolytic microorganisms	Sulfite reducing Clostridia	Thermododucric organisms	Yeast & Moulds
Enrichment															
Brain Heart Infusion Broth	1.10493.	SMWW, EPA, AOAC, BAM, APHA, ISO		✓											
Brilliant Green 2%-Bile Broth	1.05454.	SMWW, EPA, AOAC, BAM, APHA, ISO					✓	✓							
Brilliant Green 2%-Bile Broth with MUG, Fluorocult®	1.12587.						✓	✓							
Bryant & Burkey Broth	1.01617.										✓				
Differential Reinforced Clostridial (DRCM) Broth	1.11699.	DIN											✓		
EC Broth	1.10765.	APHA, SMWW, EPA, AOAC, BAM, ISO, USDA					✓	✓							
EE Broth Mossel	1.05394.	EP, APHA							✓						
Enterococci Broth, Chromocult®	1.10294.									✓					
Fluid Thioglycollate Medium	1.08191.	EP, USP, AOAC, BAM, APHA, USDA		✓											
Fluid Thioglycollate Medium G	1.16761.			✓											
Lauryl Sulfate Broth	1.10266.	SMWW, ISO, EPA, AOAC, BAM, APHA, USDA					✓	✓							
Lauryl Sulfate Broth with MUG, Fluorocult®	1.12588.	AOAC, BAM, APHA, SMWW, ISO					✓	✓							
LMX Broth, Fluorocult® + E.coli Supplement	1.10620. + 1.00898.	EPA					✓	✓							
Thioglycollate Broth	1.08190.	APHA, USP		✓											
Tryptic Soy Broth	1.05459.	EP, USP, AOAC, BAM, APHA, USDA, ISO, SMWW	✓												
Tryptic Soy Broth + Polymyxin Supplement	1.05459. + 1.09875.	ISO			✓										

HEAT PROCESSED FOOD

Isolation & Enumeration

	Merck Cat. No.	Reference Method	Aerobic organisms	Anaerobic organisms	Anaerobes	Bacillaceae	Bacillus cereus	Coliforms	E. coli	Enterobacteriaceae	Enterococcus	Lipolytic microorganisms	Proteolytic microorganisms	Thermododucric organisms	Sulfite reducing Clostridia	Yeast & Moulds
Isolation & Enumeration																
APT Agar	1.10453.	APHA, USDA	✓											✓		
Bryant & Burkey Agar (agar added to broth) Agar agar	1.01617. + 1.01614.											✓				
Calcium Caseinate Agar	1.05409.												✓			
Coliform Agar, Chromocult®	1.10426.	EPA						✓	✓							
Coliform Agar ES, Chromocult®	1.00850.	EPA						✓	✓							
Czapek Dox Agar	1.05460.	SMWW														✓
Dextrose Tryptone Agar	1.10860.	APHA, NCA				✓										
Enterococcus Agar	1.05262.	SMWW									✓					
Enterococcus Agar, Chromocult®	1.00950.										✓					
Kanamycin Esculin Azide Agar	1.05222.										✓					
KF Streptococcus Agar	1.10707.	APHA, SMWW, EPA									✓					
Malt Extract Agar	1.05398.															✓
MYP Agar + Polymixin Supplement	1.05267. + 1.09875.	AOAC, BAM, APHA, ISO, USDA				✓	✓									
Orange Serum Agar	1.10673.	APHA			✓											✓
Plate Count Agar	1.05463.	APHA, ISO, USDA, SMDP, SMWW, EPA, AOAC, BAM	✓													
Plate Count Skim Milk Agar	1.15338.	DIN, IDF	✓											✓		
Potato Dextrose Agar	1.10130.	APHA, USP, AOAC, BAM														✓
Standard I Nutrient Agar	1.07881.		✓													
Sulphite Iron Agar with Polymyxin Supplement	1.10864. + 1.09875.	ISO													✓	

HEAT PROCESSED FOOD

Isolation & Enumeration

	Merck Cat. No.	Reference Method	Aerobic organisms	Anaerobic organisms	Anaerobes	Bacillaceae	Bacillus cereus	Coliforms	E. coli	Enterobacteriaceae	Enterococcus	Lipolytic microorganisms	Proteolytic microorganisms	Thermoduric organisms	Sulfite reducing Clostridia	Yeast & Moulds	
Tryptic Soy Agar	**1.05458.**	**EP, USP, AOAC, BAM, APHA, ISO, SMWW, USDA**	✓														
Tryptone Glucose Extract (TGE) Agar	**1.10128.**	**SMWW, APHA, API, AOAC**				✓											
TSC Agar	**1.11972.**	**APHA, DIN, ISO**		✓												✓	
TSC agar + Polymixin Supplement	**1.11972. + 1.09875.**	**APHA, DIN, ISO**														✓	
VRB Agar	**1.01406.**	**APHA, SMDP, IDF, BAM, ISO**						✓	✓								
VRB Agar with MUG, Fluorocult®	**1.04030.**	**APHA, BAM**						✓	✓								
VRBD Agar	**1.10275.**	**EP, ISO, APHA**								✓							

MEAT & MEAT PRODUCTS

Detection of Pathogens

	Merck Cat. No.	Reference Method	Bacillus cereus	Pathogenic bacilli	Campylobacter	Cl. perfringens	Listeria	Salmonella	Shigella	Staph. aureus	Y. enterocolitica	E. coli 0157:H7 (VTEC)
Pre-Enrichment												
Buffered Peptone Water	1.07228.	BAM, APHA, ISO, DIN						✓				
Lactose Broth	1.07661.	SMWW, EPA, APHA, USP, EP, AOAC						✓				
Enrichment												
Bolton Broth + Bolton Supplement	1.00068. + 1.00069.	BAM			✓							
Buffered Listeria Enrichment Broth + FDA-BAM 1995 Supplement	1.09628. + 1.11781.	BAM,APHA					✓					
DIASALM + Novobiocin Supplement	1.09803. + 1.09874.							✓				
Differential Reinforced Clostridial Medium (DRCM)	1.11699.	DIN				✓						
EC Broth, Modified (w/ novobiocin)	1.14582.	USDA, APHA										✓
Fraser Broth Base + Fraser Supplement	1.10398. + 1.10399.	ISO, AFNOR					✓					
Giolitti-Cantoni Broth	1.10675.	DIN, IDF, ISO, APHA								✓		
GN Broth Hajna	1.10756.	APHA, USDA							✓			
Listeria Enrichment Broth (LEB)	1.10549.	BAM, APHA					✓					
Listeria Enrichment Broth Base + FDA BAM 1992 Supplement	1.11951. + 1.11883.	IDF, BAM					✓					
Listeria Enrichment Broth Base + FDA-BAM 1995 Supplement	1.11951. + 1.11781.	IDF, BAM					✓					
L-PALCAM Broth	1.10823.						✓					
M Broth	1.10658.	SMWW, AOAC, APHA						✓				
MKTTn Broth	1.05878.	ISO						✓				
MSRV Medium Base Modified + Supplement	1.09878. + 1.09874.	AOAC						✓				

MEAT & MEAT PRODUCTS

Detection of Pathogens

	Merck Cat. No.	Reference Method	Bacillus cereus	Pathogenic bacilli	Campylobacter	Cl. perfringens	Listeria	Salmonella	Shigella	Staph. aureus	Y. enterocolitica	E. coli 0157:H7 (VTEC)
Rappaport Broth (Salmonella enrichment Broth)	**1.10236.**							✓				
Rappaport Vassiliadis (RVS) Broth	**1.07700.**	**ISO**						✓				
Reinforced Clostridial Medium (RCM)	**1.05411.**	**EP**				✓						
Salmosyst® Broth + Salmosyst® Supplement	**1.10153. + 1.10141.**							✓				
Selenite Cystine Broth	**1.07709.**	**APHA, DIN, USP, ISO, AOAC, BAM**						✓				
Staphylococcus Broth (Baird)	**1.07899.**	**DIN**								✓		
Tetrathionate Broth Muller-Kauffmann	**1.10863.**	**ISO**						✓				
Tryptic Soy Broth + Polymyxin Supplement	**1.05459. + 1.09875.**	**ISO**	✓	✓								
Tryptic Soy Broth, Modified (w/ novobiocin)	**1.09205.**	**DIN, BAM, ISO**										✓
UVM Broth	**1.10824.**	**AOAC, APHA**					✓					
UVM Broth + UVM II Supplement	**1.10824. + 1.04039.**	**USDA**					✓					
Yersinia Selective Enrichment Broth	**1.16701.**										✓	

MEAT & MEAT PRODUCTS

Detection of Pathogens

	Merck Cat. No.	Reference Method	Bacillus cereus	Pathogenic bacilli	Campylobacter	Cl. perfringens	Listeria	Salmonella	Shigella	Staph. aureus	Y. enterocolitica	E. coli 0157:H7 (VTEC)
Isolation & Enumeration												
Baird Parker Agar Base + Egg-Yolk Tellurite Emulsion	1.05406. + 1.03785.	AOAC, BAM, APHA, EP, ISO, SMWW, USDA, USP								✓		
Bismuth Sulfite Agar	1.05418.	AOAC, APHA, BAM, SMWW, USP						✓				
BPL Agar	1.07236.							✓				
BPLS Agar	1.07237.							✓				
Brilliant Green Agar Modified	1.10747.	ISO						✓				
Campylobacter Agar Base + Skirrow Supplement	1.02248. + 1.02249.	APHA, ISO, SMWW			✓							
Campylobacter Blood-Free Agar Base (modified CCDA) + CCDA Supplement	1.00070. + 1.00071.	ISO			✓							
Chapman Agar	1.05469.									✓		
Desoxycholate Lactose Agar	1.02894.	APHA						✓				
Hektoen Enteric Agar	1.11681.	AOAC, BAM, APHA, ISO						✓				
Mannitol Salt Agar	1.05404.	USP, BAM								✓		
MYP Agar + Polymixin Supplement	1.05267. + 1.09875.	AOAC, BAM, APHA, ISO, USDA	✓	✓								
Oxford Agar Base + Oxford Supplement	1.07004. + 1.07006.	AOAC, BAM, APHA, ISO, USDA					✓					
PALCAM Listeria Agar Base + PALCAM Supplement	1.11755. + 1.12122.	BAM, APHA, ISO					✓					
Rambach® Agar	1.07500.	FDA (510K)						✓				

MEAT & MEAT PRODUCTS

Detection of Pathogens

	Merck Cat. No.	Reference Method	Bacillus cereus	Pathogenic bacilli	Campylobacter	Cl. perfringens	Listeria	Salmonella	Shigella	Staph. aureus	Y. enterocolitica	E. coli 0157:H7 (VTEC)
Sorbitol MacConkey Agar (SMAC)	**1.09207.**	**DIN, BAM, APHA, ISO, SMWW, USDA**										✓
SPS Agar	**1.10235.**					✓						
SS Agar	**1.07667.**	**APHA**						✓	✓			
TSC Agar + TSC Supplement	**1.11972. + 1.00888.**	**APHA, DIN, ISO**				✓						
Vogel Johnson Agar	**1.05405.**	**USP, BAM, EP**								✓		
XLD Agar	**1.05287.**	**APHA, EP, USP, AOAC, BAM, SMWW**						✓				
Yersinia Agar (CIN) + CIN Supplement	**1.16434. + 1.16466.**	**APHA, ISO, BAM**									✓	

MEAT & MEAT PRODUCTS

Marker Organisms Testing

	Merck Cat. No.	Reference Method	Aerobic Plate Count	Anaerobic Plate Count	Bacillaceae	Coliforms	E. coli	Enterobacteriaceae	Enterococcus	Listeria	Lactic acid bacteria	Staphylococci	Sulfite reducing Clostridia	Yeast & Moulds
Isolation & Enumeration														
APT Agar	1.10453.	APHA, USDA	✓											
Baird Parker Agar Base + Egg-Yolk Tellurite Emulsion	1.05406. + 1.03785.	AOAC, BAM, APHA, EP, ISO, SMWW, USDA, USP										✓		
Bile Esculine Azide Agar	1.00072.	ISO							✓					
Chapman Agar	1.05469.											✓		
China-Blue Lactose Agar	1.02348.		✓			✓		✓						
Coliform Agar ES, Chromocult®	1.00850.					✓	✓							
Coliform Agar, Chromocult®	1.10426.	EPA				✓	✓							
Count Agar Sugar-Free	1.10878.	IDF	✓											
Dichloran Glycerol (DG 18) Agar	1.00465.													✓
Dichloran Rose Bengal Chloramphenicol (DRBC) Agar	1.00466.	BAM, APHA												✓
E.coli Direct Agar with MUG, Fluorocult®	1.04038.						✓							
Enterococcus Agar, Chromocult®	1.00950.								✓					
Kanamycin Esculin Azide Agar	1.05222.								✓					
KF Streptococcus Agar	1.10707.	APHA, SMWW, EPA							✓					
MacConkey Agar	1.05465.	EP, USP, SMWW, EPA, AOAC, BAM, APHA				✓	✓	✓						
Mannitol Salt Agar	1.05404.	USP, BAM										✓		
MRS Agar	1.10660.	DIN, APHA									✓			
OGYE Agar Base + OGY Supplement	1.05978. + 1.09877.	APHA, ISO												✓
Oxford Agar Base + Oxford Supplement	1.07004. + 1.07006.	AOAC, BAM, APHA, ISO, USDA								✓				

MEAT & MEAT PRODUCTS

Marker Organisms Testing

	Merck Cat. No.	Reference Method	Aerobic Plate Count	Anaerobic Plate Count	Bacillaceae	Coliforms	E. coli	Enterobacteriaceae	Enterococcus	Listeria	Lactic acid bacteria	Staphylococci	Sulfite reducing Clostridia	Yeast & Moulds
PALCAM Listeria Agar Base + PALCAM Supplement	1.11755. + 1.12122.	BAM, APHA, ISO								✓				
Plate Count Agar	1.05463.	APHA, ISO, USDA, SMDP, SMWW, EPA, AOAC, BAM	✓											
Plate Count Skim Milk Agar	1.15338.	DIN, IDF	✓											
Potato Dextrose Agar	1.10130.	APHA, USP, AOAC, BAM												✓
Reinforced Clostridial Medium (RCM)	1.05411.	EP		✓									✓	
Rogosa Agar	1.05413.	APHA									✓			
Rose Bengal Chloramphenicol Agar (RBC)	1.00467.	SMWW												✓
SPS Agar	1.10235.			✓									✓	
Standard I Nutrient Agar	1.07881.		✓											
Tryptone Glucose Extract (TGE) Agar	1.10128.	SMWW, APHA, API, AOAC	✓											
TSC Agar	1.11972.	APHA, DIN, ISO		✓									✓	✓
Vogel Johnson Agar	1.05405.	USP, BAM, EP										✓		
VRB Agar	1.01406.	APHA, SMDP, IDF, BAM, ISO				✓	✓							
VRB Agar with MUG, Fluorocult®	1.04030.	BAM, APHA				✓	✓							
VRBD Agar	1.10275.	EP, ISO, APHA						✓						
Yeast Extract Agar	1.03750.													✓
Yeast Extract Chloramphenicol Agar YGC	1.16000.	IDF, ISO, DIN												✓

MEAT & MEAT PRODUCTS

Marker Organisms Testing

	Merck Cat. No.	Reference Method	Coliforms	E. coli	Enterobacteriaceae	Enterococcus	Listeria	Staphylococci	Sulfite reducing Clostridia	Yeast & Moulds
Enrichment										
Brilliant Green 2%-Bile Broth	1.05454.	SMWW, EPA, AOAC, BAM, APHA, ISO	✓	✓						
Brilliant Green 2%-Bile Broth with MUG, Fluorocult®	1.12587.		✓	✓						
Bromocresol Purple Azide Broth	1.03032.					✓				
Buffered Listeria Enrichment Broth Base + FDA-BAM 1995 Supplement	1.09628. + 1.11781.	BAM,APHA					✓			
Differential Reinforced Clostridial (DRCM) Broth	1.11699.	DIN							✓	
EC Broth	1.10765.	USDA, APHA	✓	✓						
EE Broth Mossel	1.05394.	EP, APHA			✓					
Enterococci Broth, Chromocult®	1.10294.					✓				
Fraser Broth Base + Fraser Supplement	1.10398. + 1.10399.	ISO, AFNOR					✓			
Giolitti-Cantoni Broth	1.10675.	DIN, IDF, ISO, APHA						✓		
Lauryl Sulfate Broth	1.10266.	SMWW, ISO, EPA, AOAC, BAM, APHA, USDA	✓	✓						
Lauryl Sulfate Broth with MUG, Fluorocult®	1.12588.	AOAC, BAM, APHA, SMWW,ISO	✓	✓						
Listeria Enrichment Broth (LEB)	1.10549.	BAM, APHA					✓			
Listeria Enrichment Broth Base + FDA BAM 1992 Supplement	1.11951. + 1.11883.	IDF, BAM					✓			
Listeria Enrichment Broth Base + FDA-BAM 1995 Supplement	1.11951. + 1.11781.	IDF, BAM					✓			
LMX Broth, Fluorocult® + E.coli Supplement	1.10620. + 1.00898.	EPA	✓	✓						

MEAT & MEAT PRODUCTS

Marker Organisms Testing

	Merck Cat. No.	Reference Method	Coliforms	E. coli	Enterobacteriaceae	Enterococcus	Listeria	Staphylococci	Sulfite reducing Clostridia	Yeast & Moulds
L-PALCAM Broth	**1.10823.**						✓			
M Broth	**1.10658.**	**SMWW, AOAC, APHA**			✓					
Reinforced Clostridial Medium (RCM)	**1.05411.**	**EP**							✓	
Sabouraud 2%-Dextrose Agar	**1.07315.**	**SMWW**								✓
Staphylococcus Broth (Baird)	**1.07899.**	**DIN**						✓		
UVM Broth	**1.10824.**	**AOAC, APHA**					✓			
UVM Broth + UVM II Supplement	**1.10824. + 1.04039.**	**USDA**					✓			

MEAT & MEAT PRODUCTS

Spoilage Organisms

	Merck Cat. No.	Reference Method	Antibiotic residues	Bacillaceae ("flat sour")	Clostridia	Proteolytic organisms	Pseudomonades	Lipolytic organisms	Lactic acid bacteria/Lactobacillaceae	Streptococci	Yeast & Moulds
Enrichment											
Bryant & Burkey Broth	1.01617.							✓			
Differential Reinforced Clostridial (DRCM) Broth	1.11699.	DIN			✓						
M-17 Broth	1.15029.									✓	
MRS Broth	1.10661.	DIN, APHA							✓	✓	
Reinforced Clostridial Medium (RCM)	1.05411.	EP			✓						
Tryptic Soy Broth + Polymyxin Supplement	1.05459. + 1.09875.	ISO		✓							
Isolation & Enumeration											
Antibiotic Agar No. 1	1.05272.	EP, USP, AOAC	✓								
APT Agar	1.10453.	APHA, USDA							✓		
Bryant & Burkey Agar (agar added to broth) Agar agar	1.01617. + 1.01614.							✓			
Calcium Caseinate Agar	1.05409.					✓					
Cetrimide Agar	1.05284.	DIN, EP, USP, BAM, AOAC					✓				
Dextrose Tryptone Agar	1.10860.	APHA, NCA		✓							
DG 18 Agar	1.00465.										✓
DRBC Agar	1.00466.	BAM, APHA									✓
GSP Agar	1.10230.						✓				
M-17 Agar	1.15108.	APHA								✓	
MRS Agar	1.10660.	DIN, APHA							✓	✓	
MYP Agar + Polymixin Supplement	1.05267. + 1.09875.	AOAC, BAM, APHA, ISO, USDA		✓							
OGYE Agar Base + OGYE Supplement	1.05978. + 1.09877.	APHA, ISO									✓
Potato Dextrose Agar	1.10130.	APHA, USP, AOAC, BAM									✓
Pseudomonas Agar Base + Pseudomonas CFC Selective Supplement	1.07620. + 1.07627	ISO					✓				
Reinforced Clostridial Agar (RCA)	1.05410.				✓						

MEAT & MEAT PRODUCTS

Spoilage Organisms

	Merck Cat. No.	Reference Method	Antibiotic residues	Bacillaceae ("flat sour")	Clostridia	Proteolytic organisms	Pseudomonades	Lipolytic organisms	Lactic acid bacterial/Lactobacillaceae	Streptococci	Yeast & Moulds
Rogosa Agar	**1.05413.**	**APHA**							✓		
Rose Bengal Chloramphenicol Agar (RBC)	**1.00467.**	**SMWW**									✓
SPS Agar	**1.10235.**				✓						
Trybuturin Agar Base	**1.01957.**							✓			
TSC Agar	**1.11972.**	**APHA, DIN, ISO**			✓						
Yeast Extract Agar	**1.03750.**										✓
Yeast Extract Chloramphenicol Agar (YGC)	**1.16000.**	**IDF, ISO, DIN**									✓

SPICES

Pre-Enrichment & Enrichment

	Merck Cat. No.	Reference Method	Bacillus cereus/Pathogenic Bacilli	Cl. perfringens	Listeria	Moulds	Salmonella/Shigella
Pre Enrichment							
Buffered Peptone Water	1.07228.	BAM, APHA, ISO, DIN					✓
Lactose Broth	1.07661.	SMWW, EPA, APHA, USP, EP, AOAC					✓
Enrichment							
Buffered Listeria Enrichment Broth + FDA-BAM 1995 Supplement	1.09628. + 1.11781.	IDF, BAM			✓		
DIASALM + Novobiocin Supplement	1.09803. + 1.09874.						✓
Differential Reinforced Clostridial Medium (DRCM)	1.11699.	DIN		✓			
Fraser Broth Base + Fraser Supplement	1.10398. + 1.10399.	ISO, AFNOR			✓		
GN Broth Hajna	1.10756.	APHA, USDA					✓
Listeria Enrichment Broth (LEB)	1.10549.	BAM, APHA			✓		
Listeria Enrichment Broth Base + FDA BAM 1992 Supplement	1.11951. + 1.11883.	IDF, BAM			✓		
Listeria Enrichment Broth Base + FDA-BAM 1995 Supplement	1.11951. + 1.11781.	IDF, BAM			✓		
L-Palcam Broth	1.10823.				✓		
M Broth	1.10658.	SMWW, AOAC, APHA					✓
MKTTn Broth	1.05878.	ISO					✓
MSRV Medium + Novobiocin Supplement	1.09878. + 1.09874.	AOAC					✓
Rappaport Salmonella Enrichment Broth	1.10236.						✓
Rappaport Vassiliadis (RVS) Broth	1.07700.	ISO					✓
Reinforced Clostridial Medium (RCM)	1.05411.	EP		✓			

SPICES

Pre-Enrichment & Enrichment

	Merck Cat. No.	Reference Method	Bacillus cereus/Pathogenic Bacilli	Cl. perfringens	Listeria	Moulds	Salmonella/Shigella
Sabouraud 2%-Dextrose Broth	1.08339.	EP, USP, AOAC				✓	
Salmosyst® Broth + Salmosyst® Supplement	1.10153. + 1.10141.						✓
Selenite Cystine Broth	1.07709.	APHA, DIN, USP, ISO, AOAC, BAM					✓
Tetrathionate Broth Muller-Kauffmann	1.10863.	ISO					✓
Tryptic Soy Broth + Polymyxin Supplement	1.05459. + 1.09875.	ISO	✓				
UVM Broth	1.10824.	AOAC, APHA, USDA			✓		
UVM Broth + UVM II Supplement	1.10824. + 1.04039.	USDA			✓		

SPICES

Pathogens Isolation & Enumeration

	Merck Cat. No.	Reference Method	Bacillus cereus/Pathogenic Bacilli	Clostridia	Listeria	Moulds	Salmonella
Isolation & Enumeration							
Bismuth Sulfite Agar	1.05418.	AOAC, APHA, BAM, SMWW, USP					✓
BPL Agar	1.07236.						✓
BPLS Agar	1.07237.						✓
Brilliant Green Agar Modified	1.10747.	ISO					✓
Czapek Dox Agar	1.05460.					✓	
DCLS Agar	1.10270.						✓
Differential Clostridial Agar (DCA) acc. to Weenk	1.10259.			✓			
DG 18 Agar	1.00465.					✓	
DRBC Agar	1.00466.	BAM, APHA				✓	
Hektoen Enteric Agar	1.11681.	AOAC, BAM, APHA, ISO					✓
Oxford Agar Base + Oxford Supplement	1.07004. + 1.07006.	AOAC, BAM, APHA, ISO, USDA			✓		
PALCAM Listeria Agar Base + PALCAM Supplement	1.11755. + 1.12122.	BAM, APHA, ISO			✓		
MYP Agar + Polymixin Supplement	1.05267. + 1.09875.	AOAC, BAM, APHA, ISO, USDA	✓				
OGYE Agar Base + OGYE Supplement	1.05978. + 1.09877.	APHA, ISO				✓	
Potato Dextrose Agar	1.10130.	APHA, USP, AOAC, BAM				✓	
RambachR Agar	1.07500.	FDA(510K)					✓
Rose Bengal Chloramphenicol Agar (RBC)	1.00467.	SMWW				✓	

SPICES

Pathogens Isolation & Enumeration

	Merck Cat. No.	Reference Method	Bacillus cereus/Pathogenic Bacilli	Clostridia	Listeria	Moulds	Salmonella
SS Agar	1.07667.	APHA					✓
SPS Agar	1.10235.			✓			
TSN Agar	1.05264.			✓			
TSC Agar	1.11972.	DIN, APHA, ISO		✓			
XLD Agar	1.05287.	APHA, EP, USP, AOAC, SMWW, BAM					✓
Yeast Extract Chloramphenicol Agar (YGC)	1.16000.	IDF, ISO, DIN				✓	

SPICES

Marker Organisms Enrichment

	Merck Cat. No.	Reference Method	Bacillus cereus/Bacillaceae	Coliforms	E. coli	Enterobacteriaceae	Enterococcus	Listeria	Sulfite reducing Clostridia
Enrichment									
Brilliant Green 2%-Bile Broth	1.05454.	SMWW, EPA, AOAC, BAM, APHA, ISO		✓	✓				
Brilliant Green 2%-Bile Broth with MUG, Fluorocult®	1.12587.			✓	✓				
Differential Reinforced Clostridial Medium (DRCM)	1.11699.	DIN							✓
EC Broth	1.10765.	APHA, SMWW, EPA, AOAC, BAM, ISO, USDA		✓	✓				
EE Broth Mossel	1.05394.	EP, APHA				✓			
Enterococcus Broth, Chromocult®	1.10294.						✓		
Fraser Broth Base + Fraser Supplement	1.10398. + 1.10399.	ISO, AFNOR						✓	
Lauryl Sulfate Broth	1.10266.	SMWW, ISO, EPA, AOAC, BAM, APHA, USDA		✓	✓				
Lauryl Sulfate Broth with MUG, Fluorocult®	1.12588.	AOAC, BAM, APHA, SMWW, ISO		✓	✓				
Listeria Enrichment Broth (LEB)	1.10549.	BAM, APHA						✓	
Listeria Enrichment Broth Base + FDA BAM 1992 Supplement	1.11951. + 1.11883.	IDF, BAM						✓	
Listeria Enrichment Broth Base + FDA-BAM 1995 Supplement	1.11951. + 1.11781.	IDF, BAM						✓	

SPICES

Marker Organisms Enrichment

	Merck Cat. No.	Reference Method	Bacillus cereus/Pathogenic Bacilli	Coliforms	E. coli	Enterobacteriaceae	Enterococcus	Listeria	Sulfite reducing Clostridia
LMX Broth with MUG, Fluorocult®	1.10620.	EPA		✓	✓				
L-PALCAM Broth	1.10823.							✓	
Reinforced Clostridial Medium (RCM)	1.05411.	EP							✓
Tryptic Soy Broth with Polymyxin Supplement	1.05459. + 1.09875.	ISO	✓						
UVM Broth, Modified	1.10824.	AOAC, APHA, USDA						✓	
UVM Broth + UVM II Supplement	1.10824. + 1.04039.	USDA						✓	

SPICES

Marker Organisms Isolation & Enumeration

	Merck Cat. No.	Reference Method	Standard Aerobic Plate Count	Standard Anaerobic Plate Count	Bacillaceae	Coliform bacteria	E. coli	Enterobacteriaceae	Enterococcus	Listeria	Sulfite reducing Clostridia
Isolation & Enumeration											
Bile Esculin Azide Agar	1.00072.	ISO							✓		
China-Blue Lactose Agar	1.02348.		✓			✓		✓			
Coliform Agar ES, Chromocult®	1.00850.					✓	✓				
Count Agar Sugar-Free	1.10878.		✓								
E.coli Direct Agar with MUG (ECD), Fluorocult®	1.04038.						✓				
Enterococcus Agar, Chromocult®	1.00950.								✓		
Kanamycin Esculin Azide Agar	1.05222.								✓		
KF Streptococcus Agar	1.10707.	APHA, SMWW, EPA							✓		
MacConkey Agar	1.05465.	EP, USP, EPA, SMWW, AOAC, BAM, APHA				✓	✓	✓			
Oxford Agar Base + Oxford Supplement	1.07004. + 1.07006.	AOAC, BAM, APHA, ISO, USDA								✓	
PALCAM Listeria Agar Base + PALCAM Supplement	1.11755. + 1.12122.	BAM, APHA, ISO								✓	
Plate Count Agar	1.05463.	APHA, ISO, USDA, SMDP, SMWW, EPA, AOAC, BAM	✓								
Reinforced Clostridial Agar (RCA)	1.05410.										✓
SPS Agar	1.10235.			✓							✓
Standard Count Agar	1.01621.		✓								

SPICES

Marker Organisms Isolation & Enumeration

	Merck Cat. No.	Reference Method	Standard Aerobic Plate Count	Standard Anaerobic Plate Count	Bacillaceae	Coliform bacteria	E. coli	Enterobacteriaceae	Enterococcus	Listeria	Sulfite reducing Clostridia
Tryptone Glucose Extract (TGE) Agar	**1.10128.**	**SMWW, APHA, API, AOAC**	✓								
TSC Agar	**1.11972.**	**DIN, APHA, ISO**		✓							
VRB Agar	**1.01406.**	**APHA, SMDP, IDF, BAM, ISO**				✓	✓				
VRB Agar with MUG, Fluorocult®	**1.04030.**	**APHA, BAM**				✓	✓				
VRBD Agar	**1.10275.**	**EP, ISO, APHA**						✓			

SPICES

Spoilage Organisms - Enrichment, Isolation & Enumeration

	Merck Cat. No.	Reference Method	Clostridia	Yeast & Moulds
Enrichment				
Sabouraud 2%-Dextrose Broth	1.08339.			✓
Isolation & Enumeration				
DG 18 Agar	1.00465.			✓
DRBC Agar	1.00466.	BAM, APHA		✓
OGYE Agar Base + OGYE Supplement	1.05978. + 1.09877.	APHA, ISO		✓
Potato Dextrose Agar	1.10130.	APHA, USP, AOAC, BAM		✓
Reinforced Clostridial Medium (RCM)	1.05411.	EP	✓	
Rose Bengal Chloramphenicol Agar (RBC)	1.00467.	SMWW		✓
Yeast Extract Agar	1.03750.			✓
Yeast Extract Chloramphenicol Agar (YGC)	1.16000.	IDF, ISO, DIN		✓

	Merck Cat. No.	Reference Method	Bacillus cereus/Pathogenic Bacilli	Clostridium perfringens	L. monocytogenes	Salmonella/Shigella	Staphylococcus aureus	VTEC/E. coli 0157:H7
Pre Enrichment								
Buffered Peptone Water	1.07228.	BAM, APHA, ISO, DIN				✓		
Lactose Broth	1.07661.	SMWW, EPA, APHA, USP, EP, AOAC				✓		
Enrichment								
Buffered Listeria Enrichment Broth + FDA-BAM 1995 Supplement	1.09628. + 1.11781.	IDF, BAM			✓			
DIASALM + Novobiocin Supplement	1.09803. + 1.09874.					✓		
Differential Reinforced Clostridial Medium (DRCM)	1.11699.	DIN		✓				
EC Broth, Modified (w/ novobiocin)	1.14582.	USDA, APHA						✓
Fraser Broth Base + Fraser Supplement	1.10398. + 1.10399.	ISO, AFNOR			✓			
Giolitti-Cantoni Broth	1.10675.	DIN, IDF, ISO, APHA					✓	
GN Broth Hajna	1.10756.	APHA, USDA				✓		
Listeria Enrichment Broth (LEB)	1.10549.	BAM, APHA			✓			
Listeria Enrichment Broth Base + FDA BAM 1992 Supplement	1.11951. + 1.11883.	IDF, BAM			✓			
Listeria Enrichment Broth Base + FDA-BAM 1995 Supplement	1.11951. + 1.11781.	IDF, BAM			✓			
L-PALCAM Broth	1.10823.				✓			
M Broth	1.10658.	SMWW, AOAC, APHA				✓		
MKTTn Broth	1.05878.	ISO				✓		
MSRV Medium + MSRV Supplement	1.09878. + 1.09874.	AOAC				✓		
Rappaport Broth (Salmonella Enrichment Broth)	1.10236.					✓		

VEGETABLE TESTING

Detection of Pathogens

	Merck Cat. No.	Reference Method	Bacillus cereus/Pathogenic Bacilli	Clostridium perfringens	L. monocytogenes	Salmonella/Shigella	Staphylococcus aureus	VTEC/E. coli 0157:H7
Rappaport Vassiliadis (RVS) Broth	1.07700.	ISO				✓		
Reinforced Clostridial Medium (RCM)	1.05411.	EP		✓				
Salmosyst Broth + Salmosyst Supplement	1.10153. + 1.10141.					✓		
Selenite Cystine Broth	1.07709.	APHA, DIN, USP, ISO, AOAC, BAM				✓		
Staphylococcus Broth (Baird)+ Egg-Yolk Tellurite Emulsion	1.07899. + 1.03785.						✓	
Tetrathionate Broth Muller-Kauffmann	1.10863.	ISO				✓		
Thioglycollate Broth	1.08190.	APHA, USP	✓					
Tryptic Soy Broth + Polymyxin Supplement	1.05459. + 1.09875.		✓					✓
Tryptic Soy Broth Modified (w/novobiocin)	1.09205.	DIN, BAM						✓
UVM Broth, Modified	1.10824.	AOAC, APHA, USDA			✓			
UVM II Broth (UVM Broth + UVM II Supplement)	1.10824. + 1.04039.	USDA			✓			

VEGETABLE TESTING

Detection of Pathogens

	Merck Cat. No.	Reference Method	Aeromonas	Bacillus cereus/Pathogenic Bacilli	Clostridium perfringens	L. monocytogenes	Salmonella	Shigella	S. aureus	VTEC/E. coli 0157:H7
Isolation & Enumeration										
Ampicillin Dextrin Agar with Vancomycin (ADA-V)	1.07621. + 1.07625.	EPA	✓							
Baird Parker Agar Base + Egg-Yolk Tellurite Emulsion	1.05406. + 1.03785.	AOAC, BAM, APHA, EP, ISO, SMWW, USDA, USP							✓	
Bismuth Sulfite Agar	1.05418.	AOAC, APHA, BAM, SMWW, USP					✓			
Brilliant Green Agar Modified	1.10747.	ISO					✓			
Chapman Agar	1.05469.								✓	
Desoxycholate Lactose Agar	1.02894.	APHA					✓			
Differential Clostridial Agar (DCA) acc. to Weenk	1.10259.						✓			
GSP Agar	1.10230.		✓							
Hektoen Enteric Agar	1.11681.	AOAC, BAM, APHA, ISO					✓			
Mannitol Salt Agar	1.05404.	USP, BAM							✓	
MYP Agar + Cereus Supplement	1.05267.+ 1.09875.	AOAC, BAM, APHA, ISO, USDA		✓						
Oxford Agar Base + Oxford Supplement	1.07004. + 1.07006.	AOAC, BAM, APHA, ISO, USDA				✓				
PALCAM Listeria Agar Base + PALCAM Supplement	1.11755. + 1.12122.	BAM, APHA, ISO				✓				
Rambach Agar	1.07500.	FDA(510K)					✓			
Sorbitol MacConkey Agar (SMAC)	1.09207.	DIN, BAM, APHA, ISO, SMWW, USDA								✓

VEGETABLE TESTING

Detection of Pathogens

	Merck Cat. No.	Reference Method	Aeromonas	Bacillus cereus/Pathogenic Bacilli	Clostridium perfringens	L. monocytogenes	Salmonella	Shigella	S. aureus	VTEC/E. coli 0157:H7
SPS Agar	**1.10235.**				✓					
SS Agar	**1.07667.**	**APHA**					✓	✓		
SSDC (Salmonella Shigella Agar w/sodium desoxy-cholate and calcium chloride)	**1.16724.**	**ISO**								✓
TSC Agar + TSC Supplement	**1.11972. + 1.00888.**				✓					
TSN Agar	**1.05264.**				✓					
Vogel Johnson Agar	**1.05405.**	**EP, USP, BAM**							✓	
XLD Agar	**1.05287.**	**APHA, EP, USP, AOAC, SMWW, BAM**					✓	✓		

VEGETABLE TESTING

Marker Organisms

	Merck Cat. No.	Reference Method	Aerobic Plate Count	Coliforms	E. coli	Enterobacteriaceae	Enterococcus	Heat resistent moulds	Lactic acid bacteria	Staphylococci	Sulfite reducing Clostridia
Enrichment											
Azide Dextrose Broth	1.01590.	EPA, SMWW					✓				
Brilliant Green 2%-Bile Broth	1.05454.	SMWW, EPA, AOAC, BAM, APHA, ISO		✓	✓						
Brilliant Green 2%-Bile Broth with MUG, Fluorocult®	1.12587.			✓	✓						
EC Broth	1.10765.	APHA, SMWW, EPA, AOAC, BAM, ISO, USDA		✓	✓						
EE Broth Mossel	1.05394.	EP, APHA				✓					
Enterococcus Broth, Chromocult®	1.10294.						✓				
Giolitti-Cantoni Broth	1.10675.	DIN, IDF, ISO, APHA								✓	
Lauryl Sulfate Broth	1.10266.	SMWW, ISO, EPA, AOAC, BAM, APHA, USDA		✓	✓						
Lauryl Sulfate Broth with MUG, Fluorocult®	1.12588.	AOAC, BAM, APHA, SMWW		✓	✓						
LMX Broth, Fluorocult® + E.coli Supplement	1.10620. + 1.00898.	EPA		✓	✓						
MRS Broth	1.10661.	DIN, APHA							✓		
Staphylococcus Broth (Baird)	1.07899.									✓	
Isolation & Enumeration											
Baird Parker Agar Base + Egg-Yolk Tellurite Emulsion	1.05406. + 1.03785.	AOAC, BAM, APHA, EP, ISO, SMWW, USDA, USP								✓	
Bile Esculin Azide Agar	1.00072.	ISO					✓				
Chapman Agar	1.05469.									✓	
China-Blue Lactose Agar	1.02348.					✓					
Coliform Agar ES, Chromocult®*	1.00850.				✓						
Coliform Agar, Chromocult®*	1.10426.	EPA			✓						
Count Agar Sugar-Free	1.10878.		✓								

VEGETABLE TESTING

Marker Organisms

	Merck Cat. No.	Reference Method	Aerobic Plate Count	Coliforms	E. coli	Enterobacteriaceae	Enterococcus	Heat resistent moulds	Lactic acid bacteria	Staphylococci	Sulfite reducing Clostridia
Differential Clostridial Agar (DCA) acc. to Weenk	1.10259.										✓
E.coli Direct Agar with MUG (ECD), Fluorocult®	1.04038.			✓	✓						
Enterococcus Agar, Chromocult®	1.00950.						✓				
Kanamycin Esculin Azide Agar	1.05222.						✓				
KF Streptococcus Agar	1.10707.	APHA, SMWW, EPA					✓				
MacConkey Agar	1.05465.	EP, USP, EPA, SMWW, AOAC, BAM, APHA		✓	✓	✓					
Malt Extract Agar	1.05398.							✓			
Mannitol Salt Agar	1.05404.	USP, BAM								✓	
MRS Agar	1.10660.	DIN, APHA							✓		
Orange Serum Agar	1.10673.	APHA						✓			
Plate Count Agar	1.05463.	APHA, ISO, USDA, SMDP, SMWW, EPA, AOAC, BAM	✓								
Reinforced Clostridial Agar (RCA)	1.05410.										✓
Rose Bengal Chloramphenicol Agar (RBC)	1.00467.	SMWW									
SPS Agar	1.10235.										✓
Standard Count Agar	1.01621.		✓								
Test Agar for the Residue Test KUNDRAT	1.10662.						✓				
Tryptone Glucose Extract (TGE) Agar	1.10128.	SMWW, APHA, API, AOAC	✓								
Vogel Johnson Agar	1.05405.	EP, USP, BAM								✓	
VRB Agar	1.01406.	APHA, SMDP, IDF, BAM, ISO				✓					
VRB Agar with MUG, Fluorocult®	1.04030.	APHA, BAM		✓	✓						
VRBD Agar	1.10275.	EP, ISO, APHA		✓	✓	✓					

VEGETABLE TESTING

Spoilage Organisms

	Merck Cat. No.	Reference Method	Aerobic Plate Count	Alicyclobacillus	Coliforms	Enterobacteriaceae	Heat resistant moulds	Lactobacillus spp.	Lactic acid bacteria	Pseudomonas spp.	Sulfite reducing Clostridia	Yeast & Moulds
Isolation & Enumeration												
APT Agar	1.10453.	APHA, USDA						✓	✓			
BAT Medium	1.07994.	IFU		✓								
Cetrimide Agar	1.05284.	DIN, EP, USP, BAM, AOAC								✓		
Coliform Agar ES, Chromocult® *	1.00850.				✓							
Count Agar Sugar-Free	1.10878.		✓									
Czapex Dox Agar	1.05460.						✓					
DG 18 Agar	1.00465.											✓
Differential Clostridial Agar (DCA) acc. to Weenk	1.10259.										✓	
DRBC Agar	1.00466.	BAM, APHA										✓
Malt Extract Agar	1.05398.						✓					
MRS Agar	1.10660.	DIN, APHA							✓			
OGYE Agar Base + OGY Supplement	1.05978. + 1.09877.	APHA, ISO										✓
Orange Serum Agar	1.10673.	APHA					✓		✓			
Plate Count Agar	1.05463.	APHA, ISO, USDA, SMDP, SMWW, EPA, AOAC, BAM	✓									
Potato Dextrose Agar	1.10130.	APHA, USP, AOAC, BAM					✓					✓
Reinforced Clostridial Agar (RCA)	1.05410.										✓	
Rogosa Agar	1.05413.	APHA						✓				
Rose Bengal Chloramphenicol Agar (RBC)	1.00467.	SMWW										✓
SPS Agar	1.10235.										✓	
Standard Count Agar	1.01621.		✓									

VEGETABLE TESTING

Spoilage Organisms

	Merck Cat. No.	Reference Method	Aeromonas hydrophila	Bacillus cereus	Cl. perfringens	L. monocytogenes	Pl shigelloides	Salmonella	Shigella	S. aureus	Y. enterocolitica	Vibrio spp.
Tryptone Glucose Extract (TGE) Agar	**1.10128.**	**SMWW, APHA, API, AOAC**	✓									
TSC Agar + Polymyxin Supplement	**1.11972. + 1.09875.**										✓	
VRB Agar	**1.01406.**	**APHA, SMDP, IDF, BAM, ISO**			✓							
VRB Agar with MUG, Fluorocult®	**1.04030.**	**APHA, BAM**			✓							
VRBD Agar	**1.10275.**	**EP, ISO, APHA**				✓						
Yeast Extract Agar	**1.03750.**											✓
Yeast Extract Chloramphenicol Agar (YGC)	**1.16000.**	**IDF, ISO, DIN**										✓

* only sold in Canada until further notice

	Merck Cat. No.	Reference Method	Aerobic Plate Count	Anaerobic Plate Count	Aeromonas spp.	Cl. perfringens	Coliforms	E. coli	E. coli 0157 (VTEC)	Enterococcus	Fecal coliforms	Fecal Streptococci	Fungi	Legionella	Pseudomonas aeruginosa	Pseudomonas spp.	Sulfite reducing Clostridia	Vibrio cholerae/parahaemolyticus
Enrichment																		
A 1 Medium	1.00415.	SMWW, EPA, APHA									✓							
Azide Dextrose Broth	1.01590.	SMWW, EPA								✓								
Brain Heart Infusion Broth	1.10493.	SMWW, EPA, AOAC, BAM, APHA, ISO, USDA										✓						
Brilliant Green 2%-Bile Broth	1.05454.	SMWW, EPA, AOAC, BAM, APHA, ISO					✓	✓			✓							
Brilliant Green 2%-Bile Broth with MUG, Fluorocult®	1.12587.						✓	✓			✓							
EC Broth	1.10765.	SMWW, EPA, APHA, AOAC, BAM, ISO, USDA					✓	✓										
EC Broth Modified (w/ novobiocin)	1.14582.	USDA, APHA							✓									
Endo Broth MF	1.10750.	SMDP, SMWW, EPA, APHA					✓											
Lactose Broth	1.07661.	SMWW, EPA, APHA, USP, EP, AOAC					✓											
Lauryl Sulfate Broth	1.10266.	SMWW, EPA, ISO, AOAC, BAM, APHA, USDA					✓	✓										
Lauryl Sulfate Broth with MUG, Fluorocult®, ISO	1.12588.	AOAC, BAM, APHA, SMWW					✓	✓										
LMX Broth with MUG, Fluorocult®	1.10620.	EPA					✓	✓										
MacConkey Broth	1.05396.	EP					✓	✓										
Malachite Green Broth	1.10329.	DIN														✓		
Presence-Absence Broth	1.00414.	SMWW, EPA						✓										
ReadyCult® 100	1.01298.	EPA					✓	✓										

WATER Testing

Enrichment, Isolation, Enumeration & Identification

Isolation & Enumeration	Merck Cat. No.	Reference Method	Aerobic Plate Count	Anaerobic Plate Count	Aeromonas spp.	Cl. perfringens	Coliforms	E. coli	E. coli 0157 (VTEC)	Enterococcus	Fecal coliforms	Fecal Streptococci	Fungi	Legionella	Pseudomonas aeruginosa	Pseudomonas spp.	Sulfite reducing Clostridia	Vibrio cholerae/parahaemolyticus
m-Aeromonas Agar + m-Aeromonas Supplement	1.07621. + 1.07625.	EPA			✓													
Bile Esculine Azide Agar	1.00072.	ISO								✓								
Cetrimide Agar	1.05284.	DIN, EP, USP, BAM, AOAC														✓	✓	
Coliform Agar ES, Chromocult®	1.00850.						✓	✓										
Coliform Agar, Chromocult®	1.10426.	EPA					✓	✓										
Czapek-Dox Agar	1.05460.	SMWW											✓					
E.coli 0157:H7 Agar with MUG, Fluorocult®	1.04036.								✓									
E.coli Direct Agar with MUG (ECD), Fluorocult®	1.04038.							✓										
Endo Agar	1.04044.	SMDP, APHA, SMWW					✓	✓										
Enterococcus Agar	1.05262.	SMWW								✓		✓						
Enterococcus Agar w/o TTC	1,05289	SMWW								✓		✓						
Enterococcus Agar, Chromocult®	1.00950.									✓		✓						
GSP Agar	1.10230.				✓												✓	
Kanamycin Esculin Azide Agar	1.05222.									✓								
KF Streptococcus Agar	1.10707.	APHA, SMWW, EPA								✓								
Lactose TTC Agar with Tergitol	1.07680.	ISO					✓	✓										
Legionella Combi Pack	1.10425.	ISO												✓				
Levine EMB Agar	1.01347.	SMWW					✓	✓										
m Endo LES	1.11277.	SMWW, EPA, APHA						✓										
m FC Agar	1.11278.	SMWW, EPA, AOAC									✓							
MacConkey Agar	1.05465.	SMWW, EPA, EP, USP, AOAC, BAM, APHA					✓	✓										

	Merck Cat. No.	Reference Method	Aerobic Plate Count	Anaerobic Plate Count	Aeromonas spp.	Cl. perfringens	Coliforms	E. coli	E. coli 0157 (VTEC)	Enterococcus	Fecal coliforms	Fecal Streptococci	Fungi	Legionella	Pseudomonas aeruginosa	Pseudomonas spp.	Sulfite reducing Clostridia	Vibrio cholerae/parahaemolyticus
Plate Count Agar	1.05463.	SMDP, SMWW, EPA, AOAC, BAM, APHA, ISO, USDA	✓	✓														
Pseudomonas Agar F	1.10989.	DIN, USP, BAM														✓		
Pseudomonas Agar P	1.10988.	DIN, USP, BAM														✓		
Pseudomonas Agar Base + Pseudomonas CN Selective Supplement	1.07620. + 1.07624.	ISO														✓		
R2A Agar	1.00416.	SMWW, APHA, EPA, EP	✓	✓														
Reinforced Clostridial (RCA) Agar	1.05410.					✓											✓	
SPS Agar	1.10235.			✓		✓												
TCBS Agar	1.10263.	APHA, WHO, AOAC, BAM, ISO, SMWW																✓
Tryptone Glucose Extract (TGE) Agar	1.10128.	SMWW, APHA, API, AOAC	✓	✓														
TSC Agar	1.10263.	APHA, DIN, ISO		✓		✓											✓	
VRB Agar	1.01406.	APHA, SMDP, IDF, BAM, ISO					✓	✓										
VRB agar with MUG, Fluorocult®	1.12588.	BAM, APHA					✓	✓										
Yeast Extract Agar	1.13116.	ISO	✓	✓														
Identification (Biochemical tests)																		
Aminopeptidase, Bactident®	1.13301.				✓	✓	✓	✓	✓	✓	✓			✓	✓	✓		
Catalase, Bactident®	1.11351.																	
Gram Stain Kit	1.11885.				✓	✓	✓	✓	✓	✓	✓			✓	✓	✓		
Indole, Bactident®	1.11350.							✓										

	Merck Cat. No.	Reference Method	Aerobic Plate Count	Anaerobic Plate Count	Aeromonas spp.	Cl. perfringens	Coliforms	E. coli	E. coli 0157 (VTEC)	Enterococcus	Fecal coliforms	Fecal Streptococci	Fungi	Legionella	Pseudomonas aeruginosa	Pseudomonas spp.	Sulfite reducing Clostridia	Vibrio cholerae/parahaemolyticus
Kovacs Reagent (indole reaction)	1.09293.							✓										
Oxidase, Bactident®	1.13300.				✓										✓	✓		✓
Rapid Identification																		
Bactident® E.coli	1.13303.							✓										
Singlepath® E.coli 0157	1.04141.	AOAC							✓									
Duopath® Verotoxins	1.04144.	AOAC							✓									
Diluent																		
Buffered Peptone Water	1.07228.	BAM, DIN, ISO, APHA																
Maximum Recovery Diluent	1.12535.	ISO																
Membrane Filter Rinse Fluid (USP)	1.05286.	USP																
Ringer's Tablets	1.15525.	ISO																

PHARMA & COSMETICS

Products	Merck Cat. No.	Autoclave control	Anaerobes	Aerobes	Aeromonas	Bacillus cereus/Bacillus spp.	Coliforms	Candida albicans	Diluents	E. coli	Environmental tests	Gram-negative screening	Enterobacteriaceae	Microbial Limit Test	Media Fill	Preservative effectiveness	Pseudomonas aeruginosa	Pseudomonas spp.	Staphylococci/S. aureus	Sterility Testing	Water	Yeast & Moulds
Baird Parker Agar Base + Egg-Yolk Tellurite Emulsion	1.05406. + 1.03785.														✓				✓			
Bismuth Sulfite Agar	1.05418.														✓							
Brain Heart Infusion Broth	1.10493.		✓																✓			
Brilliant Green Agar Modified	1.10747.														✓							
NaCl Peptone Broth	1.10582.								✓													
Candida Agar Nickerson	1.10456.							✓														✓
Cetrimide Agar	1.05284.														✓		✓	✓				
Coliform 100, ReadyCult®	1.01298.						✓			✓											✓	
Coliform Agar, Chromocult®	1.10426.						✓			✓											✓	
DNase Test Agar	1.10449.																		✓			
EMB Agar	1.01347.												✓									
EMB Agar Levine	1.01342.											✓			✓							
Fluid Thioglycollate Medium	1.08191.		✓																	✓		
Fluid Thioglycollate Medium G	1.16761.		✓																	✓		
King Agar B	1.10991.												✓					✓			✓	
Lactose Broth	1.07661.		✓	✓							✓				✓							
Letheen Agar, modified	1.10404.										✓					✓						
Letheen Broth, modified	1.10405.															✓						
LMX Broth, Fluorocult®	1.10620.						✓			✓												
MacConkey Agar	1.05465.											✓	✓		✓							
MacConkey Agar with MUG, Fluorocult®	1.04029.						✓			✓		✓	✓									
Malt Extract Agar	1.05398.																					✓
Malt Extract Broth	1.05397.																					✓
Mannitol Salt Agar	1.05404.														✓				✓			
Membrane Filter Rinse Fluid (USP)	1.05286.								✓													
MYP Agar + Polymixin Supplement	1.05267. + 1.09875.					✓																
Nutrient Agar	1.05450.		✓	✓												✓						
Nutrient Broth	1.05443.		✓	✓							✓											
OF Basal Medium	1.10282.		✓	✓						✓		✓					✓	✓	✓			
Plate Count Agar	1.05463.																				✓	

PHARMA & COSMETICS

	Merck Cat. No.	Autoclave control	Anaerobes	Aerobes	Aeromonas	Bacillus cereus/Bacillus spp.	Coliforms	Candida albicans	Diluents	E. coli	Environmental tests	Gram-negative screening	Enterobacteriaceae	Media Fill	Microbial Limit Test	Preservative effectiveness	Pseudomonas aeruginosa	Pseudomonas spp.	Staphylococci/S. aureus	Sterility Testing	Water	Yeast & Moulds
Potato Dextrose Agar	1.10130.														✓							✓
Pseudomonas Agar F	1.10989.														✓		✓	✓				
Pseudomonas Agar P	1.10988.														✓		✓	✓				
R2A Agar	1.00416.																				✓	
Ringer tablets	1.15525.								✓													
Sabouraud 2%-Dextrose Agar	1.07315.															✓						✓
Sabouraud Dextrose Agar	1.05438.														✓	✓						✓
Sabouraud 2%-Dextrose Broth	1.08339.															✓						✓
Sabouraud Maltose Agar	1.05439.															✓						✓
Selenite Cystine Broth	1.07709.														✓							
Sterikon® plus bioindicator	1.10274.	✓																				
TAT Broth	1.11723.															✓				✓		
TBX Agar, Chromocult®	1.16122.									✓												
Thioglycollate Broth	1.08190.		✓																	✓		
Triple Sugar Iron Agar (TSI)	1.03915.									✓		✓	✓									
Tryptic Soy Agar	1.05458.		✓	✓											✓	✓					✓	
Tryptic Soy Agar w/ Lecithin & Tween® 80	1.07324.										✓				✓					✓		
Tryptic Soy Broth	1.05459.	✓	✓	✓										✓	✓	✓				✓		
Tryptic Soy Broth, irradiated	1.00800.		✓	✓										✓						✓		
Tryptic Soy Broth, non animal	1.00525.		✓	✓										✓						✓		
Tryptic Soy Broth, non animal, irradiated	1.00550.		✓	✓										✓						✓		
VJ (Vogel & Johnson) Agar	1.05405.														✓				✓			
VRB Agar	1.01406.						✓			✓												
VRB Agar with MUG, Fluorocult®	1.04030.						✓			✓												
VRBD Agar	1.10275.												✓									
Wort Agar	1.05448.																			✓		✓
XLD Agar	1.05287.														✓							

EUROPEAN PHARMACOPOEIA 5TH EDITION (2005)

Medium	EP-Description	MERCK equivalent product	Merck Cat. No.	Clostridia	E. coli	Enterobacteriaceae	Ps. aeruginosa	Salmonella	Staph. aureus	Total Aerobic Microbial Count	Yeast & Moulds	Sterility Testing
A	Broth medium (Casein soya bean digest broth)	Tryptic Soy Broth	1.05459.					✓				✓
B	Agar medium (Casein soya bean digest agar)	Tryptic Soy Agar	1.05458.							✓		
C	Agar medium (Sabouraud-glucose agar with antibiotics)	SABOURAUD 4% Dextrose Agar	1.05438.								✓	
D	Broth medium (Lactose monohydrate broth)	Lactose Broth	1.07661.			✓		✓				
E	Enrichment broth medium (Entero-bacteria enrichment broth-Mossel)	Enterobacteriaceae Enrichment Broth acc.to MOSSEL	1.05394.			✓						
F	Agar medium (Crystal violet, neutral red, bile agar with glucose)	VRBD Agar	1.10275.			✓						
G	Broth medium (MacConkey broth)	MacCONKEY Broth	1.05396.		✓							
H	Agar medium (MacConkey agar)	MacCONKEY Agar	1.05465.		✓							
I	Broth medium (Tetrathionate bile brilliant green broth)	TBG Broth modified	1.05178.					✓				
J	Agar medium (Deoxycholate citrate agar)	LEIFSON Agar	1.02896.					✓				
K	Agar medium (Xylose, lysine, deoxycholate agar)	XLD Agar	1.05287.					✓				
L	Agar medium (Brilliant green, phenolred, lactose monohydrate, sucrose agar)	BPLS Agar (USP)	1.07232.					✓				
M	Agar medium (Triple sugar, iron agar)	Triple Sugar Iron Agar	1.03915.					✓				

EUROPEAN PHARMACOPOEIA 5TH EDITION (2005)

			Merck Cat. No.	Clostridia	E. coli	Enterobacteriaceae	Ps. aeruginosa	Salmonella	Staph. aureus	Total Aerobic Microbial Count	Yeast & Moulds	Sterility Testing
N	Agar medium (Cetrimide agar)	Pseudomonas Selective Agar (CETRIMIDE Agar)	1.05284.				✓					
0	Agar medium (Baird-Parker agar)	BAIRD-PARKER-Agar Egg-yolk tellurite emulsion	1.05406. 1.03785.						✓			
P	Medium (Reinforced Medium for clostridia)	RCM Agar	1.05411.	✓								
Q	Medium (Columbia agar)	COLUMBIA Agar	1.10455.	✓								
R	Medium (Lactose monohydrate sulphite medium)	not commercially available from MERCK		✓								
S	Agar medium R2A	R2A Agar	1.00416.							✓		

EUROPEAN PHARMACOPOEIA 5TH EDITION (2005)

Sterility tests

Medium	EP-description	MERCK´s equivalent product	Merck Cat. No.	Media Fill Test	Sterility Testing	Dilutions	Antibiotic Assay
	Fluid thioglycollate medium	Fluid Thioglycollate Medium	1.08191.		✓		
	Alternative thioglycollate Medium for devices having tubes w/small lumina	Thioglycollate Broth	1.08190.	✓	✓		
	Soya-bean casein digest medium	Tryptic Soy Broth Tryptic Soy Broth, irradiated Tryptic Soy Broth, non-animal origin Tryptic Soy Broth, non-animal origin, irradiated	1.05459. 1.00800. 1.00525. 1.00550.	✓ ✓ ✓ ✓	✓ ✓ ✓ ✓		

Diluting & Rinsing fluids

Medium	EP-description	MERCK´s equivalent product	Merck Cat. No.	Media Fill Test	Sterility Testing	Dilutions	Antibiotic Assay
solution	Buffered sodium chloride-peptone solution pH 7.0	Sodium chloride peptone broth (buffered)	1.10582.			✓	

Antibiotic Assay

Medium	EP-description	MERCK´s equivalent product	Merck Cat. No.	Media Fill Test	Sterility Testing	Dilutions	Antibiotic Assay
2.72 (EP 4.6)	Medium A	Antibiotic Agar 1 (for pH 6.5 +/- 0.2)* Antibiotic Agar 11 (for pH 7.9 +/- 0.2)	1.05272. 1.05269.				✓
	Medium B	Antibiotic Agar 10 not commercially available from Merck Can be prepared from Caso Broth + 12g/litre Agar-Agar + 10g/litre Tween 80	1.05459. 1.01614. 8.22187				✓
	Medium C	not commercially available from Merck					✓
	Medium D	not commercially available from Merck					✓
	Medium E	not commercially available from Merck					✓
	Medium F	not commercially available from Merck					✓
	Medium G	not commercially available from Merck					✓

* final pH for Medium A specified in EP is 6.6

USP 28/2005 PHARMACEUTICAL TESTINGS

USP Media#	USP 28/2005 description	EMD's equivalent product	Merck Cat. No.	<61> MICROBIAL LIMITS TEST						
				E. coli	Ps. aeruginosa	Salmonella	Staph. aureus	Total Aerobic Microbial Count	Yeast & Moulds	Sterility Testing
# I	Fluid Casein Digest-Soy Lecithin-Polysorbate 20 Medium	TAT Broth Base additive: Polyoxyethylene-Monolaurate (Tween® 20)	1.11723. + 8.17072.					✓		✓
# II	Soybean-Casein Digest Agar Medium	Tryptic Soy Agar	1.05458.					✓		✓
# III	Fluid Soybean-Casein Digest Medium	Tryptic Soy Broth	1.05459.					✓		✓
# IV	Mannitol-Salt Agar Medium	Mannitol Salt Agar	1.05404.				✓			
# V	Baird-Parker Agar Medium	BAIRD-PARKER Agar Base add: Egg-Yolk Tellurite Emulsion	1.05406. + 1.03785.				✓			
# VI	Vogel Johnson Agar Medium	VOGEL JOHNSON Agar Base add: Potassium tellurite	1.05405. + 1.05164.				✓			
# VII	Cetrimide Agar Medium	Pseudomonas Agar Base (CETRIMIDE Agar)	1.05284.		✓					
# VIII	Pseudomonas Agar Medium for Detection of Fluorescin	Pseudomonas Agar F, Base add: Glycerin	1.10989. + 1.04091.		✓					
# IX	Pseudomonas Agar Medium for Detection of Pyocyanin	Pseudomonas Agar P, Base add: Glycerin	1.10988. + 1.04091		✓					
# X	Fluid Lactose Medium	Lactose Broth	1.07661.	✓		✓				
# XI	Fluid Selenite-Cystine Medium	Selenite Cystine Broth	1.07709.			✓				
# XII	Fluid Tetrathionate Medium	Tetrathionate Broth Base add: Potassium iodide, Iodine, Brilliant green	1.05285. 1.05043. 1.04761. 1.01310.			✓				
# XIII	Brilliant Green Agar Medium	Brilliant Green Agar	1.07232.			✓				
# XIV	Xylose-Lysine-Desoxycholate Agar Medium	XLD Agar	1.05287.			✓				
# XV	Bismuth Sulfite Agar Medium	Bismuth Sulfite Agar	1.05418.			✓				
# XVI	Triple Sugar-Iron-Agar Medium	Triple Sugar Iron Agar	1.03915.		✓	✓				
# XVII	MacConkey Agar Medium	MacCONKEY Agar	1.05465.	✓						

USP 28/2005 PHARMACEUTICAL TESTINGS

			Merck Cat. No.	E. coli	Ps. aeruginosa	Salmonella	Staph. aureus	Total Aerobic Microbial Count	Yeast & Moulds	Sterility Testing
# XVIII	Levine Eosin-Methylene Blue Agar Medium	LEVINE EMB Agar	1.01342.	✓						
# XIX	Sabouraud Dextrose Agar Medium	SABOURAUD Dextrose Agar	1.05438.						✓	
# XX	Potato Dextrose Agar Medium	Potato Dextrose Agar add: Tartaric acid	1.10130. + 1.00804						✓	
Coagulase	Coagulase Test Plasma	Bactident® Coagulase plasma (Rabbit) w/EDTA	1.13306.				✓			
Oxidase	N,N-dimethyl-p-phenylen-ediamine dihydrochloride	Bactident® Oxidase (test strips)	1.13300.		✓					
Pigment T.	Pigment Test f. Pseudomonas aerug.	UV-lamp (4W/366 nm)	1.13203.		✓					
Gram	Gram Stain Reagents	Gram Staining Kit	1.11885.	✓	✓	✓	✓			

USP 28/2005

<71> STERILTY TESTS

USP Media#	USP 28/2005 description	Merck equivalent product	Merck Cat. No.	Media Fill Test	Sterility Testing	Dilutions	Vitamin Test
# I	Fluid Thioglycollate Medium	Fluid Thioglycollate Medium	1.08191.		✓		
# II	Alternative Thioglycollate Medium for Devices Having Tubes w/small lumina	Thioglycollate Broth	1.08190.	✓	✓		
# III	Soybean-Casein Digest Medium	Tryptic Soy Broth	1.05459.	✓	✓		
		Tryptic Soy Broth, irradiated	1.00800.	✓	✓		
		Tryptic Soy Broth, non-animal origin	1.00525.	✓	✓		
		Tryptic Soy Broth, non-animal origin, irradiated	1.00550.	✓	✓		

DILUTING & RINSING FLUIDS

USP Media#	USP 28/2005 description	Merck equivalent product					
	Fluid A	Peptone from Meat (peptic)	1.07224.			✓	
	Fluid D	Peptone from Meat (peptic) additive: Polyoxyethylene Monooleate (Tween® 80)	1.07224.			✓	
	Fluid K	Nutrient Broth	1.05443.			✓	
# I	Fluid Casein Digest-Soy Lecithin-Polysorbate 20 Medium	TAT Broth Base additive: PolyoxyethyleneMonolaurate (Tween® 20)	1.11723. + 8.17072.			✓	

VITAMIN ASSAY TEST

USP section	USP 28/2005 description	Merck equivalent product					
(91)	Calcium Pantothenate Assay	Vitamin Pantothenic Acid Assay Broth, Base additive: Polyoxyethylene Monooleate (Tween® 80)	1.11993.				✓
(171)	Vitamin B12 Activity Assay	Vitamin B12 Lactobacillus Assay Broth, Base additive: Polyoxyethylene Monooleate (Tween® 80)	1.11988.				✓
(411)	Folic Acid Assay	Vitamin Folic Acid Assay Broth, Base *	1.11990.				✓
	Biotin Assay	Vitamin Biotin Assay Broth	1.11989.				✓
	Nicotinic Acid Assay	Vitamin Nicotinic Acid Assay Broth	1.11992.				✓

* additive: Polyoxyethylene Monooleate (Tween® 80)

USP 28/2005 <81> ANTIBIOTICS - MICROBIAL ASSAYS

USP section	USP 28/2005 description	Merck equivalent product	Merck Cat. No.	Antibiotic Assay
(81)	Medium 1	Antibiotic Agar No. 1	1.05272.	✓
	Medium 2	Antibiotic Agar No. 2	1.05270.	✓
	Medium 3	Antibiotic Broth	1.05273.	✓
	Medium 4	Antibiotic Agar No. 2 add: 1 g/L D(+)glucose	1.05270. 1.08342.	✓
	Medium 5	Antibiotic Agar No.5	1.05271.	✓
	Medium 6	Tryptic Soy Broth add: 0.03 g/L Manganese sulfate pH: 7.0 ± 0.2	1.05459. 1.05941.	✓
	Medium 7	Antibiotic Agar No. 2 (pH: 7.0 ± 0.2)	1.05270.	✓
	Medium 8	Antibiotic Agar No. 2 (pH: 5.9 ± 0.2)	1.05270.	✓
	Medium 9	Tryptic Soy Broth add: 20 g/L Agar Agar	1.05459. 1.01614.	✓
	Medium 10	Tryptic Soy Broth add: 12 g/L Agar Agar additive: Polyoxyethylene Monooleate (Tween® 80)	1.05459. 1.01614.	✓
	Medium 11	Antibiotic Agar No. 11 (pH: 8.3 ± 0.2)	1.05269.	✓
	Medium 12	Antibiotic Agar No. 12	1.10672.	✓
	Medium 13	SABOURAUD Dextrose Broth	1.08339.	✓
	Medium 19	-		✓
	Medium 32	Antibiotic Agar No. 1 add: 0.3 g/L Manganese Sulfate	1.05272. 1.05941.	✓
	Medium 34	-		✓
	Medium 35	-		✓
	Medium 36	Tryptic Soy Agar	1.05458.	✓
	Medium 39	Antibiotic Broth (pH: 7.9 ± 0.2)	1.05273.	✓
	Medium 40	-		✓
	Medium 41	-		✓

Review of Dehydrated Culture Media and Additives


Priceless
quality.

Culture Media

How to use Dehydrated Culture Media

Safe products - Granulated and TSE "free"

Merck KGaA, Darmstadt, Germany supplies more than 450 dehydrated culture media formulations including about 100 custom made formulations. The individual ingredients and the final media are carefully quality-controlled to assure consistency and high quality. All Merck's dehydrated culture media and peptones comply with the European Directorate for the Quality of Medicines (EDQM) (the EC guideline on Requirements in Relation to Active Substances and Directive 1999/82/EEC of 8 September 1999 amending the annex to Council Directive 75/318/EEC) and the European Pharma-copoeia section 5.2.8 Minimizing the risk of transmitting animal spongiform encephalopathy agents via medicinal products.

Merck is a pharmaceutical and chemical company known worldwide and also a worldwide recognized manufacturer of unique granulated dehydrated culture media. The use of granulated culture media protects against dust coming from powders which contain hazardous or even toxic substances. While working with such powder these substances could spread around and could be inhaled. Granulated culture media dissolve more quickly than powders, do not form lumps and have superior flow properties. For more convenience, some dehydrated culture media already contain the selective supplement e.g. antibiotic mixtures.

Dehydrated culture media are generally offered in plastic containers, i.e. 500 g and 5 kg. Other sizes i.e. 1 kg, 2.5kg, 10 kg are also available and additionally a 25kg card-board box for bulk users upon request.

Receiving culture media

Upon arrival of a shipment of dehydrated culture media and/or supplement, the laboratory staff checks the name of the medium, the lot#, and the expiration date. The product documentation accompanying the shipment is also checked i.e. Certificate of Analysis (CofA), BSE/TSE Certificate (country of origin of the material), Material Safety Data Sheet (MSDS), the CE label (for Europe: if a medium is used for clinical testing), and the technical data sheet.

A comparison of documentation, in particular certificates from different manufacturers/suppliers help in selecting a brand supplier.

The laboratory staff signs off on the receipt check and documents the following information, for example, in an inventory log book and also on the label of the product: the date of receipt and a code that product passed the receipt check. The results of the monitoring and seal check is documented in the inventory log book.

Storage / Expiration date

The products are stored according to the manufacturer's instructions.

The dehydrated culture media (marked with date of receipt) are stored ensuring a first in first out. The inventory should be such that it allows a regular turn around. The temperature and humidity in the storage area should be monitored.

Figure: **Merck's safe screw capped supplement vial**

Dehydrated culture media

Although the packaging of Merck's dehydrated culture media protects the contents against light and humidity, it is advised to store dehydrated culture media in the dark and dry place. The optimum storage temperature is 15-25 °C (59-77 °F). Dehydrated culture media are hygroscopic. The containers should always be tightly closed after use to prevent entry of moisture. Absorption of water leads to pH shifts and eventually clumping. Media that have formed lumps must be discarded as they may have undergone chemical changes. A pH value which may have changed on prolonged storage can be corrected.

Under ideal conditions, dehydrated culture media in the original containers can be stored, depending on the type of medium, for at least 3 to 5 years.

Supplements

Merck provides its supplements in small glass bottles with a safe rubber stopper and screw cap. The supplements must be stored in the refrigerator (2-8°C). Under such conditions supplements can be stored in original sealed bottles, depending on the contents, for 3-5years.

Egg yolk / Egg yolk tellurite emulsion

The egg yolk and egg yolk tellurite emulsions must be stored at 2-8°C. Under ideal conditions egg yolk and egg yolk tellurite emulsion can be stored in its original sealed bottles for at least 1year.

Prepared culture media

Prepared culture media dispensed in plates, tubes, or bottles, which are not used immediately should be protected against light and desiccation, so that the composition and the performance is not changing during storage. The stability of prepared culture media is limited. For most of the prepared agar plates and bottled media the optimal storage temperature is 4-12°C in the dark, and wrapped in a bag to avoid contamination and dehydration. Prepared culture media containing blood, egg yolk, egg yolk tellurite emulsion and antibiotics should be stored in the refrigerator at 2-8°C.

If plating agars are to be stored for a longer period of time, they must be prevented from drying out by sealing each Petridish with adhesive tape along the joint between the lid and base or by packing several dishes into airtight plastic bags. Before packaging the plates are not dried. They should be cooled down, because hot to warm agar plates produces condense water. Excessive condense water may ultimately result in contamination of the plates. The storage of agar plates before drying limits the adverse effects of drying of the agar surface during storage. A loss of more than 15% of the water content (during storage and incubation) can adversely affect the growth of microorganisms, particularly Gram-negative. Liquid media in test tubes or flasks should also be sealed airtight. Loss of water can result in precipitation and crystallization of certain substances in the culture media.

The extent of water loss depends on the composition, the amount of medium in the plates, the type of incubator i.e. fan-assisted or otherwise, the humidity of the atmosphere in the incubator, the position and number of the plates in the incubator and the incubation temperature.

In the case of culture media which contain unstable additives, it is often better to store the prepared medium without required additives and to add these later when the final medium is needed.

Some culture media contain ingredients which are extremely light sensitive, e.g. rose bengal in yeast agars like Rose Bengal Chloramphenicol Agar. Upon exposure to light sensitive an inhibitory substance is formed. Rose bengal containing culture media must be kept in the dark both during storage and incubation.

The expiration date of prepared culture media depends on the quality of the basic ingredients, the formulation, the quality of the preparation procedures, the sterilization, the packaging and the storage conditions. Each laboratory should assess the expiration date of each prepared culture medium for its typical preparation, packaging and storage conditions.

ISO 11133 part 1 (2000-06-01) recommends that media to which final components are added immediately before use shall be kept in a refrigerator for not more than 3 months, at room temperature for not more than 1 month and in sealed bags for a maximum period of one week.

First opening

The date of first opening a container should be noted (e.g. in inventory log book and label). The content of the containers at the first opening is visually checked for consistency, color, signs of clumping or caking. The quality of the medium depends on the storage environment. A loss in quality of dehydrated media is realized with changes in flow characteristics of the powder, homogeneity, caking, color changes etc. Any dehydrated medium which has absorbed moisture or shows obvious changes in physical appearance should be discarded.

It is advised to conduct visual checks on the container contents regularly and microbial quality controls on the performance of each prepared medium. The findings should be documented allowing a tracebility of test results back to the container of the culture medium, its performance and its storage conditions. The contents of a container should be used within one year after opening.

Preparation

Culture media are for professional use only!
Preparation see also under literature.

Weighing

Document all relevant data, i.e. type of medium, lot#, weights and volume, date of weighing, scale, and operator.

Dehydrated culture media are in principle not safe. They contain hazardous/ toxic substances like bile salts, azide, selenite, dyes, etc. and powder. It is advised to take the precautions preventing the exposure to powdered dehydrated culture media. The inhalation of dust from powder spread during weighing may be hazardous and must be prevented. The use of Merck granulated media protects against health risks when working with culture media.

A face mask gives some protection against airborne powder. It is recommended to use a hood when weighing out media portions. It gives a good protection against airborne powder. The operation of a hood should be checked and certified by a qualified inspector annually and recorded in the equipment QA/QC log book.

Before weighing check the container contents, the date of first opening label, the expiry date, the full identity of the medium i.e. the catalog number, the name of the medium and its typical composition. Follow precisely the manufacturer's instructions for the preparation on the label.

It is advised to weigh no greater amounts than that required for the preparation of max. 1liter medium.

Dehydrated culture media should be weighed in a weighing boat or clean beaker. Top scales with an accuracy of ±0.1 g should be used. Analytical balances with an accuracy of ±0.001g should be used for weighing out selective components, dyes, etc. Scales and analytical balances are checked and calibrated annually by the manufacturer, and the results of the check are recorded in the equipment QA/QC logbook. Scales and balances must rest on a firm, level surface.

Clean up after weighing. Powder left on scales may contaminate the internal parts. This will impair the accuracy of the scale. For cleaning, water or a surface disinfectant, e.g. 70% ethanol, should be used.

Glass- and plastic ware

Glass- and plastic ware should be inert and not leach components, and should be free of surface alkali. Glassware should be of high-quality low-alkaliborosicilate and designed for applicable heating processes (100°C and 121°C at 1 Pascal) used in the preparation of culture media. Ask your glass ware supplier for advice.

The calibration markings on pipettes, and, if present on vessels too, should be validated. Edged or chipped glassware should be discarded.

Only chemically clean glassware should be used when preparing media. Re-usable glassware is sterilized, washed with hot (90°C) water with a suitable detergent, and dried before storage. Rinse the clean containers to be used (usually conical flasks) thoroughly with purified water to remove any traces of other substances (e.g. detergents). Detergent residues affect adversely the performance of culture media.

Occasionally it may be necessary to remove stubborn residues by soaking glassware in potassium dichromate cleaning solution.

Re-usable caps should be cleaned in a detergent solution and rinsed thoroughly.

Cleaning agents are mostly acidic or alkaline. A simple test to check for residues is rinsing a vessel with a bromothymol blue indicator solution. In a pH range of 6.5-7.3 the solution changes its color from yellow (acidic) to blue-green/blue (alkaline).

The vessel volume should be double the amount of media being prepared. This allows shaking the medium, especially if other components need to be added and to prevent the medium from oversterilizing in the autoclave.

Water

Water used in the preparation of dehydrated culture media must be purified and/or deionized water free from any nutritive and/ or toxic (inhibitory) substances. Purified water shall have a resistivity of at least 300 000Wcm and the conductivity should be less than 10 mS (microSiemens).

Tap water should not be used. In some areas tap water may be contaminated and may contain relatively high amounts of heavy metals and /or chlorine. These can cause precipitation problems and may inhibit the growth of microorganisms.

If the distilled water is prepared from chlorinated water, it is necessary to neutralize the chlorine prior to distillation. This is achieved by adding sodium thiosulphate.

The distilled water can be stored in containers. These should be produced from inert materials (e.g. neutral glass, polyethylene etc.). The containers must be free of any inhibitory substances prior to their initial use. If, during storage, no precautions are taken, atmospheric CO_2 will dissolve, making the water acidic. Also algae may grow quickly in water tanks and their metabolites can inhibit growth of microorganisms.

In some cases it may be necessary to use freshly prepared water, free of dissolved carbon dioxide.

Water processed through an ion exchanger (de-ionized), may have high microorganism content. De-ionized water should not be used without verifying that it does not contain microorganisms. Filtering the water is not enough as water may contain substances inhibitory to the growth of particularly fastidious microorganisms.

In Standard Methods for the Examination of Water and Wastewater a test for the bacteriological suitability of laboratory water is described.

Dissolving (rehydrate) dehydrated culture media

A Good Laboratory Practice (GLP) preparation of culture media requires that during heating evaporation of the reconstituted medium is prevented. Evaporation does not only change the concentration of the ingredients in the reconstituted medium but vapor coming from the media may contain hazar-dous/toxic substances.

The dissolution or rehydration of the dehydrated culture base medium is done as follows:

1. **Measuring water**
   It is necessary to measure exact volumes of distilled or deionized or purified water. The measuring cylinders should have an accuracy in proportion to the volume to be measured. E.g. 500 ml of water should be measured using a 500ml or 1L cylinders but should not be measured in a cylinder of 2 L or greater.
2. **Selecting and labelling a vessel (flask)**
   The right sized vessel should be 2 to 3 times the volume of the culture medium to be prepared. Volumes of no more than 1L are preferred. If larger sizes are needed follow the same rule (check first if autoclave fits the needed sized vessel). Overheating of the medium may result when preparing volumes of more than 1L. Label the vessel (flask) with at least the preparation date, expiry date (helps to identify media immediately that should not be used any more) and identity.
3. **Adding small amount water**
   Approximately a third of the required volume of water is added to a vessel first (this avoids sticking of medium to the bottom and reduces the occurrence of clumping).
4. **Transfer of weighed dehydrated medium**
   The medium should be transferred completely from the weighing boat or clean beaker to the vessel (flask), avoiding airborne dust, and sticking of medium to vessel opening, -walls, and -bottom.
   Granulated culture media will dissolve easily. Just a gently stirring will suffice. A powdered medium however, forms clumps quickly and requires vigorously shaking until it dissolves.
5. **Adding remaining water**
   Progressively add the remaining amount of water and carefully rinse down any material adhering to the walls of the vessel.

6. **Check on sticking**
   All components, except agar-agar and gelatin contained in a dehydrated culture medium, are water soluble. An agar containing medium is dissolved when a transparent agar layer remains on the bottom. A powdered medium sticks quickly to the bottom and components do not completely go into solution even with vigorous shaking. Check before heating the medium - undissolved portions could burn and change the concentrations of the formulation!
   Culture media without agar-agar or gelatin can be dissolved usually in **cold water**, or only require gentle heating. Use should be made of this fact to ensure that the medium is prepared under mild conditions.
7. **Soaking agar containing media**
   Media containing agar should be allowed to soak for several minutes prior to heating (e.g. with mixing).
8. Heating under avoidance of evaporation
   Before heating the medium precautions need to be taken against evaporation of water. Vessels (flasks) should be capped e.g. by using non absorbent cotton prop topped with aluminium foil, a loosely tight metal or screw cap. Tightly closed vessels may "explode", particularly when the reconstitution occurs in a magnetron. It is important that correct glass ware is used.
   Check if the medium contains heat labile ingredients. Avoid overheating the media. Nearly all culture media contain peptones or extracts which are heat sensitive. Overheating of media with a high sugar content and peptones produces Maillard reactions (caramelizing) with formation of growth inhibitory substances and darker colors. These media cannot be used as they were prepared incorrectly.
   Heating should be done with frequent agitation to ensure an even heat distribution. Direct contact of a vessel on a heating plate should be avoided as components may get burned before going into solution. Either use a water bath or a cooking pot. Just before a medium begins to boil it should be removed from the heating source. Agar media, particularly those with low agar content, may boil unexpectedly and may flow out of the flask.

   Boiling water bath / flowing steam
   Culture media containing agar or gelatin must be heated in order to dissolve completely. Heating should be carried out in a boiling water bath or free-flowing steam (e.g. in a steam pot or a not closed autoclave without excess pressure).

   Heating plate
   It is common practice to use a heating plate. Direct contact of a vessel on a heating plate should be avoided as components may get burned before going into solution.The medium must be frequently stirred while gently increasing the temperature. **Boiling of the medium must be avoided.** Overheated media must be discarded.

   Magnetron
   The medium can be dissolved in the magnetron, when the water soluble components, except for agar, are completely dissolved. The magnetron heating process should be validated, meaning, the optimal time should be assessed for a given type of magnetron, a given load, a given type of vessel, and the volume of medium to be prepared. Load the magnetron with vessels of equal media volumes.
   Because a magnetron produces high short bursts of heat (a short overheating) it is not considered to be the most ideal way to dissolve a medium. The process is quick and therefore attractive, particularly when non planned small quantities of medium (e.g. Friday late afternoon) have to be prepared. Only the right glassware and caps should be used and vessels not closed too tightly!
9. **Check for complete dissolution**
   Culture media, which are only heated and not autoclaved, must be checked for complete dissolution! This is achieved when the viscous solution flows smoothly and if no agar particles are to be seen sticking to the walls of the vessel after shaking.
   For some culture media a visual turbidity is necessary and wanted (e.g. Bismuth Sulfite Agar). It is essential that the insoluble components should then be distributed as fine as possible to ensure that the turbidity is homogeneous.
10. **Cooling**
    Allow media containing agar or gelatin to cool to 47 ± 2°C before sterilization in the autoclave.

Dissolving volumes greater than 1 L

If media, containing agar or gelatin, are needed in volumes greater than 1 liter per vessel, these should be dissolved under mild conditions in the following manner:

- Dehydrated media needs rapid dispersion by instant and repeated stirring followed by heating if necessary, to dissolve.
- Agar needs to swell before going into solution. Let media containing agar stand for several minutes and mix to dissolve completely prior to heating.
- For media prepared from scratch each individual component should be added separately and allowed to dissolve before filling up with the complete volume.

Reconstitution of supplements

Supplements commonly contain hazardous and/or toxic agents which must be handled with care. The dispersion of a powder may give allergic or other reactions to the laboratory personnel.

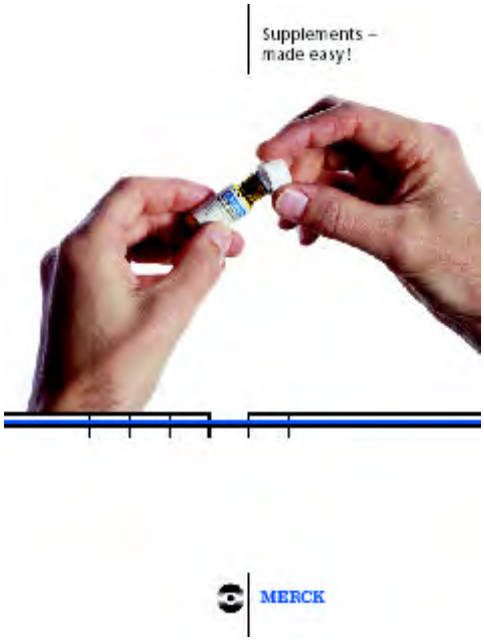


Figure: Merck's safe screw capped supplement vial

Merck's supplements have rubber stoppers and are screw capped and the content is freeze-dried to a tablet (cake). Check the content of the vial and if the tablet is broken, open the vial with care. Avoid dispersion in the case the tablet is broken. Follow the instructions on the label and add the reconstitution fluid. Close the cap and suspend the contents by gently swirling the vial. Contact of the vial contents with the cap during swirling should be avoided. Check the vial for complete dissolution.

After reconstitution, the active substance is generally not stable and the shelf life is mostly limited to the same day but never more than a week at 2-8°C. The dissolving time (day) and the day of use should be marked e.g. on the vial (only when a vial is stored).

Antibiotic solutions may be stored frozen in suitable small aliquots but should then not be frozen again. The potential loss of activity due to freezing should be tested by the user.

pH check and adjustment

The pH value of reconstituted Merck dehydrated culture media should be identical to the pH values specified in the certificates of analysis (CofA's).

The pH value very much depends on the composition of the culture medium, the temperature at which the pH is measured (generally 25 °C) and the treatment which the culture medium has been subjected to during reconstitution (dissolving) and sterilization. If the pH does not meet the manufacturer's specification, this may be due to the water used and /or possible errors made during the preparation. In the section trouble shooting the potential causes for deviations in pH are listed.

In general it is not necessary to adjust the pH of a commercially available culture medium. Dehydrated culture media have typical compositions and the pH may have been adjusted to suit the performance. For culture media prepared from individual components, a pH adjustment may be necessary. The pH should be adjusted so that after sterilization and cooling to 25°C the medium has the required pH ± 0,2pH units unless otherwise stated in the manufacturer's instructions.

The pH is best determined with a calibrated pH-meter (take care to compensate for temperature when standardizing the electrode). A quick pH check of a commercial culture medium is made possible by using special indicator test strips pH 4.0-7.0 (Merck cat. no. 1.09542.) and pH 6.5-10.0 (Merck cat. no. 1.09543.).

The pH should be adjusted to the value specified, if necessary. The pH should be corrected by adding 1N or 1/10N hydrochloric acid (1N or 1mol is 36,5 g HCl in 1 liter water) or 1N or 1mol sodium hydroxide solution (40g in 1liter water) to a sample of known volume taken from the reconstituted culture medium (e.g. 50 ml). The volume of acid or alkali added to the sample can then be used to calculate the quantity necessary to adjust the pH of the final culture medium (acid and alkali solution must be sterile when added to the already sterilized medium). The medium therefore should be kept liquid during the pH measurement of the sample.

Sterilization / "pasteurization"

Sterilization is a procedure designed to entirely eliminate viable microorganisms from a material or medium. Sterilization can be accomplished by:

- **Sterilization by moist**
  Sterilization by moist (saturated steam) has the advantage of a rapid heat penetration and requires lower temperatures. The temperature and time combination for moist sterilization depends on the type of medium:
  1. 121 °C for 15 min. e.g. most media
  2. 115 °C for 15 min. e.g. Baird Parker Agar base, RVS
  3. 110 °C for 10 min. e.g. media with 20% sugar content

- **"Pasteurization" by moist**
  100 °C for 30 min e.g. media containing bile
- **Sterilization by dry heat**
  160 °C for 120 min. e.g. glassware or 170°C to 180°C for >60min.
- **Sterilization by filtration**
- **Sterilization by irradiation**

Sterilization by moist

Sterilization by moist should be performed in a validated and certified autoclave or a media preparator. The equipment must be periodically re-validated and re-certified. Culture media and their additives may decompose at higher temperatures and prolonged cycles. It is therefore important to follow manufacturer's instructions, or to look up the characteristics of the product/substance to be sterilized.

For the sterilization of culture media volumes up to 1 L, the standard sterilization temperature is 121°C for 15 minutes and 1 Pascal (15 pounds) saturated steam (inside autoclave) per square inch. Some culture media, such as Baird Parker Agar Base and Rappaport-Vassiliadis types (RV and RVS) are sterilized at 115°C for 15 min and 10 pounds steam per square inch. Very heat sensitive culture media, e.g. media containing a high concentration of sugar and peptones are sterilized at 110°C for 10minutes and 5 pounds of steam per square inch.

The relations of steam pressure inside the chamber and temperature given that all air is expelled is shown in the following table:

Table: Relation between temperature and steam pressure inside an autoclave chamber and temperature

Steam pressure	Temperature	
(in pounds)	°C	°F
5	109	228
10	115	240
15	121	250
20	126	259
25	130	267

If a larger volume is to be sterilized in one container, a longer cycle should be employed. The cycle must be adapted by authorized staff.

The cycle times above do not include the time required for heating up and cooling. Information on heating and cooling times can either be read from the process chart or must be obtained from the manufacturer of the autoclave or the validation papers for the autoclave.

Complete sterility can only be guaranteed if the steam chamber and the vessels are completely degassed. This is achieved by passing a larger amount of free-flowing steam through the autoclave, e.g. with the valve open at the beginning of the heating up phase. If all air is not replaced with steam, "cold spots" (insufficient heating) and "hot spots" (overheating) occur.

Culture media are heat sensitive and over-sterilization, prolonged heating and cooling and improper loading may change the composition of the medium. Overheating can result in a range of media defects e.g. incorrect pH, caramelization, abnormal color, failure to solidify etc. Therefore it is important that the overall heat penetration of a medium is controlled.

The space between the flasks determines the flow of steam and therefore the evacuation of air and the heat penetration. Consequently autoclaves should not be overloaded. Flask should be positioned, so that free passage of steam is possible. Tubes or flasks containing liquids are plugged with non absorbent cotton or capped loosely. Tubes should be placed in racks or packed loosely in baskets. Flasks should never be more than two-thirds full.

During an autoclave run loss of medium occurs. The volume loss differs with the type of autoclave and loading. The volume loss should be determined for a given autoclave and loading. When tubes with 9ml broth used for ten-fold dilution series are sterilized it is important to compensate for this loss. The dispensing volumes should be adapted to compensate for the loss.

The performance of the autoclave should be monitored for temperature using thermometers inside the chamber and thermocouples inside simulation vessels representative for the load. In choosing simulation vessels it should be taken into account that the heat penetration varies greatly with the media volumes and the type of medium (agar versus liquid). The heat penetration of 1L medium, can be up to 20% longer than for 500 ml of medium, depending on the type of autoclave. As agar is a poor heat conductor, the heat penetration in agar media is considerably longer than for a liquid medium. In an ideal world a run is loaded with similar dispensed volumes and agar media separately autoclaved from liquid media. This is, however, impracticable in most laboratories. Therefore, it is advised to preheat agar containing media before autoclaving.

Sterikon® plus bioindicator is used to validate the efficacy of the sterilization process (121 °C for 15min.) and can be added to each different simulation vessel or tube in a run. The vial contains the test strain *Geobacillus stearothermophilus* in a nutrient broth.

Figure: **Sterikon® plus bioindicator for steam sterilization (121°C for 15min.)**

The door of an autoclave should only be opened after the sterilization cycle when the pressure is equalized and the water cooled down to approx 65-70 °C. If the chamber pressure is too quickly reduced to atmospheric pressure, the load inside still has a temperature above atmospheric and will boil over.

Staff must wear protective clothing (gloves, visor and apron) when removing vessels from the autoclave. It is also advised not to carry more than one hot vessel or tube rack at a time.

Sterilization in an autoclave or media preparator is the most optimal way of sterilizing culture media. In an emergency situation culture media can be heated in a household pressure cooker.

"Pasteurization" by moist

Bile salt containing media like VRBD Agar or VRB Agar, XLD, MacConkey Agar, MacConkey Broth, Brilliant Green 2%-Bile Broth or Hektoen Enteric Agar should not be autoclaved. Heat to boiling (100°C for 30 min.) suffices. This "pasteurization" will kill, apart from some spores, most microorganisms. The "pasteurization" resistant microorganisms will not interfere with the reading during the incubation period (24h) for these media.

Sterilisation by dry heat

Dry heat is used for the sterilization of glassware and metalic instruments that could corrode. The dry heat sterilization cycle is commonly 160°C for 120min. Dry heat sterilization can be validated by using the test strain Dry heat Bacillus*subtilis var.niger*.

Sterilization by filtration

Filtration excludes rather than kills microorganisms. It is used for the sterilization of stock solutions of sugars, additives (antibiotics or chemotherapeutics but not blood or egg yolk) that are heat labile and sometimes for (small volumes) liquid media. In culture media preparation membrane filter are widely used with a pore size of 0,22µ.

The effectiveness of membrane filters depends largely on the size of the pores. For removing bacteria a pore size of 0.22 µ with a pressure of not less than 30 psi is commonly used, whereas for the retention of viruses and mycoplasma membrane filters with pore sizes of 0.01-0.1µ are recommended.

Before a filter sterilization is started, the solution should be checked for complete reconstitution. A filter should be pre-wetted with sterile water, so that the loss due to adherence to the filter is minimized.

Filtration of dehydrated culture media is tedious and not recommended. The filters may clot when larger volumes of media (e.g. for media fill test) are prepared. Furthermore there is a great risk that medium becomes contaminated during membrane filtration. It is documented that small sized bacteria e.g. *Pseudomonas diminuta*, mycoplasma's as well as, L-forms of bacteria can pass through 0,22µ and even penetrate 0,1µ membrane filters.

For the media fill test the use of gamma-irradiated culture media is recommended. These media also offer an alternative when larger volumes of dilution media must be prepared.

Sterilization by filtration can be performed under vacuum or pressurized conditions.

Re-usable filter systems are sterilized in the different parts of the filtration apparatus, assembled or not, in the autoclave at 121 °C for 15 minutes. If necessary, aseptic assembly can be performed in a laminar flow cabinet after autoclaving.

The sterilization by filtration can be validated by filtration of a culture of 10^7 *Pseudomonas diminuta*.

Sterilization by gamma-irradiation

Gamma rays are generated by radioactive cobalt-60 isotopes. The dose for sterilization of specifically designed dehydrated culture media can be as high as 48-62 kGy. This dose is used e.g. to sterilize triple sealed tubes of Tryptic Soy Broth (TSB). A great advantage of the gamma-irradiation to sterilize granulated dehydrated media is that the culture media sterilization is eliminated. The sterile granules can be reconstituted (without sticking and vigorous shaking) with sterile water under aseptic conditions. For media fill applications large volumes of media can be quickly prepared with no risk of a false positive due to filter sterilization e.g. due to mycoplasma's passing through the filter membrane.

The gamma-irradiation process and packaging is validated and certified. It is ensured via quality control before and after sterilization that the irradiation does not adversely affect the growth performance of media.

Preparation of acidic culture media

Agar media with a pH value below 6.0 must be prepared under very mild conditions. Heating acidic media hydrolyzes agar-agar, reducing the gel strength and may promote other chemical reactions affecting adversely the performance characteristics.

To avoid hydrolysis of agar-agar before heating, the pH should be set at about 7.0. After sterilization or final heating the pH is than adjusted to the acidic value.

If agar-agar hydrolysis is the problem, Agar-Agar (Merck cat. no. 1.01614.) can be added to the culture medium before dissolution. Approximately 5.0g/liter is generally sufficient.

Cooling of culture media after heating

After heating for dissolution and steam sterilization, the hot media must be cooled quickly to a temperature of 44-47 °C. This is best done in a circulating thermostatically controlled water-bath. The time needed to reach 44-47 °C depends on the type of media, the volume and the number of units in the waterbath. The loading of the water bath should allow a quick cooling of the medium. Therefore avoid adding still hot media to a waterbath with already cooled media.

Broths may be cooled under running tap water (i.e. when contamination with tap water is avoided).

Media should not be hold for prolonged periods at elevated temperatures (>44-47°C). This will affect adversely the performance, produce precipitates, and can reduce the gel strength of the media.

Addition of supplements and sterile additives

Sterile heat sensitive additives, e.g. blood or egg yolk emulsion, filter sterilized antibiotic solutions or antibiotic supplements are added after the sterilization to the media. Before solutions are transferred they should be checked for complete dissolution and in case of blood and egg yolk emulsion visually for the absence of microorganisms. Supplements should be added to media at a temperature of around 44-47 °C.

The solutions to be added should be adapted to room temperature (25°C). Cold solution straight from the refrigerator may produce flakes in agar media, or may cause gelling. This will hinder a thorough mixing.

Pouring agar plates

Before pouring agar plates, the medium should be cooled a temperature of 44-47°C. Pouring plates at higher temperatures produces excessive formation of condensed water in the lids of the Petridishes.

The medium should be swirled before pouring to ensure that it is homogenous. A volume of 15-18ml of liquid agar medium is poured into Petridishes so that an agar layer thickness of at least 2-3 mm is obtained.

Allow the agar to cool and solidify by placing the Petridishes with lids in place on a cool, horizontal surface. Do not stack plates as this delays the solidification. Check the plates on solidification by gently ticking to the edge. The gelling occurs in the center at the very last.

Air bubbles in the plates can be removed by briefly fanning them with the luminous flame of a Bunsen burner. Invert plates and mark the preparation date and the type of medium on the bottom.

Use of instruments

Attention must be paid to the package inserts of the manufacturers when instruments are used for the media preparation, inoculation, incubation, interpretation etc.

Drying of agar plates

Before surface inoculation agar plates are dried until the surface is visually dry. Wet agar surfaces promote microbial swarming and colony liquefaction. **Do not shake off condensation water from the lid! This water must go back into the medium!** Plates can be dried at 55 °C for 20-30min. in an incubator with circulating air. The time required for drying should be validated and depends on the type of incubator, the circulation, the loading, the thickness of agar layer, the composition of the agar medium, the type of Petridish etc.

For drying in an incubator the lid is removed and dish is inversed. The lid is placed on the edge of the inversed plate. This ensures that the complete surface is equally dried. Alternatively plates can be dried in a laminar-flow cabinet until the droplets have disappeared from the surface of the medium.

Do not over-dry agar plates. If plate surface is too dry ("wrinkled") the performance is strongly impaired (starting with inhibition of Gram-negatives). However, if plates are too wet a motile organism can move while multiplying and form more than one colony.

Slant agar tubes

It has proved useful in certain applications in microbiology to carry out surface cultures in tubes (e.g. strain preservation). For this purpose large culture media surfaces are necessary which can be obtained using "slant agar". Test tubes with sterilized, still liquid agar medium are brought into a slanting position so that a 3cm slanting surface develops on top of a covering layer also about 3cm long. The medium is then allowed to solidify in this position. Slant racks are commercially available to ease this procedure.

De-aeration of media for anaerobic culture

Just prior to use, if necessary, heat the culture medium in boiling water or under flowing steam for 15 min. with lids or caps loose; after heating, tighten the caps and cool the medium rapidly to the operating temperature.

Re-melting of prepared agar media

Melt an agar medium by placing the vessel or tube with loosely cap in a boiling water (waterbath), steam flow-through auto-clave, or magnetron.

The melting in the magnetron at a given load and volume of media should be validated.

Media that have previously been autoclaved should be re-heated for a minimum time to maintain media quality. Overheating must be avoided. The medium is completely melted when upon swirling air bubbles go through the center. Cool the medium to 44-47°C in a thermostatically controlled waterbath.

Molten medium should be used as soon as possible. **Do not remelt the medium a second time!**

Test strains for quality control

Test strains of certain microorganisms are used for the quality control of culture media. These are strains with stable characteristics representative of their species and have shown to be reliable for the demonstration of optimal performance of a particular culture medium. The test organisms for each medium may include robust positive strains with typical characteristics:

- weakly growing positive strains (i.e. of a more sensitive nature),
- biochemically non-reactive strains (e.g. those showing different fermentation or fluorescence reactions) and
- completely inhibited strains.

The European Pharmacopoeia (EP), the United States Pharmacopoeia (USP), ISO 11133 part 1, Standard Methods for the Examination of Water and Wastewater (SMWW) recommend specific test organisms to be used.

These test strains can be sourced from an internationally recognized culture collection (e.g ATCC, NCTC, DSM and others). The relevant culture characteristics of the stock culture from reference strains should be examined and recorded by the laboratory. The strain is renewed should atypical characteristics occur. The use of wild type cultures for which no subculture or handling history is available is not recommended as cultural responses are not "standardized".

The quality control of culture media is conducted with working cultures. These are subcultures from a reference- or master strain. It is also possible to use frozen inocula if shown that the microorganisms can survive long enough.

Reference or master strain

A microorganism which is used as a reference strain should be at least identified to the genus and species level. Its origin (food, soil etc.), the source (supplier) and its characteristic parameters (e.g. colony morphology, growth characteristics in/on typical media, biochemical gallery, agglutination reaction etc) must be described. Reference- or master test strains should be cataloged.

Reference stocks or primary culture

Reference or master test strains can be obtained from culture collections in freeze-dried ampules. For opening and culturing, the instructions of the supplier should be followed.

Phenotypic and genotypic changes in cultures may occur through repeated subculture and exposure to physical and/or chemical conditions that induce alterations.

For test strains used for the quality control of culture media a standard operation procedure (SOP) from reference or master strain to working culture must strictly be followed.

This is a single subculture (first generation) from a reference or master strain. This subculture is done in the laboratory. Reference stocks or primary culture strains are commercially available from different sources, e.g. VWR International.

Thawed or reconstituted reference strains are streaked on to a non-selective nutrient medium capable of supporting the organism's growth, and incubated under appropriate conditions until adequate growth is observed.

No more than two serial subcultures (secondary cultures) of a reference stock or primary culture should be prepared. After a maximum of two subcultures, the reference stock or primary culture must be replaced.

Reference stocks or primary cultures must be kept in a manner that minimizes the opportunity for any contamination, or anything which can alter its characteristics. They may be kept on beads at -70 °C or freeze-dried.

Primary cultures on culture media may be stored at 2-8°C for up to 4 weeks.

Working culture or secondary culture

A working or secondary culture is a primary subculture from a reference stock or primary culture. Working cultures or secondary cultures are prepared as pure stationary phase cultures. Anaerobic cultures are grown in Cooked Meat Broth or on Meat Liver Agar, Schaedler Agar, Clostridium Agar or another suitable anaerobic medium.

Frozen anaerobic cultures (e.g. on commercially available beads) may also be prepared.

A working culture on a medium is prepared from the reference stock or primary culture as follows:

1. Agar slant or plate is inoculated with a reference stock or primary culture. The inoculated medium is incubated to stationary phase growth using the culture conditions appropriate for the strain.
2. The freshly prepared culture is stored either at 2-8 °C or at room temperature (25°C) for up to 4weeks.
3. The working culture is checked for purity and colony morphology. In case of doubt discard the working culture.

Working cultures are used to prepare cell suspensions for inoculation of test media. A frozen culture is used directly.

From the working culture no more than 3 serial subcultures for testing culture media can be prepared.

Working cultures may be stored at 2-8 °C for up to 4 weeks. A working culture is discarded after 8weeks.

Quality control of prepared media

Quality control is conducted for each new batch or lot# of media prepared in-house. A new lot# is at least tested in parallel with an approved lot# of the medium, or an approved batch of the medium, a non-selective reference medium (e.g. Blood Agar or Tryptic Soy Agar or Broth). It is recommended to compare the new batch or lot# of a medium with an approved batch of the medium and a non-selective reference medium.

Quality control of media prepared in-house includes tests to verify sterility, correct growth performance and testing on physical parameters that may compromise the utility of the medium.

Physical quality control testing includes checking pH and appearance, quantity filled and/or agar layer thickness, color, clearness, absence of optical artifacts (e.g. particles demonstrating incomplete dissolution before heating), gel strength, consistency and moistness.

The **growth performance** testing assesses:

- **productivity** (recovery of wanted strain)
- **selectivity** (inhibition of unwanted strain(s)
- **functioning of the diagnostic or differential system** (e.g. lecithinase reaction)

The inoculum employed may consist of robust, weakly growing, biochemically unreactive, or sublethally injured cells. Selective media are tested with both, wanted and unwanted organisms.

Sterility testing should always be undertaken when the culture medium is aseptically dispensed. For sterilized liquid media which are not further dispensed, the validation of the sterilization process may be sufficient.

Growth Performance Testing

Performance testing can be quantitative, semi-qualitative, or qualitative. A qualitative test meets the minimum requirement for quality control of culture media. A greater assurance on the performance of culture media is obtained when a quantitative test is done.

The **quantitative methods** for agar media include: spiral plating, modified Miles and Misra method, surface plating, or pour plating.

For liquid media it includes:
growth rate assessments in impedance - or turbidity instrument, the official French dilution test (ten-fold dilution series with subculture from tubes with growth around extinction), quantitative dilution with single or mixed cultures, and colony counting after enrichment.

The **semi quantitative** methods for agar media include ecometric methods or the semi-quantitative single tube method for wanted, unwanted and mixed organisms.

The **qualitative testing** methods for agar media include a qualitative streaking method for agar media and a single tube method for liquid media.

Detailed protocols and interpretation of the test result for each method are described in EP, USP, Deutches Arzneimittelbuch DAB, **ISO 11133 part 2** and the Culture Media Pharmacopoeia.

For **complex culture** procedures e.g. Salmonella testing, it is recommended to test the complete procedure also using commercially available analytes with low known and disclosed numbers of capsules.

Trouble shooting

Physical quality problems such as appearance, precipitates and pH-shift are common findings and in most cases due to errors made during preparation. The most common sources of mistakes are: bad water quality, defect or malfunctioning pH electrode, incomplete dissolution (visible particles) and overheating.

Possible sources for errors during preparation and handling

Clumping of dehydrated culture media

- Humidity was too high during storage
- Container was left open too long
- Container was not tightly closed after it had been opened
- Dehydrated culture medium was too old

pH-shift

- Water was not neutral
- Container was not tightly closed after it had been opened
- Culture medium was overheated during preparation
- Dehydrated culture medium was too old
- Residues of rinsing solutions

Turbidity, precipitation

- Turbidity of the prepared culture medium should only be considered as an error if it appears in the culture vessel (e.g. Petridish, test tube etc.). Any turbidity observed in the vessel used for preparation due to the presence of a considerably thicker layer of culture medium is of no consequence. Precipitates which settle out to form sediments indicate, however, that an error has been made. Exception: obligatorily turbid culture media!
- Water was not adequately demineralized
- Vessel used for preparation was not clean
- pH-value was incorrect (see "pH adjustment")
- Culture medium was overheated during preparation
- In the case of self-mixed culture media, the basic components contained precipitating impurities
- Caused by the sample material
- Loss of water of the prepared culture medium due to evaporation

Solidification point too high

- Important when sample material or heat sensitive substances are to be mixed into the culture media when they are still fluid
- Too much dehydrated culture medium was weighed out
- Agar-agar not suitable

Gel stability too low

- Insufficient dehydrated culture medium was weighed out
- Dehydrated culture medium was not completely dissolved
- Culture medium was overheated, possibly at a low pH value, during preparation (see "pH adjustment")
- Vessel was not swirled before pouring the plates
- In the case of self-mixed culture media, unsuitable or too little agar-agar
- Acidic culture medium was not prepared under mild conditions (see "Acidic culture media")

Colour change

- In the case of culture media containing indicators, the pH was incorrect (see "pH adjustment")
- Culture medium was overheated during preparation: culture medium dark, coloured pigments destroyed, sugar caramelized
- Vessel used for preparation was not clean

Ready-to-use culture medium contaminated

- Inadequate sterilization
- Drying of the plates in a contaminated (spores) incubator
- Contaminated after sterilization, e.g. while pouring the plates, contaminated Petridishes
- Storage in a contaminated place (refrigerator)
- Addition of unsterile supplements

Growth to poor

- Residues of growth inhibiting substances present in the vessels used for preparation culture (e.g. detergent) in the water used (e.g. substances from the air), in the sample material
- Microorganisms in the sample material already damaged
- pH shift in the case of culture medium
- In the case of culture medium bases, additives dosed incorrectly
- pH shift caused by acid (or basic) sample material
- Culture medium was overheated during preparation
- In the case of pour-plates, temperature was too high

Growth too strong

- Culture medium was overheated during preparation causing destruction of selective inhibitors
- In the case of culture medium bases, additives dosed incorrectly
- Culture medium was inoculated with too much sample material

Colonies liquefy or swarm

- Surface of the culture medium was too moist
- Surface of the culture medium was inoculated with too much sample material
- Culture medium was overheated during preparation causing destruction of inhibitors

A-typical growth

- Culture medium prepared incorrectly
- Dehydrated culture medium was too old
- Prepared culture medium was too old or unfit for use
- Wrong conditions were used for cultivation
- Residues of foreign substances present in the vessel used for preparation or culture (e.g. detergents), in the water used, in the sample material
- Lethally injured cells damaged by the sample material

Inoculation and incubation

The culture media should be pre-warmed to the required incubation temperature by placing them in the incubator several hours before use.

Working safe with microorganisms

Make sure aseptic techniques and established precautions against microbiological hazards throughout all procedures are being followed, since it must be assumed that all specimens/ samples collected may contain infectious microorganisms. After use, each prepared plate/tube, specimen- or sample container and other contaminated materials must be sterilized before discarding. Directions for use should be read and followed carefully.

The regulations and guidelines when working with microbiological hazardous material must be followed at all times.

Microbiology laboratories, which are working with hazardous and infectious test strains, specimens and samples that may contain hazardous microorganisms are advised to handle all material according to the biosafety levels for the suspected microorganism. There are 4 biosafety levels:

- **Biosafety Level 1**
  work with defined and characterized strains of viable organisms not known to consistently cause disease in healthy adult humans.
  Example: *Serratia marcescens*
- **Biosafety Level 2**
  for laboratories in which work is done with the broad spectrum of indigenous moderate-risk agents which are associated with human disease; activities can be performed on the open bench provided the potential for producing splashes or aerosols is low.
  Example: *Salmonella spp.*
- **Biosafety Level 3**
  work with agents with a potential for respiratory transmission and which may cause serious and potentially lethal infection. All laboratory work must be performed in a biological safety cabinet or other enclosed equipment to protect personnel and the environment from exposure to potentially infectious aerosols.
  Example: *Mycobacterium tuberculosis, Coxiella burnetii*
- **Biosafety Level 4**
  work with highly dangerous agents which may be transmitted via aerosol and for which there is no available vaccine or therapy. Specialized equipment and facilities are required.
  Example: *Marburg or Congo-Crimean hemorrhagic fever*

Contaminated or potentially contaminated surfaces can be decontaminated using approved disinfectants known to be bactericidal and/or virucidal.

During analysis, decontamination by immersion in freshly prepared disinfectant solution/dilution for small-sized and corrosion resistant equipment (e.g. pipettes) is recommended. Pasteur pipettes shall only be used once.

Disposing media and cultures

Both, contaminated and not used culture media must be disposed in a way which is safe and meets state or national regulations. The material safety data sheet (MSDS) provides detailed information on disposal of each medium.

There are particular references to dealing with substances suspected of being contaminated with pathogenic microorganisms. According to these recommendations, heat treatment disinfection is particularly important before cleaning or disposal are carried out. A chemical disinfection should only be carried out in exceptional cases.

A **thermic disinfection** of cultures in disposable vessels, in particular plastic, is simply and most effectively carried out using an autoclave (121 °C for 30 min.) **Note: autoclave used for disposal of material should not be used to sterilize culture media or other material.** All material must be in autoclavable plastic bags with a high melting point. When the microorganisms are killed these plastic bags and its contents can be discarded with the regular waste. If suitable incinerators are available, the cultures can also be killed and destroyed by burning. Cultures in re-usable glass vessels (e.g. conical flasks, culture test tubes) must first be killed in the autoclave (121 °C for 30 min.).

Also slightly contaminated glass vessels or heat-stable equipment are firstly autoclaved (134 °C for 20min) or sterilized in a hot-air cabinet (180 °C for at least 30min.). Then the vessels and equipment can be cleaned. If necessary, sterilization can then take place using the autoclave or hot-air cabinet.

Disposables shall be decontaminated prior to its disposal.

The laboratory should operate an identification and separation system of contaminated materials and their containers. This applies for:

- non-contaminated waste that can be disposed with regular waste
- scalpels, needles, knives, broken glass
- contaminated material for autoclaving and recycling
- contaminated material for disposal
- anatomical waste, e.g. animal tissue

Chemical disinfection is carried out with appropriate disinfectants. Most disinfectants have some toxic effects - wear gloves and eye protection.

Rooms and equipment can be decontaminated by fumigation with formaldehyde gas, ozone or UV radiation.

The active ingredients in chemical agents are usually only effective against vegetative microorganisms but not against bacterial spores. Certain bacteria and certain viruses are more resistant to certain active substances than other microorganisms. In chemical disinfection all objects must be thoroughly wetted with the disinfectant. Therefore, adherent air bubbles must be removed. To adequately cover a culture in a Petridish (100 mm diameter) 10-15ml of disinfectant is necessary. The disinfectant should be allowed to act for at least 6 hours (e.g. overnight).

Wash equipment only after it has been decontaminated. After washing, rinse all equipment with deionized water.

Additional users instructions

- Concerning the enrichment of a sample follow the instructions of the manufacturer of the culture medium
- Decision to perform an enrichment only applies to a skilled and authorized person
- Additional biochemical/serological tests must be performed after the isolation step in order to guarantee the diagnostic result
- Interpretation of the diagnostic result only applies to a skilled and authorized person
- National guidelines for the transport and storage of microbiological samples/specimen must be strictly followed
- National guidelines for handling samples/specimen of human resp. biological origin must be strictly followed
- Samples/specimen must be clearly marked with the name of the patient according to the national guidelines
- Contaminated as well as unused media must be disposed according to the local or national guidelines
- A quality assurance programm should be implemented and performed in the laboratory according to the existing standards (e.g. Good Laboratory Practice)
- Standards of clinical microbiology must be followed
- Microbiological examinations are to perform only by trained staff

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Culture media by consistency

Liquid medium
A liquid culture medium consisting of an aqueous solution of one or more constituents (e.g. Buffered peptone water, Nutrient broth).

Solid culture medium
A culture medium containing solidifying or gelling agent (e.g. agar-agar) in concentrations varying from 1 to 2%.

Semi-solid culture medium
A sloppy semi solid medium containing 0.15% of agar-agar. Commonly employed for motility testing (e.g. SIM Agar) or using motility as selective feature (e.g. Diasalm, MSRV).

Culture media by intend of use

The composition of a culture medium formulation determines its purpose.

Preservation medium
A preservation culture medium preserves and maintain the viability of microorganisms over an extended period. During long-term storage the preservation medium protects microorganisms against the adverse influences (e.g. Dorset egg medium).

Resuscitation medium
A resuscitation medium is a non selective nutrient rich medium enabling stressed and damaged microorganisms to repair and to recover their capacity for normal growth (e.g.Tryptic soya agar with 0.3 % yeast extract or Tryptic soy broth).

Enrichment medium
A liquid culture medium provides nutrients for multiplication of microorganisms (e.g. Buffered peptone water or Nutrient broth).

Fermentation medium
A liquid culture medium formulated to achieve the nutrients for an optimal yield of a specific microorganisms (e.g. Yeast) or metabolism product (e.g. toxin).

Selective enrichment medium
A selective enrichment medium is formulated to support the multiplication of target microorganism or a group of microorganisms whilst partially or totally inhibiting the growth of accompanying interfering organisms (e.g. Muller-Kauffmann Tetrathionate broth with novobiocin or L-PALCAM broth).

Isolation medium
A solid culture medium which supports the growth of microorganisms (e.g. Plate Count Agar).

Selective isolation medium
A selective isolation medium which supports the growth of specific target microorganisms, whilst inhibiting other interfering microorganisms (e.g. PALCAM agar or MacConkey agar).

Differential medium
A culture medium which permits the testing of one or more physiological/biochemical characteristics of a microorganisms for their identification (e.g. Fluorocult LMX broth or Simmons Citrate Agar).

Identification medium
A culture medium designed to produce a specific identification reaction which does not require any further confirmatory test (e.g.Triple Sugar (TSI) Agar).

General-purpose media

Some culture media may be assigned to several categories. Blood Agar, for example can be used as a resuscitation medium, as isolation medium or as a differential medium for the detection of haemolysis.

Nutrient Requirements of Microorganisms

For its maintenance and the multiplication microorganisms must draw from its environment the substances required for the synthesis of its cell material and the generation of energy. The substances microorganisms require are termed **nutrients**. The nutrient requirement of microorganisms varies with type of microorganism and can be very complex. Escherichia coli is very simple in its nutritional requirement, whereas Lactobacilus spp. are very demanding (fastidious). A culture medium must supply not only the nutrients a specific microorganism requires, but these must also be present in the appropriate concentration. A too high concentration of a nutrient e.g. amino acid, may inhibit the growth.

Composition of microorganisms-macromolecules

The solid matter of microorganisms contains in addition to hydrogen and oxygen (derivable from water) carbon, nitrogen, phosphorus and sulfurs. These six element account for 95% of the cellular dry weight.

Microorganisms consists of water and macromolecules. Apart from lipids, the macromolecules are build from monomers. Monomers are the precursors of the macromolecules. Examples of macromolecules are:

- protein
- polysaccharides
- lipid
- lipopolysaccharide
- DNA
- RNA

Proteins are the most abundant class of macromolecules and consists of a polymers of the monomers amino acids. After protein ribonucleic acid (RNA) is the most abundant macromolecule. Ribonucleic acid is a polymer of nucleotides and occurs in ribosomes, messenger and transfer RNA's, the key players in the protein synthesis. Lipids rank in abundance as third. Fatty acids are the main constituent of lipids. The simplest form of a lipid is a tri-glyceride and the more complex forms are phospholipid and glycolipid. Lipids are crucial for the membrane structure and serve also storage depots of excess of carbon.

The smaller fractions of cell constitute polysaccharide, lipopolysacharide and DNA. Polysaccharides are polymers of sugars and are primarily present in cell walls. They also serve as carbon and energy source (e.g. glycogen) Lipopolysachharides, such as glycolipid and glycoprotein, play an important role in cell membrane and cell surface receptor molecules. DNA is the other polymer of nucleotides and its contribution to the bacterial cell weight is small. Its function as the repistory of genetic information is, however, crucial to microorganisms.

Nutrient requirements

Microorganisms differ in the specific form under which carbon, nitrogen, sulfur and oxygen must be provided as nutrients. Nutritional studies have shown that microorganism that do not perform photosynthesis or bacteria that obtain energy from the oxidation of inorganic compounds obtain carbon simply from organic nutrients. These include i.e. amino acids, fatty acids, organic acids, sugars, nitrogen bases, aromatic compounds. The **C source** has a dual function and serves both as source of carbon and source of energy. Carbon is the major element in all classes of macromolecules. Some organism require a single organic compound whereas other can not grow with only one compound. Microorganism are extremely divers in the kind and the number of organic compounds they require as C source. After carbon nitrogen is the most important element and is found i.e. in proteins as amino acids and in nucleic acids.

The **nitrogen (N) source** for most organisms are inorganic compounds, that is ammonia, and nitrate or, organic compounds, that is, amino acids, nitrogen bases of nucleotides and many N-containing organic compounds. Nitrogen fixing microorganisms require nitrogen gas.

In addition to C and N source a microorganisms requires **macronutrients** such as phosphor, sulfur, potassium, magnesium, calcium, sodium and iron. Phosphor is required for the synthesis of nucleic acids and phospholipids. Sulfur is required in the amino acids cysteine and methionine and in vitamins such as thiamine, biotin, lipoic acid, and co-enzyme A. Most cell sulfur originates from inorganic source such as sulfate or sulfide. Potassium is required for the protein synthesis and plays an important role in the homoeostasis. Magnesium functions to stabilise ribosomes, cell and nucleic acids. It is also required for the activity of many enzymes. Calcium helps to stabilize the cell wall and plays a key role in the heat stability of endospores. Sodium plays a role in the homeostasis. Iron plays a major role in the cellular respiration and is a key component of cytochromes and iron-sulfur proteins involved in the electron transport.

Microelements or **trace elements** are elements such as cobalt, nickel, chromium, copper, manganese, selenium tungsten, vanadium and zinc. Many of the trace elements play a structural role in enzymes.

Any compound that a microorganism can not synthesise from simpler carbon sources must be provided as a nutrient. Such organic compounds are termed **growth factors**. These include vitamins, amino acids, purines and pyrimidines. Growth factors fulfill specific needs in biosynthesis and they are required in only small amounts. Vitamins function as co-enzymes. Lactic acid bacteria are renowned for their complex vitamin requirement.

Culture Media as Nutrient Source

Synthetic versus complex media

Culture media are the nutrient solutions to grow microorganisms. A culture medium can be a **synthetic** or chemically defined medium prepared by adding precise defined chemicals. They are occasionally used when microorganism with simple nutrient requirements are cultured.

In many instances the nutrient requirements of microorganisms are complex. Organisms with relatively simple nutrients demands like Escherichia coli require complex media. **Complex media** employ peptones and /or extracts supplemented with a sugar (mostly glucose) as energy and C source and a buffer to maintain an optimal pH for growth as the nutritional base.

Balance of nutrients

In the construction of culture media the goal is to provide a balanced mixture of the required nutrients at concentrations that optimise growth of the target organisms. The approach of making a medium as rich as possible by providing all nutrient in great excess does not result in an optimal culture medium. An imbalance among amino acids, for example, such as the excess of one amino acid can inhibit the utilization for growth of a structural related amino acid. Furthermore peptides can surpass their component activity in growth activity. The requirement for peptides results from the fact that a peptide can supply several limiting amino acids in a form that can be absorbed and utilized more rapidly than the free amino acids.

It is not only the presence of a broad range of nutrients but also their concentration and the ratio in which they are present that determines whether the growth of an organism is optimal. Many nutrients become inhibitory or toxic as their concentration is too high.

Complex culture media

A nutrient base must be custom made and optimised for its application. The composition of a medium varies with its application. A nutrient base for the growth of a fastidious microorganism may not be suitable for an optimal growth of a non fastidious microorganism or an other type of fastidious organisms. Similarly nutrient bases employed successfully for the growth of an given organism may not be optimal for the production of toxins.

Despite the diversity in applications and requirement complex culture media have a core structure. The core consituents are:

1. **N source**

   The amino nitrogen base of a complex culture medium can provided by an amino acid or by peptones and extracts derived from enzymatic digested meat, caseine, yeast and vegetables. The amino nitrogen base is not well chemically defined. It provides water-soluble peptides, free amino acids, vitamins, carbohydrates single and complex, growth factors, vitamins, metals.

2. C source or energy source

   Altough peptones can be utilized as C and N source, sugars, such as glucose (dextrose), lactose or other monosaccharides and polysaccharides are commonly added to peptones.

3. Buffer salts

   Buffer salts are used to avoid drastic pH shifts in the medium that are caused by the dissimilation of sugars or the utilisation of protein.

   The most commonly used buffer is the phosphate buffer consisting of a combination of Potassium or Sodium hydrogen phosphate and Potassium or Sodium dihydrogen phosphate. Occasionally other buffers such at MOPS or Tris buffers are employed.

   Buffer components may chelate or sequester essential metal trace elements.

4. Mineral salts and metals

   The basal medium of complex media for fastidious microorganisms for example for Lactobacillaceae, may be supplemented with micro levels of metals (e.g. Ca^{++}, Mg^{++}, Fe^{+++} or Fe^{++}, Mn^{++}) and/or mineral salts (PO_4, SO_4). In most complex media these elements are provide by peptones, infusions or extracts.

5. Growth factors

   Most fastidious organisms require the presence of growth factors. These are not only required as nutrients but also for the protection against toxic agents such as hydrogen peroxide or superoxides.

   Growth factors can be supplemented by adding blood, serum, yeast extract, haemin or vitamins.

6. Sodium chloride

   Sodium chloride is added to maintain the osmotic balance in the medium.

7. Gelling agent

   Agar-agar is the most widely used gelling agent. In some instances silica gel, alginate or gelatin my be used as gelling agent. Gelling agents are employed for the preparation of plating agars or media used for plate counting.

   Agar-agar is not inert. It also contributes metals, minerals and pyruvate and therefore can influence the nutrient composition of a medium.

8. Selective agents

   Selective agents are added to inhibited the accompanying microorganisms without affecting the growth of a target organisms. As selective agents may interact with the nutrient base, the optimal dose can vary with the composition of a complex medium. This interaction may enhance or diminish the selectivity.

9. Indicator dyes

   For the identification or differentiation of organisms pH indicator dyes can be employed. These indicate pH changes due to the dissimilation of a carbohydrate.

Why Granulated Culture Media?

"Clear the air"

Safety, convenience and cost saving

As a customer you have a choice: powdered media or granulated media. A choice for granulated media is a choice for a protection against the health hazards of working with powders containing hazardous/toxic chemicals, ease of handling (quick dissolving, no sticking), cost saving (selective components in base media; no need to purchase expensive supplements) and a choice for Merck. Merck is a pharmaceutical company and this is unique in culture media manufacturing. Merck has more than a century experience in microbiology and is the oldest manufacturer of dehydrated culture media.

Granulated culture media

A **granule** is a small particle of compressed powdered medium. The granules are firm but loosely bound powdered medium. Granules can loose their outer shell, for example, during vigorously shaking of a bottle, due the physical rubbing of granules against each other and the container wall.

Safer

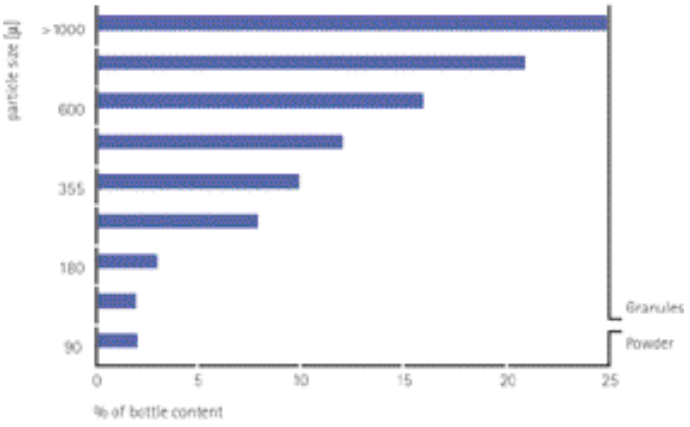
The powder of routinely used culture media often contains hazardous/toxic substances. The handling of powdered culture media usually results in the air borne spread in the working area.

Some hazardous/toxic substances in routinely used culture media bases (For details see Brochure Granulated Culture media)

Hazardous/ toxic ingredient	An example of a culture media base
Acriflavine	Fraser Broth
Bile salts	Violet Red Bile Glucose Agar
Brilliant Green	Brila Broth
Chloramphenicol	DG18 agar
Cycloheximide	Oxford Agar
Dichloran	DRBC agar
Fuchsin	Endo Agar
Lithium Chloride	Baird Parker Agar
Malachite Green	Rappaport Vassiliadis Broth
Rose Bengal	RBC Agar
Selenite	Selenite Cysteine Broth
Sodium Azide	KAA agar
Tergitol	XLT-4 Agar

Inhalation of powder containing hazardous/toxic substances is unhealthy. During culture media preparation one is commonly not aware of the inhalation of fine powder. However, when bile salt containing culture media are weighted or transferred from weighing boot to flasks, inhalation of powdered culture media is noticed. Bile salt irritates the mucosa and triggers on inhalation an immediate coughing. Powdered media also contaminate the skin, eyes, ears and often leads to allergic reactions.

The use of granulated media significantly reduces the spread of powder. Consequently the hazards of inhaling hazardous/toxic substance is reduced leading to a safer, cleaner working environment.



The spread of powder during weighing (Take figure from brochure)

A choice for cost reduction

Purchasing Merck's granulated culture media reduces your costs. The most obvious cost saving is the purchase of culture media to which the selective ingredients are incorporated in the basal medium. There is than no need to purchase expensive supplements. However, there are other less obvious ways to save costs.

- You can reduce the costs of the quality control/validation of the culture media. Each batch of Merck dehydrated culture medium gets a final quality control by the central quality control laboratory acc. to ISO 11133 part 2. Merck provides you with meaningful certificates of analysis that give quantitative information on the performance for the most frequently used culture media.
- Merck produces its culture media in batch sizes varying from 100 to 4500 kg. As the shelf life of granulated dehydrated culture media is 5 years (for some media 3 years) Merck can deliver you one batch for your culture medium consumption over up to 4-5 years.
- Working with Merck granulated culture media saves labour costs. Granulated culture media do not stick, they dissolve quickly. There is no clumping and no sticking of powder to the bottom. A simple gentle swirling dissolve all ingredients except agar or gelatine in minutes.
- Finally, there is no separation or lumping even under warm or humid conditions prolonging the shelf life of products. There is less risk that you have to discard an expensive bottle of culture medium due to clumping!

A choice for convenience

Working with granulated culture media is much more convenient than handling powdered dehydrated culture media. The environment is cleaner and the scales are hardly contaminated.

The granules are easily transferred from weighing booth to flask. They do not stick to the flask opening. There is no need to clean the top of your flask. Your hands remain free of powder thus avoiding getting allergic reactions.

Powder

Granulated

Disadvantages?

Perhaps the only draw back of working with Merck's granulated culture media is that you develop a dis-liking to working with powdered dehydrated culture media.

Granulated culture media combine safety, optimal performance and clear convenience and cost saving advantages.

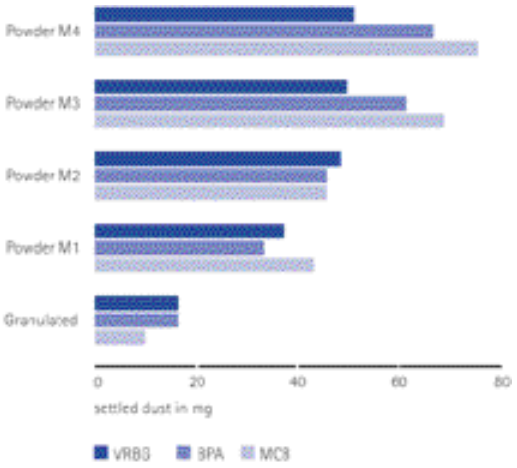
Misconceptions

The advantages of working with granular form of dehydrated culture media are so great that "arguments" have been created and spread to discourage their use. These include:

- *The composition of a granulated dehydrated culture medium base is in-homogeneous. Look into a bottle of granulated medium!*
- *Granulated medium is expensive! You have to waste the powder part of the container!?*
- *Granulation process exposes the powder to excessive heat*

When you open a container of granulated culture medium you see indeed different sizes of granules and when the container content is finishing an increasing amount of powdered medium.

Typically a bottle of Merck's granulated medium contains granules of different sizes and some powdered medium. At a customer the powder amount in a bottle of granulated dehydrated culture medium can vary from 1% to maximally 15%. It is dependant on type of media.



A typical example of the particle frequency in a container of agar medium at end user

The customer is led to believe that therefore a granulated dehydrated culture media is in-homogenous in composition. The composition of granules and powder in a bottle is identical. There is only a difference in the particle sizes ranging from powder (mesh size < ca. 100 micron) to granules (> ca. 100-1000micron).

It is gossiped that the performance between powder and granules differs. A misconception. The manufacturing and scientifically supported facts are clear. Merck produce first similar to any dehydrated culture manufactures homogenous dehydrated powder media. However, Merck goes one step further than other powdered culture media manufacturers. Merck compresses the homogenous dehydrated powder media by a temperature controlled compressing to granules. Comparison studies demonstrate that media prepared from powder and granules perform identically. Therefore, do not discard the small amount of powder remaining in a nearly finished bottle. It can be used for media preparation.

Performance of culture media prepared from granules and powder

Medium	Test organism	Recovery (log_{10} Cfu/ml) Granules	Powder	Colony Diameter (mm) Granules	Powder
Solid (ca. 85% granules + 15% powder)					
Plate Count Agar	L.acidophilus	8.1	8.1	1-2	1-2
Cat. No. 1.05463	S.pyogenes	7.2	7.2	1-2	1-2
	E.coli	9.2	9.2	4-6	4-6
Baird Parker Agar	Staph. aureus	9.3	9.3	1-2	1-2
Cat. No. 1.05406	E.coli	< 1.0	< 1.0		
VRBD Agar	S.gallinarium	9.2	9.2	1	1
Cat. No. 1.10275	E.coli	9.3	9.3	1-2	1-2
	E.faecalis	< 1.0	< 1.0		

Medium	Test organism	Recovery (µS–CO_2/h)* Granules	Powder	End log_{10} Cfu/ml Granules	Powder
Liquid (ca. 90% granules + 10% powder)					
Tryptic Soy Broth	E.coli	280	260	6.5	6.5
Cat. No. 1.05459					
MacConkey Broth	E.coli	310	290	6.6	6.5
Cat. No. 1.05396	S.typhimurium	290	300	6.2	6.2
Selenite Cystine	S.poona	51	51	4.5	4.5
Broth	S.enteriditis	42	45	4.3	4.3
Cat. No. 1.07709					

*Measurement using an impedance growth analyser

Vigorous shaking of the container causes the outer shells of the granules to form powder. The granules themselves are firm but are composed of loosely bound powdered medium, so that the granules will dissolve quickly in water. If the granules could resist the mechanical disintegration, they would not dissolve quickly in water.

More than 50 years of granulated culture media

Merck demonstrates the high quality of its granulated culture media in providing over decades the customer with explicit and meaningful certificates of analysis. For most culture media the growth performance is given quantitatively. In its certificate of analysis Merck specifies the highest performance criteria in the market. Recently ISO 11133 part 2 adopted partly Merck's quality control procedures for dehydrated culture media. The criteria for the selective media in ISO 11133 part 2 with a recovery ratio of 0.1 (10%) are less strict than Merck's quality control criteria for selective medica (0.3 or 30 %).

Benefits of granulated dehydrated culture media

Safer
- Considerably less dust is formed when handling the media. The dangers of allergic reactions and inhalation of toxic substances are thus largely eliminated.

Accurate
- No separation of components and lump formation even under humid or warm conditions.
- No contamination of scales resulting in inaccurate weighing.

Fast
- Better coating of the granulate with water reduces the time required for suspending and dissolving the media. Formation of clumps which are hard to dissolve is thus prevented.

Easier/ Economical
- Better flow properties, the media do not adhere to the wall of vessels or apparatus and are thus easier to weigh out.
- No need for expensive supplements.
- Large batches: savings on your Quality Control.
- Certificates of analysis acc. to ISO 11133 part2.
- Quantitative performance data on most Certificates of analysis.

Reliable
- Homogeneous distribution of the package contents is ensured even after long storage. The components do not therefore separate out.
- Longer shelf life.

Table An overview of granulated dehydrated culture media where selective components are included in basal medium: No need to purchase expensive supplements!

Parameter	Culture medium	Cat. number
Cl. perfringens	SPS Agar	1.10235
Dermatophytes	DTM Agar	1.10896
E.coli O157	m-EC Broth m-TSB Broth	1.14582 1.09205
Enterococcus	KAA Agar KF Streptococcus Agar	1.05222 1.10707
Listeria	Listeria Enrichment Broth (LEB) UVM I Broth	1.10549 1.10824
Salmonella	Selenite Cystine Broth	1.07709 1.07717
Yeast & moulds	DG 18 Agar DRBC Agar RBC Agar YGC Agar	1.00465 1.00466 1.00467 1.16000
Y.enterocolitica	Yersinia Selective Erichment Broth acc. to Ossmer	1.16701

Sterility Control - Media Fill

For ensuring the microbiological safety and quality of the products pharmaceutical companies can not compromise on accuracy and precision. An area of pharmaceutical manufacturing that requires diligent surveillance is aseptic processing.

The media fill test is employed to validate the aseptic processing. It incorporates a sterile, growth medium in place of actual drug products. Media fill procedure is representative of procedures encountered under the most rigorous conditions during normal work assignments. The length of the media fill should be representative of the aseptic process time and include the interruptions and personnel activities that occur during the process.

The simulated product(s) are examined for turbidity (growth) after 14days of incubation. Clear products indicate that no contamination was introduced during compounding procedures. Turbid products indicate that contamination of the product has occurred, and the process should be repeated following analysis and correction of the probable cause(s) of such contamination.

Observation is accomplished by holding the products up to a light source and examining for turbidity or sedimentation. A suggested schedule of observation is at 24 hours, 3 days, 7days, 10days, and 14 days. This assures detection of both fast-growing organisms which may "bloom" and collapse, as well as slow-growing organisms.

The temperature for media fill incubation is recommended at 20-25°C for seven days, then 30-35°C for seven days. There are two schools of thought on this subject , since others think 30-35°C should be first and 20-25°C second.

Superior filtrability

The aseptic filling process is simulated by filling with sterile Tryptic Soy Broth (Cat. No. 1.05459) or Thioglycolate Broth (Cat. No. 1.08190). For media fills larges volumes of sterile culture medium is required, The preparation of large volumes by membrane filtration is not easy, time consuming and costly. Particulates from sterility test broth often plug aseptic filters and these consequently have to be changed frequently. It is difficult to maintain aseptic conditions at media fill testing. The occurrence of false positive media fill test results can not be excluded.

Recognising the problems with culture media for media fill Merck designed ultrafiltrable sterility testing broths. The high quality of special selection of peptones minimises the plugging of aseptic filters. The prepared sterility broths have superior flow properties and growth performance.

The mycoplasma hazard

Membrane filter sterilisation does not necessary result in a sterile medium. *H. pseudoflava*, for example, was shown to penetrate 0.2/0.22micron rated filter tested, with log titer reduction (LTR) values ranging from 3.5 to 7.7 logs. Titer reductions provided by 0.2/0.22micron rated filters for *H. pseudoflava* are comparable to those reported for A. *laidlawii mycoplasma*, although under different conditions. A 0.1 micron rated filter type was also found to be penetrated by *H. pseudoflava*. The penetration of 0.22 µ as well as 0.1µ filter membranes by culture media borne mycoplasma bear the risk of a positive media fill test. Furthermore, this may lead to the contamination of the filling system tested and ultimately of the products.

Gamma-irradiated

Merck responded to the potential hazards at media fill by marketing triple sealed gamma-irradiated (48kGy) sterility testing media. This dose is an overkill to bacteria, spores and mycoplasma which have D-values of about 3 kGy. Sterility of the medium itself is assured. Gamma-irradiated dehydrated sterility testing media is ready to use without autoclaving or membrane filtration. Just add the water. The granulated medium does not produce dust and dissolves in minutes.

The seals of the triple packed dehydrated sterility testing media are hydrogen peroxide resistant, thus allowing decontamination before transfer to the isolator and at opening.

The gamma-irradiation process is validated and certified. The growth promotion of gamma-irradiated sterility medium is similar to that of standard non gamma-irradiated medium.

Animal-peptone free sterility testing media

If it is necessary to avoid the use of peptones of animal origin Merck offers animal-peptone free sterility testing media. Certificates of analysis guarantee a performance similar to that of standard sterility testing broths.

Benefit from granulated sterility testing media

The sterility media are sold in **granulated form**.

Granulated dehydrated sterility testing media combine safety, optimal performance and offer clear advantages. These include: no dust, no sticking of medium to vessels etc., quick dissolving, and superior flow properties.

MPF
A
WW

A 1 Medium

Selective culture medium for the detection of faecal coliforms in water.

The medium conforms with the recommendations of standard methods (US-EPA) for the examination of water.

Mode of Action

Peptone from casein, lactose and salicin are nutrients and guarantee good growth of microorganisms. Sodium chloride provides the osmotic balance. Triton® X 100 is contained as a detergent.

Typical Composition (g/litre)

Peptone from casein 20.0; lactose 5.0; sodium chloride 5.0; salicin 0.5; Triton® X 100 1.0 ml

Preparation

Completely dissolve 31.5 g in 1 litre demin. water and fill into tubes containing inverted fermentation vials (Durham-tubes). Autoclave for 10 min. at 121°C

pH : 6.9 ± 0.2 at 25°C

The prepared medium is clear to slightly opalescent and yellowish in colour. The prepared medium can be stored for up to 1 week at room temperature (store in the dark).

For the examination of 10 ml water samples a double-strength concentrated broth is used.

Experimental Procedure

1. Inoculate tubes according to the Standard Methods MPN-Method.
2. Incubate for 3 hours at 35 ± 0.5°C and then continue incubation in a water bath at 44.5 ± 0.2 °C for 21 ± 2hours

The water level in the bath must be above the level of the liquid in the test tubes!!!

Evaluation

Gas formation in the Durham tubes indicates the presence of faecal coliforms.

The number of faecal coliforms is determined using the MPN-table.

Literature

Andews, W. H., Pressnell, M. W. 1972. Rapid Recovery of Escherischia coli from estuarine water. Appl. Microbiol. **23**:521-523.

Andrews, W. H., Diggs, C. D., and Wilson, C. R. 1975. Evaluation of a medium for the rapid recovery of Escherischia coli from shellfish. Appl. Mikrobiol. **29**: 130-131.

Standridge and Delfino. 1981. Appl. Environ. Microbiol. **42**: 918

Eaton, A. D., Clesceri, L. S. and Greenberg, A. E. (ed.). 1995. Standard methods for the examination of water and wastewater, 19th. Ed. APHA, Washington D.C.

Vanderzant, C., and Splittstoesser, D. F. (ed.). 1992. Compendium of methods for the microbiological examination of food, 3rd ed. APHA Washington D.C.

Association of Official Analytical Chemists. 1995. Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD

Ordering Information

Product	Merck Cat. No	Pack size
A 1 Medium	1.00415.0500	500 g

Escherichia coli

Uninoculated tube

Quality control

Test strains	Inoculum approx. CFU	Growth	Gas formation
Escherichia coli ATCC 25922	100	good/very good	+
Escherichia coli ATCC 8739	100	good/very good	+
Enterococcus faecalis ATCC 19433	100	none / medium	none
Enterobacter aerogenes ATCC 13048	100	none / medium	+/-
Bacillus subtilis ATCC 6633	100	none	none
Aeromonas hydrophila ATCC 7966	100	none	none

Alkaline Peptone Water

For the enrichment of Vibrio cholera and Vibrio spp. in foodstuffs and other materials.

Mode of Action

The medium complies with the recommendations of BAM, 8th Edition 1995, Chapter 9: Vibrio cholera, V. parahaemolyticus, V.vulnificus and other Vibrio spp.; American Public Health Association (APHA), Chapter 28: VIBRIO; AOAC Official Method 988.20 Detection of Vibrio cholera in Oysters; ISO 8914, 1990 Detection of Vibrio parahaemolyticus.

The growth of a broad spectrum of Vibrio spp. is promoted by Peptones, a sodium chloride concentration of 10 g/litre and a high pH of 8.5.

Typical Composition (g/litre)

Peptone 10.0; Sodium chloride 10.0

Preparation

Suspend 20 g in 1 litre of sterile demin. water and autoclave (15min at 121°C).

pH: 8.5 ± 0.2 at 25 °C.

The prepared medium is clear and yellow-brown.

Experimental Procedure and Evaluation

Inoculate Alkaline Peptone Water (usually add 25 g test portion of the sample to 225ml broth) and incubate 6-8 h and 16-24 h at 35-37°C.

After 6-8 h and 16-24 h of incubation streak 0.1 ml on the surface of TCBS Agar, Cat.No. 1.10263 in a way that single colonies are well isolated.

Further tests for differentiation and identification of Vibrio spp. are described in the different methods/standards.

Literature

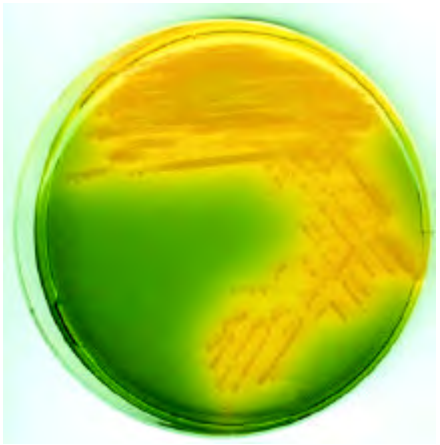
FDA-BAM, 8th Edition 1995, Chapter 9: Vibrio cholera, V. parahaemolyticus, V. vulnificus and other Vibrio spp.; American Public Health Association (APHA), Chapter 28: VIBRIO; AOAC Official Method 988.20 Detection of Vibrio cholera in Oysters; ISO 8914, 1990 Detection of Vibrio para-haemolyticus.

Ordering Information

Product	Merck Cat. No.	Pack size
Alkaline Peptone Water	1.01800.0500	500 g
DCLS Agar (Deoxycholate Citrate Lactose Sucrose Agar)	1.10270.0500	500 g
TCBS Agar (vibrio Selective Agar)	1.10263.0500	500 g

Quality control after 16-24 h incubation (Inoculum: < 15 c.f.u / ml)

Test strains	Growth (cfu/ml)
Vibrio vulnificus ATCC 33149	≥ 10^6
Vibrio cholerae El Tor Inaba CH 38	≥ 10^6
Vibrio cholerae El Tor Ogawa CH 60	≥ 10^6
Vibrio parahaemolyticus ATCC 17802	≥ 10^6



Vibrio cholerae

Vibrio parahaemolyticus

Anaerobic Agar acc. to BREWER

For the surface cultivation of clostridia and other anaerobic microorganisms according to BREWER (1940, 1942).

IVD *in vitro diagnosticum –*
For professional use only

See also General Instruction of Use
Warnings and precautions see ChemDAT®
(www.chemdat.info)

Principle

Microbiological method

Mode of Action

The medium contains a series of reducing agents (thioglycollate, formaldehydesulfoxylate, cystine) which ensure adequate anaerobiosis (QUASTEL and STEPHENSON 1926, AUBERTIN etal. 1928). Methylene blue serves as a redox indicator, its decolouration indicates anaerobiosis.

Typical Composition (g/litre)

Peptone from casein 10.0; peptone from soymeal 5.0; yeast extract 5.0; L-cystine 0.4; D(+)glucose 10.0; sodium chloride 5.0; sodium thioglycollate 2.0; sodium formaldehydesulfoxylate 1.0; methylene blue 0.002; agar-agar 12.6.

Preparation and Storage

Usable up to the expiry date when stored dry and tightly closed at +15 to +25 °C. Protect from light.After first opening of the bottle the content can be used up to the expiry date when stored dry and tightly closed at +15 to +25°C.

Suspend 51g/litre, autoclave (15 min at 121°C), pour plates to give thick layers.

pH: 7.2 ± 0.2 at 25 °C.

The plates are clear and light green.

Specimen

e.g. Isolated bacteria stool, blood, abscess. Clinical specimen collection, handling and processing, see general instructions of use.

Inoculate the culture medium using pour-plate method. For the identification of spore-forming microorganisms add the sample material at a temperature of 80-100 °C.

Incubation: incubate up to 48 hours at 35°C in an anaerobic atmosphere under optimal conditions (e.g. with Anaerocult® A, Anaerocult® P or Anaerocult® A mini).

Literature

AUBERTIN, E., AUBEL, E., et GENEVOIS, L.: A propos de la culture des anaérobies strict en milieu, aérobie. - **Compt. rend. Soc. Biol. (PARIS), 98**; 957-959 (1928).

BREWER, J.H.: Clear liquid medium for the "aerobic" cultivation of anaerobes. - **J. Amer. Med. Ass., 115**; 598-600 (1940).

BREWER, J.H.: A new Petridish and technique for use in the cultivation of anaerobes and microaerophiles. - **Science, 95**; 587 (1942).

QUASTEL, J.H., a STEPHENSON, M.: Experiments on "strict" anaerobes: I. The relationship of B. sporogenes to oxygen . - **Biochem. J.**, 20; 1125-1137 (1926).

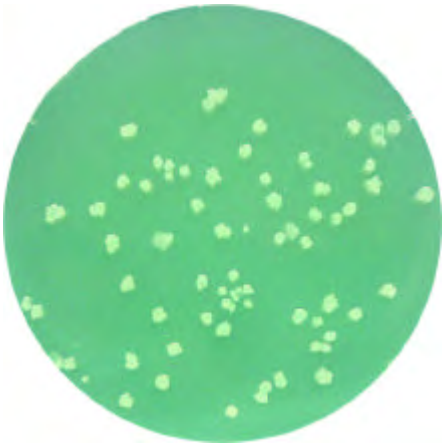
Ordering Information

Products	Merck Cat. No.	Pack size
Anaerobic Agar acc. to BREWER	1.05452.0500	500 g
Anaerocult® A	1.13829.0001	1 x 10
Anaerocult® A mini	1.01611.0001	1 x 25
Anaerocult® P	1.13807.0001	1 x 25
Anaerobic jar	1.16387.0001	1 ea
Anaerotest®	1.15112.0001	1 x 50
Anaeroclip®	1.14226.0001	1 x 25
Plate basket	1.07040.0001	1 ea

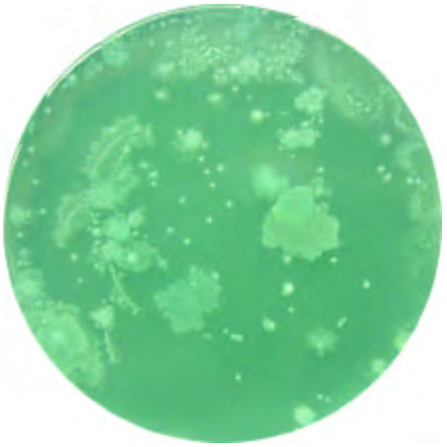
Anaerobic Agar acc. to BREWER

Quality control

Test trains	Growth
Clostridium tetani ATCC 19406	fair / good
Clostridium botulinum	good / very good
Clostridium perfringens ATCC 10543	good / very good
Clostridium putrificum ATCC 25784	good / very good
Clostridium septicum ATCC 12464	good / very good
Clostridium novyi 1795	good / very good
Staphylococcus aureus ATCC 25923	fair / very good
Escherichia coli ATCC 25922	good / very good



Clostridium perfringens ATCC 10543



Clostridium septicum ATCC 12464

Anaerobic jar

For cultivation of anaerobic and microaerophilic microorganisms in defined atmospheric conditions.

IVD

in vitro diagnosticum –
For professional use only

CE

See also General Instruction of Use
Warnings and precautions see ChemDAT®
(www.chemdat.info)

Mode of Action

Using Anaerocult® A (Cat. No. 1.13829.) and Anaerotest® (Cat. No. 1.15112.) for anaerobic microorganisms. Using Anaerocult® C (Cat. No. 1.16275.) for microaerophilic microorganisms.

Experimental Procedure

The anaerobic jar is to be used togehter with Cat. No. 1.07040. Petridish rack (for up to 12 Petridishes).

See also General Instruction of Use.

Ordering Information

Product	Merck Cat. No.	Pack size
Anaerobic jar	1.16387.0001	1 jar
Anaerocult® A	1.13829.	
Anaerotest®	1.15112.	
Anaerocult® C	1.16275.	
Petridish rack	1.07040.	

Anaerocult® A

For the production of an anaerobic milieu in the anaerobic jar (content 2.5litres) for the cultivation of obligatory and facultative anaerobes.

IVD *in vitro diagnosticum –*
For professional use only

See also General Instruction of Use
Warnings and precautions see ChemDAT®
(www.chemdat.info)

Mode of Action

Anaerocult® A contains components which chemically bind oxygen quickly and completely, creating an oxygen-free (anaerobic) milieu an a CO_2 atmosphere.

Typical Composition

Kieselguhr
Iron powder
Citric Acid
Sodium Carbonate

The chemical mixture inside the sachet contains free crystalline silica. In case of damage to the sachet do not inhale dust. Repeated inhalations can cause severe harm to health. Contact with the eyes may cause irritations.

Experimental Procedure

Anaerocult® A is put into the anaerobic jar (Cat. No. 1.16387.).

See also General Instruction of Use.

Moisten the Anaerotest® strip (Cat. No. 1.15112.) with a drop of water and fasten to the tab of the plate basket (Cat. No. 1.07040.). The reaction zone of the Anaerotest® strip should hang freely in the air space. Place the plate basket in the anaerobic jar*.

Slowly pour 35ml of water evenly over the Anaerocult® A special paper over a period of 15-20 seconds, holding the Anaerocult® A as horizontal as possible and pouring with the measuring cylinder paper.

Place the moist Anaerocult® A in the anaerobic jar without delay with the printed side of the Anaerocult® A facing the plates.

Tightly seal the anaerobic jar** immediately.

Storage

Seal tightly and protect from moisture.

Recommended storage temperature: +15 °C ↔ +25°C.

Note

* If the Petri-dish rack of another producer is used, please don't place Anaerocult® A directly above an overhanging piece of metal in the anaerobic jar in order to avoid damage of Anaerocult® A.

** Anaerobiosis is indicated by the colour change of the Anaerotest® strip from blue to white after about 4hours

Ordering Information

Product	Merck Cat. No.	Pack contents
Anaerocult® A	1.13829.0001	10 Anaero-cult® A
Anaerobic jar	1.16387.	
Anaerotest® strip	1.15112.	
Plate basket	1.07040.	

Anaerocult® A mini

Gas generator system for the incubation of one to four Petridishes in an anaerobic atmosphere for the cultivation of obligate and facultative anaerobes.

IVD *in vitro diagnosticum –*
For professional use only

See also General Instruction of Use
Warnings and precautions see ChemDAT®
(www.chemdat.info)

Mode of Action

Anaerocult® A mini contains components which chemically bind oxygen quickly and completely, creating an oxygen-free (anaerobic) milieu and a CO_2 atmosphere.

Typical Composition

Kieselguhr
Iron powder
Citric Acid
Sodium Carbonate

The chemical mixture inside the sachet contains free crystalline silica. In case of damage to the sachet do not inhale dust. Repeated inhalations can cause severe harm to health. Contact with eyes may cause irritations.

Experimental Procedure

Place Anaerocult® A mini together with one to four Petridishes and an anaerobiosis indicator Anaerotest® (Cat. No. 1.15112.) into a special incubation bag.

See also General Instruction of Use.

Moisten the reaction zone of the Anaerotest® strip (Cat. No. 1.15112.) with water.

Stick the Anaerotest® strip on to the lid of the inoculated Petri-dish (the reation zone must point downwards and hang freely in the open space).

Place Anaerocult® A mini into a special incubation bag.

Moisten Anaerocult® A mini with 8.0ml of water.

Place the Petridishes immediately into the special incubation bag and close with Anaeroclip® (Cat. No. 1.14226.) or seal with an ordinary plastic welder (it is advisable to seal with a double weld)*.

The bag must be welded closed approx. 2 cm from the opening.

Stability

See expiry date.

Storage

Seal tightly and protect from moisture (seal the plastic bag well after removing Anaerocult® A mini)

Recommended storage temperature: +15 °C ↔ +25°C.

Note

* Anaerobiosis is indicated by the colour change of the Anaerotest® strip from blue to white after about 4hours.

Ordering Information

Product	Merck Cat. No.	Pack contents
Anaerocult® A mini	1.01611.0001	25 Anaero-cult® A mini 25 special incubation bags
Anaerotest®	1.15112.	
Anaeroclip®	1.14226.	

Anaerocult® C

Anaerocult® C is used to generate an oxygen-depleted and CO_2-enriched atmosphere in a 2.5litre anaerobe jar for culturing Campylobacter species and other microorganisms with fastidious requirements (e.g. Neisseria species, Capnocytophaga species, Eikenella corrodens, Haemophilus speicies). Concentrations of about 8-10 % by volume CO_2 and 5-7 % by volume oxygen are attained.

in vitro diagnosticum –
For professional use only

See also General Instruction of Use
Warnings and precautions see ChemDAT®
(www.chemdat.info)

Mode of Action

Following addition of 6ml of water a defined quantity of oxygen is chemically bound to the finely distributed iron powder while at the same time CO_2 is evolved from sodium carbonate.

Typical Composition

Kieselguhr
Iron powder
Citric Acid
Sodium Carbonate

The chemical mixture inside the sachet contains free crystalline silica. In case of damage to the sachet do not inhale dust. Repeated inhalations can cause severe harm to health. Contact with eyes may cause irritations.

Experimental Procedure

Anaerocult® C is put into the anaerobic jar (Cat. No. 1.16387.)

See also General Instruction of Use.

Place the inoculated Petridishes into the anaerobic jar (use dishes with spacers).

Gently shake an Anaerocult® C bag on the flat of the hand and evenly add 6ml of water to the printed side.

Immediately place the Anaerocult® C bag vertically in the anaerobic jar.

Close the jar tightly and place in the incubator.

Stability

See expiry date.

Storage

Seal tightly and protect from moisture.

Recommended storage temperature: +15 °C ↔ +25°C.

Ordering Information

Product	Merck Cat. No.	Pack contents
Anaerocult® C	1.16275.0001	25 Anaero-cult® C
Anaerobic jar	1.16387.	

Anaerocult® C mini

Gas generator system for the incubation of one or two Petridishes in a low-oxygen, high-CO_2 atmosphere.

IVD *in vitro diagnosticum –*
For professional use only

CE

See also General Instruction of Use
Warnings and precautions see ChemDAT®
(www.chemdat.info)

Mode of Action

Anaerocult® C mini contains components which are able to chemically bind a precisely determined proportion of the oxygen in the special incubation bag and to release a defined quantity of CO_2. The produces a low-oxygen, high-CO_2 atmosphere.

Typical Composition

Kieselguhr
Iron powder
Citric Acid
Sodium Carbonate

The chemical mixture inside the sachet contains free crystalline silica. In case of damage to the sachet do not inhale dust. Repeated inhalations can cause severe harm to health. Contact with the eyes may cause irritations.

Experimental Procedure

Anaerocult® C mini is placed in the special incubation bag with one or two Petridishes. If it is only inteded to incubate one inoculated Petridish, please insert a further non-inoculated Petridish to enable the system to work as intended.

See also General Instruction of Use.

Insert one or two Petridishes in the special incubation bag.

Moisten Anaerocult® C mini with 3ml of water.

Immediately insert Anaerocult® C mini in the special incubation bag.

Seal the special incubation bag with a foil sealing device, preferably making 2 seams about 1cm from the mouth of the bag.

Stability

See expiry date.

Storage

Seal tightly and protect from moisture (seal the plastic bag well after removing Anaerocult® C mini).

Recommended storage temperature: +15 °C ↔ +25°C.

Ordering Information

Product	Merck Cat. No.	Pack contents
Anaerocult® C mini	1.13682.0001	25 Anaerocult® C mini 25 special incubation bags

Anaerocult® IS

A gas generating system for the anaerobic incubation of identification systems and susceptibility test.

IVD

in vitro diagnosticum –
For professional use only

See also General Instruction of Use
Warnings and precautions see ChemDAT®
(www.chemdat.info)

Mode of Action

Anaerocult® IS contains components that chemically bind any oxygen present within a short space of time and also release carbon dioxide thus creating an anaerobic atmosphere.

Typical Composition

Kieselguhr
Iron powder
Citric Acid
Sodium Carbonate

The chemical mixture inside the sachet contains free crystalline silica. In case of damage to the sachet do not inhale dust. Repeated inhalations can cause severe harm to health. Contact with the eyes may cause irritations.

Experimental Procedure

Anaerocult® IS is placed in the special incubation bag together with the agar identification system or 1-2 microtitre plates and the anaerobiosis indicator Anaerotest® (Cat. No. 1.15112.).

See also General Instruction of Use.

Moisten the reaction zone of Anaerotest® with water and stick the anaerobiosis indicator on to the identification system or microtitre plate (the reaction zone must hang freely in the open space). Place the identification system (e.g. Api 20 A*) or microtitre plate (for an identification/susceptibility test) in the special incubation bag.

Moisten Anaerocult® IS with 6 ml water.

Place the moistened Anaerocult® IS immediately in the special incubation bag.

Seal the special incubation bag with a foil sealing device (it is advisable to make 2 seals)**.

Stability

See expiry date.

Storage

Seal tightly and protect from moisture (seal the plastic bag well after removing Anaerocult® IS).

Recommended storage temperature: +15 °C ↔ +25°C.

Notes

* Supplier: Api BioMérieux

** Anaerobiosis is indicated by the colour change of the Anaerotest® strip from blue to white after about 4hours

Ordering Information

Product	Merck Cat. No.	Pack contents
Anaerocult® IS	1.16819.0001	25 Anaerocult® IS 25 special incubation bags
Anaerotest®	1.15112.	

Anaerocult® P

For generating an anaerobic environment in the single Petridish to permit cultivation of obligate and facultative anaerobes.

IVD *in vitro diagnosticum –*
For professional use only

See also General Instruction of Use
Warnings and precautions see ChemDAT®
(www.chemdat.info)

Mode of Action

Anaerocult® P mini contains components which chemically bind oxygen quickly and completely, creating an oxygen-free (anaerobic) environment and a CO_2 atmosphere.

Typical Composition

Kieselguhr
Iron powder
Citric Acid
Sodium Carbonate

The chemical mixture inside the sachet contains free crystalline silica. In case of damage to the sachet do not inhale dust. Repeated inhalations can cause severe harm to health. Contact with eyes may cause irritations.

Experimental Procedure

Place Anaerocult® P together with the Petridish and an anaerobiosis indicator Anaerotest® (Cat. No. 1.15112.) into a special incubation bag.

See also General Instruction of Use.

Moisten the reaction zone of the Anaerotest® strip (Cat. No. 1.15112.) with water.

Stick the Anaerotest® strip on to the lid of the inoculated Petridish (the reaction zone must point downwards and hang freely in the open space).

Place Anaerocult® P into a special incubation bag.

Moisten Anaerocult® P with 3.0ml of water.

Place the Petridish with the attached Anaeroclip® (Cat. No. 1.14226.) or seal with an ordinary plastic welder (it is advisable to seal with a double weld)*.

The bag must be welded closed approx. 2 cm from the opening.

Stability

See expiry date.

Storage

Seal tightly and protect from moisture (seal the plastic bag well after removing Anaerocult® P)

Recommended storage temperature: +15 °C ↔ +25°C.

Note

* Anaerobiosis is indicated by the colour change of the Anaerotest® strip from blue to white after about 4hours.

Ordering Information

Product	Merck Cat. No.	Pack contents
Anaerocult® P	1.13807.0001	25 Anaero-cult® P 25 special incubation bags
Anaerotest®	1.15112.	
Anaeroclip®	1.14226.	

Anaerotest®

For the detection of an anaerobic atmosphere.

IVD *in vitro diagnosticum –*
For professional use only

See also General Instruction of Use
Warnings and precautions see ChemDAT®
(www.chemdat.info)

Mode of Action

The blue oxidized form of the dye methylene blue is converted in oxygen-free (anaerobic) medium into the (colourlesss) leucomethylene blue. In the presence of oxygen the reduced leucobase passes again into the oxidized form (blue).

Typical Composition

Methylene blue - Reducing agent - Stabilizer.

Experimental Procedure

The indicator is used together with Anaerocult® A (Cat. No. 1.13829.) in the anaerobic jar, with Anaerocult® A mini (Cat. No. 1.01611.) for 1-4 Petridishes and with Anaerocult® P (Cat. No. 1.13807.) using just one Petridish. Anaerocult® IS (Cat. No. 1.16819.) is placed in the special incubation bag together with the agar identification system or 1-2 microtitre plates and the anaerobiosis indicator Anaerotest®. Of course Anaerotest® can also be used with other anaerobiosis systems.

See also General Instruction of Use.

Moisten the reaction zone with one drop of distilled water and put Anaerotest® into the anaerobic jar.

When using Anaerocult® (Cat. No. 1.13829.), Anaerocult® IS (Cat. No. 1.16819.), Anaerocult® A mini (Cat. No. 1.01611.) and Anaerocult® P (Cat. No. 1.13807.) draw exact instructions from the respective package inserts.

Evaluation

In an anaerobic atmosphere the reaction zone is decolourized after 4-6 hours (change of colour from blue to white)

Stability

See expiry date.

Storage

Store dry and tightly closed.

Store at +15°C to +25°C.

Only remove the number of sticks required at the time!

Do not touch the reaction zones of the test sticks!

Close containers tightly again at once!

Ordering Information

Product	Merck Cat. No.	Pack contents
Anaerotest®	1.15112.0001	50 test stripes
Anaerocult® A	1.13829.	
Anaerocult® A mini	1.01611.	
Anaerocult® P	1.13807.	
Anaerocult® IS	1.16819.	

Antibiotic Media

Product	Description	Standards
Antibiotic Agar No. 1		AOAC, EP, USP
Antibiotic Agar No. 2		AOAC, USP
Antibiotic Agar No. 4		AOAC, USP
Antibiotic Agar No. 5		AOAC, USP
Antibiotic Medium No. 6	can be prepared from Antibiotic Agar No. 2 and 1 g/litre D(+)glucose	
Antibiotic Agar No. 7	corresponds to Antibiotic Agar No. 2 but with pH: 7.0 ± 0.2.	
Antibiotic Agar No. 8	corresponds to Antibiotic Agar No. 2 but with pH: 5.6 ± 0.2.	AOAC, USP
Antibiotic Agar No. 9	can be prepared from CASO Broth, 20 g/litre Agar-Agar	EP, USP
Antibiotic Agar No. 10	can be prepared from CASO Broth, 12 g/litre Agar-Agar and 10 g/litre Tween® 80	EP, USP
Antibiotic Agar No. 11		
Antibiotic Agar No. 12		
Antibiotic Broth (Medium No. 3)		AOAC, EP, USP
SABOURAUD-2 % Dextrose Broth (Medium No. 13)		AOAC, USP

For the microbiological assay of antibiotics in pharmaceutical preparations, body fluids, animal feed preparations, and other materials according to GROVE and RANDALL (1955).

These culture media comply with the recommendations of the United States Pharmacopeia XXVI (2003) and the FDA. Antibiotic agar I also corresponds to medium A of the European Pharmacopeia II.

Antibiotic Media

Principle

The sample material can be tested by dilution and diffusion methods. The most common method is the agar diffusion test which can be performed in various ways - cylinder, punched-hole or paper-disc tests. It is based on the following principle:

The culture medium is inoculated with the relevant test strain and poured into plates. Defined quantities of the antibiotic under examination and an antibiotic standard are applied as spots (cylinder, punched-hole, paper-discs). On incubation inhibition zones develop around the site of application, there is no microbial growth within these zones and their diameter is a measure of the activity of the antibiotic being tested. The activity of the antibiotic under test is determined by comparing the diameter of its inhibition zone with that of the antibiotic standard.

Typical Composition (g/litre)

Composition of the culture medium (g/l)	Medium No. 1 (MERCK)	Medium No. 2 (MERCK)	Medium No. 3 (MERCK)	Medium No. 4	Medium No. 5 (MERCK)	Medium No. 6	Medium No. 7
Meat extract	1.5	1.5	1.5	1.5	1.5	-	1.5
Yeast extract	3.0	3.0	1.5	3.0	3.0	-	3.0
Peptone from casein	4.0	-	-	-	-	17.0	-
Peptone from meat	6.0	6.0	5.0	6.0	6.0	-	6.0
Peptone from soymeal	-	-	-	-	-	3.0	-
D(+)glucose	1.0	-	1.0	1.0	-	2.5	-
Sodium chloride	-	-	3.5	-	-	5.0	-
di-Potassium hydrogen phosphate	-	-	3.68	-	-	2.5	-
Potassium dihydrogen phosphate	-	-	1.32	-	-	-	-
Agar-agar	15.0	15.0	-	15.0	15.0	-	15.0
Polysorbate 80	-	-	-	-	-	-	-
Manganse sulfate	-	-	-	-	-	0.03	-
Quantity required (g/litre)	30.5	25.5	17.5	26.5	25.5	30.0	25.5
pH at 25 °C	6.5 (± 0.2)	6.5 (± 0.2)	7.0 (± 0.2)	6.5 (± 0.2)	7.9 (± 0.2)	7.0 (± 0.2)	7.0 (± 0.2)

Composition of the culture medium (g/l)	Medium No. 8	Medium No. 9	Medium No. 10	Medium No. 11 (MERCK)	Medium No. 12 (MERCK)	Medium No. 13 (MERCK)
Meat extract	1.5	-	-	1.5	2.5	-
Yeast extract	3.0	-	-	3.0	5.0	-
Peptone from casein	-	17.0	17.0	4.0	-	-
Peptone from meat	6.0	-	-	6.0	10.0	10.0
Peptone from soymeal	-	3.0	3.0	-	-	-
D(+)glucose	-	2.5	2.5	1.0	10.0	20.0
Sodium chloride	-	5.0	5.0	-	10.0	-
di-Potassium hydrogen phosphate	-	2.5	2.5	-	-	-
Potassium dihydrogen phosphate	-	-	-	-	-	-
Agar-agar	15.0	20.0	12.0	15.0	25.0	-
Polysorbate 80	-	-	10.0	-	-	-
Manganse sulfate	-	-	-	-	-	-
Quantity required (g/litre)	25.5	50.0	52.0	30.5	62.5	30.0
pH at 25 °C	5.6 (± 0.2)	7.2 (± 0.2)	7.2 (± 0.2)	7.9 (± 0.2)	6.1 (± 0.2)	5.6 (± 0.2)

Antibiotic Media

Preparation

Suspend the required quantity of culture medium (see Table), autoclave (15 min at 121 °C), add the test strain of bacteria at 45-50 °C. Pour plates.pH: see table

The ready-to-use plates are clear and yellowish-brown.

Experimental Procedure and Evaluation

1. Cylinder test:

Procedure: Fill Petridishes with 14 ml of the medium to form the base layer, after this has set overlay with 4 ml of the inoculated seed layer. Place steel or glass cylinders on the cooled culture medium under sterile conditions. The ready-to-use test plates can be stored in the refrigerator at +4 °C. Pipette the antibiotic solutions into the cylinders and then incubate at 37 °C for 16-24hours.

Evaluation: Remove the cylinders, measure the diameters of the inhibition zones (it is best to use a "zone reading instrument") and evaluate them statistically. Draw a standard curve using the values of the standard solutions and read off the activities of the test solutions.

2. Punched-hole test:

Holes are punched out of the inoculated culture medium and the antibiotic solutions are then pipetted into them. All other steps are analogous to those described in the cylinder test.

3. Paper-disc test:

Paper-discs with a diameter of 9 mm are impregnated with the antibiotic solution and placed on the culture medium. The antibiotic can also be applied to the disc after it has been placed on the medium. Plates con-taining a single layer of medium with a thickness of 2 mm can be used for these tests. Antibiotic agars Nos. 2 or 5 may be employed depending on the pH required. All other steps are analogous to those described in the cylinder test.

4. Serial dilution test:

The antibiotic activity is determined quantitatively by using the known sensitivity of a test strain towards an antibiotic which is expressed numerically as the minimal inhibitory concentration (MIC).

Procedure: Serial dilutions of the antibiotic to be tested are pipetted into the antibiotic broth, this is then inoculated with a defined quantity of the relevant test strain.

Evaluation: The last tube which does not show any turbidity due to microbial growth contains the active antibiotic at a concentration corresponding to the MIC.

5. Turbidimetric test:

This test is more accurate and more sensitive than the serial dilution test.

Procedure: Incubate tubes containing 1 ml aliquots of the antibiotic solution and 9 ml aliquots of the inoculated antibiotic broth for 4 hours at 37 °C in a water bath. The growth of the test bacteria is then stopped by adding 0.5 ml of a dilute formaldehyde solution and the turbidity evaluated photometrically.

Evaluation: The antibiotic concentration is determined by comparing the absorbance of the test solution with that of a previously constructed standard curve.

Antibiotic Media

Use of antibiotic culture media

Antibiotic		Cylinder test			Turbidimetric test	
			Culture medium		Test strain	Culture medium
	Test strain	Seed culture	Base layer	Seed layer		
Amphomycin	Micrococcus luteus ATCC 14452	Medium No. 1	Medium No. 7	Medium No. 1	-	-
Amphotericin B	Saccharomyces cerevisiae ATCC 9763	Medium No. 13	Medium No. 12	Medium No. 12	-	-
Ampicillin	Micrococcus luteus ATCC 9341	Medium No. 1	Medium No. 11	Medium No. 11	-	-
Bacitracin	Micrococcus luteus ATCC 10240 or Micrococcus luteus ATCC 7468 D	Medium No. 1	Medium No. 2	Medium No. 1	Staph. aureus ATCC 10537	Medium No. 3
Carbomycin	Micrococcus luteus ATCC 9341	Medium No. 3	Medium No. 11	Medium No. 11	-	-
Chloramphenicol	Micrococcus luteus ATCC 9341	Medium No. 3	Medium No. 1	Medium No. 1	-	-
Cephalothin	Staphylococcus aureus ATCC 6538 P	Medium No. 1	Medium No. 2	Medium No. 1	-	-
Colistin	Bordetella bronchiseptica ATCC 4617	Medium No. 9	Medium No. 9	Medium No. 10	-	-
Erythromycin	Micrococcus luteus ATCC 9341	Medium No. 3	Medium No. 11	Medium No. 11	-	-
Gentamicin (Refobacin® Merck)	Bac. subtilis ATCC 6633	Medium No. 1	Medium No. 5	Medium No. 5	-	-
Kanamycin	Staph. aureus ATCC 6538 P	Medium No. 1	Medium No. 11	Medium No. 11	Staph. aureus ATCC 6538 P	Medium No. 3
Neomycin	Staph. aureus ATCC 6548 P	Medium No. 1	Medium No. 11	Medium No. 11	-	-
Novobiocin	Staph. epidermidis ATCC 12228	Medium No. 1	Medium No. 2	Medium No. 1	-	-
Oleandomycin	Staph. epidermidis ATCC 12228	Medium No. 1	Medium No. 11	Medium No. 11	-	-
Paromomycin	Staph. epidermidis ATCC 12228	Medium No. 1	Medium No. 11	Medium No. 11	Klebsiella pneumoniae ATCC 10031	Medium No. 3
Polymyxin B	Bordetella bronchiseptica ATCC 4617	Medium No. 9	Medium No. 9	Medium No. 10	-	-
Penicillin, oxacillin, methicillin, nafcillin	Staph. aureus ATCC 6538 P	Medium No. 3	Medium No. 2	Medium No. 1	-	-
Streptomycin, dihydro-streptomycin	Bac. subtilis ATCC 6633	Medium No. 1	Medium No. 5	Medium No. 5	Klebsiella pneumoniae ATCC 10031	Medium No. 3
Tetracycline, oxytetracy-cline, chlorotetracycline	Bac. cereus ATCC 11778	Medium No. 1	Medium No. 8	Medium No. 8	Staph. aureus ATCC 6538 P	Medium No. 3
Viomycin	Bac. subtilis ATCC 6633	Medium No. 1	Medium No. 5	Medium No. 5	-	-

Antibiotic Media

Manufacturer	Product
American Type Culture Collection 12301 Parklawn Drive, Rockville Maryland 20852, USA	Test strains
USP Reference Standards 4630 Montgomery Avenue Bethesda, MD 20014, USA	Antibiotic Standards
Schleicher & Schüll GmbH 37586 Dassel, FRG	Paper-discs No. 2628

Literature

ABRAHAM, E.P., CHAIN, E., FLETCHER, C.M., FLOREY, H.W., GARDNER, A.D., HEATLEY, N.G., a. JENNINGS, M.A.: Further observations on penicillin. - **Lancet, 241**; 177-189 (1943).

European Pharmacopeia II, Chapter VIII, 4.

FORSTER, J.W., a. WOODRUF, H.B.: Microbial aspects of penicillin. - J**. Bact., 46**; 187-202 (1943).

GROVE, D.C., a. RANDALL, W.A.: Assay Methods of antibiotics. - **Medical Encyclopedia, N.Y.** (1955).

SCHMIDT, H.W., a. MOYER, A.J.: Penicillin I. Methods of assay. - **J. Bact., 47**; 199-208 (1944).

United States Pharmacopeia XXVI, Chapter "Biological Tests and Assays", 1995.

WALLHÄUSER, K.H., u. SCHMIDT, H.: Sterilisation, Desinfektion, Konservierung, Chemotherapeutica (G. Thieme-Verlag, Stuttgart, 1967).

Ordering Information

Product	Merck Cat. No.	Pack size
Antibiotic Agar No. 1	1.05272.0500	500 g
Antibiotic Agar No. 2	1.05270.0500	500 g
Antibiotic Agar No. 4		
Antibiotic Agar No. 5	1.05271.0500	500 g
Antibiotic Agar No. 6		
Antibiotic Agar No. 7		
Antibiotic Agar No. 8		
Antibiotic Agar No. 9		
Antibiotic Agar No. 10		
Antibiotic Agar No. 11	1.05269.0500	500 g
Antibiotic Agar No. 12	1.10672.0500	500 g
Antibiotic Broth (Medium No. 3)	1.05273.0500	500 g
SABOURAUD-2 % Dextrose Broth (Medium No. 13)	1.08339.0500	500 g
D(+)Glucosemonohydrate	1.08342.1000	1 kg
Agar-agar purified	1.01614.1000	1 kg
Manganese(II) sulfate monohydrate	1.05963.0100	100 g
Tween® 80	8.22187.0500	500 ml
Tryptic Soy Broth	1.05459.0500	500 g

Quality control of Antibiotic Agar No. 1

Test strains	Growth	Inhibition zones with
Micrococcus luteus ATCC 9341	good /very good	Cephalotin, Chloramphenicol and Penicillin/Methicillin
Staphylococcus aureus ATCC 6538-P	good /very good	
Bacillus subtilis ATCC 6633	godd /very good	-
Staphylococcus epidermidis ATCC 12228	good /very good	-
Bacillus cereus ATCC 11778	good /very good	-

Quality control of Antibiotic Agar No. 2

Test strains	Growth	Inhibition zones with
Micrococcus luteus ATCC 10240	fair /good	
Staphylococcus aureus ATCC 6538-P	good / very good	
Staphylococcus epidermidis ATCC 12228	good / very good	

Antibiotic Media

Quality control of Antibiotic Agar No. 5

Test strains	Growth	Inhibition zones with
Bacillus subtilis BGA	good / very good	Gentamicin, Streptomycin

Quality control of Antibiotic Agar No. 11

Test strains	Growth	Inhibition zones with
Micrococcus luteus ATCC 9341	good / very good	Ampicillin, Erythromycin
Staphylococcus aureus ATCC 6538-P	good /very good	Kanamycin, Neomycin
Staphylococcus epidermidis ATCC 12228	good /very good	Oleandomycin, Paromomycin

Staphylococcus aureus
ATCC 6538-P

Quality control of Antibiotic Agar No. 12

Test strains	Growth	Inhibition zones with
Saccharomyces cerevisiae ATCC 9763	good /very good	Amphotericin B (20 µg)

Quality control of Antibiotic Broth No. 3

Test strains	Growth	Inhibition zones with
Micrococcus luteus ATCC 9341	good / very good	-
Staphylococcus aureus ATCC 6538-P	good	Kanamycin, Tetracyclin
Klebsiella pneumoniae ATCC 10031	good	Streptomycin

Antibiotic Sulfonamide Sensitivity-test Agar (ASS Agar)

ASS agar (D.S.T. Agar)

For testing the sensitivity of microorganisms towards antibiotics and sulfonamides using the agar diffusion method.

IVD *in vitro diagnosticum –*
For professional use only

See also General Instruction of Use
Warnings and precautions see ChemDAT®
(www.chemdat.info)

Antibiotic sulfonamide sensitivity-test agar meets the requirements set for sensitivity test agars by the "Expert Committee on Antibiotics" of the World Health Organisation (WHO).

This culture medium can also be employed for testing fastidious microorganisms such as pneumococci, Listeria, Neisseria, Erysipelothrix etc. ANSORG et al. (1975) and SØGAARD et al. (1978) demonstrated that it could be successfully used to detect antibacterial substances in urine, renal tissue and milk.

Methods for accurate quantitative sensitivity determination have been developed by ERICSSON and SHERRIS (1971) on behalf of the WHO and the Deutsches Institut für Normung (DIN 58940) (German Institute of Standardisation).

Principle

Microbiological method.

Mode of Action

The composition of the culture medium provides favourable growth conditions. Buffering of the medium prevents pH changes from interfering with diffusion. The zones of inhibition are clearly defined. The activities of the antibiotics or sulfonamides are not inhibited or antagonized by any of the constituents of the medium.

Typical Composition (g/litre)

Peptone 20.0; D(+)glucose 2.0; sodium chloride 3.0; di-sodium hydrogen phosphate 2.0; sodium acetate 1.0; adenine 0.01; guanine 0.01; uracil 0.01; xanthine 0.01; agar-agar 12.0.

Preparation and Storage

Usable up to the expiry date when stored dry and tightly closed at +15 to +25 °C. Protect from light.

After first opening of the bottle the content can be used up to the expiry date when stored dry and tightly closed at +15 to +25°C.

Suspend 40g/litre, autoclave (15 min at 121°C), pour plates.

pH: 7.2 ± 0.2 at 25 °C.

The plates are clear and yellowish-brown.

Ready-to-use
Usable up to the expiry date when stored at +12 to +15° C.
The plates are clear and yellowish-brown.

Specimen

e.g. Isolated bacteria from urine.Clinical specimen collection, handling and processing , see general instructions of use.

Experimental Procedure and Evaluation

Perform the sensitivity testing as directed in the standard methods.

Zones of inhibition can be clearly seen and their diameters are evaluated either qualitatively or quantitatively. In the case of quantitative evaluation, the zones are measured and recorded.

Literature

ANSORG, R., ZIPPEL, H., u. THOMSSEN, R.: Bedeutung des Nachweises antibakterieller Stoffe in Urin für die bakteriologische Diagnostik und die Kontrolle der Chemotherapie von Harnwegsinfektionen. – **Zbl. Bakt. Hyg., I. Orig., A 230** , 492-507 (1975).

DIN Deutsches Institut für Normung e.V.: Methoden zur Empfindlichkeitsprüfung von bakteriellen Krankheitserregern (außer Mykobakterien) gegen Chemotherapeutika.

– **DIN 58940**.

ERICSSON, H.M., a. SHERRIS, J.C.: Antibiotic sensitivity testing. Report of an international collaborative study. – **Acta path. microbiol. scand. B. Suppl. 217**, 1971.

LINZENMEIER, G., NAUMANN, P., RITZERFELD, W., u. KNOTHE, H.: Auswahl von Chemotherapeutika zur Resistenzbestimmung schnell wachsender Bakterien (Minimalforderung). – **Dtsch. med. Wschr. 97**, 303-304 (1972) oder **Ärztl. Lab. 18**, 169-172 (1972).

SØGAARD, H., ANDERSEN, M., HUUSOM, R.: En folsom methode til pavisning at sulphonamider i nyrevaev og maelk. – **Dansk. Vet. Tidsskr., 61**; 593-595 (1978).

Ordering Information

Product	Merck Cat. No.	Pack size
Antibiotic Sulfonamide Sensitivity-test Agar (ASS Agar)	1.05392.0500	500 g
Merckoplate® ASS agar (D.S.T. Agar)	1.10410.0001	20 plates

Quality control

Test strains	Growth	Inhibition zones with
Escherichia coli ATCC 25922	good	Ampicillin
Staphylococcus aureus ATCC 25923	good	Tetracyclin, Trimethoprim-Sulphamethoxazol, Gentamicin, PolymyxinB
Pseudomonas aeruginosa ATCC 27853	good	Gentamicin
Enterococcus faecalis ATCC 33186	good	Trimethoprim-Sulphamethoxazol

APT Agar

All purpose medium with Tween® proposed by EVANS and NIVEN (1951) and DEIBEL, EVANS and NIVEN (1957) for counting and cultivating heterofermentative lactic acid bacteria including Lactobacillus, Leuconostoc species, Lactococcus lactis and other microorganisms which require a high thiamine concentration in meat products, tinned foods, fruit juices and other foodstuffs.

The medium complies with the recommendations of the American Public Health Association (1992).

Mode of Action

This medium contains a rich nutrient base with additives of Tween®, thiamine and several essential elements, which provide optimal growth conditions for the abaove mentioned bacteria. The culture medium is not selective, accompanying bacteria therefore also grow very well.

Typical Composition (g/litre)

Peptone from casein 12.5; yeast extract 7.5; D(+)glucose 10.0; sodium chloride 5.0; tri-sodium citrate 5.0; di-potassium hydrogen phosphate 5.0; Tween® 80 0.2; magnesium sulfate 0.8; manganese chloride 0.14; iron(II) sulfate 0.04; thiaminium dichloride 0.001; agar-agar 13.5.

Preparation

Suspend 59.5 g/litre, fill into suitable containers, autoclave (15min at 121 °C). **Do not overheat**

pH: 6.7 ± 0.2 at 25 °C.

The prepared medium is clear and brown.

Experimental Procedure and Evaluation

When performing bacterial counts, dilute the sample material and inoculate the APT Agar by the pour-plate method.

Incubation: 2 days at 35 °C aerobically.

In order to identify lactic acid bacteria which produce a green colouration, inoculate with the suspect colonies. After incubating for 24 hours at 32 °C, transfer a sample from the culture that has developed onto the cut surface of a smoked sausage. Place the sausage in a Petridish containing a damp piece of filter paper ("moist chamber"). Incubate for 18-24 hours at 32 °C and see whether there is a green colouration. A sample of the sausage which has not been inoculated serves as a control. In order to exclude other pigment-forming bacteria (e.g. Pseudomonas), a confirmatory bacteriological test (e.g. Gram-positive rods, negative catalase test, negative nitratase test, positive peroxidase test, acetoin production from glucose, ammonia production from arginine etc.) should also be performed.

Literature

American Public Health Association: Compendium of Methods for the Microbiological Examination of Foods. - 3rd ed., 1992.

DEIBEL, R.H., EVANS, J.B., a. NIVEN, C.F.: Microbiological assay for the thiamin using Lactobacillus viridescens. - **J. Bact., 74**; 818-821 (1957).

EVANS J.B., a. NIVEN, C.F.: Nutrition of the heterofermentative Lactobacilli that cause greening of cured meat products. - **J. Bact., 62**; 599-603 (1951).

Ordering Information

Product	Merck Cat. No.	Pack size
APT Agar	1.10453.0500	500 g

Quality control

Test strains	Growth
Lactobacillus acidophilus ATCC 4356	good / very good
Lactobacillus casei ATCC 393	good / very good
Lactobacillus fermentum ATCC 9338	good / very good
Lactobacillus plantarum ATCC 14917	good / very good
Lactobacillus viridescens ATCC 12706	good / very good
Leuconostoc mesenteroides ATCC 9135	good / very good
Lactococcus lactis spp. lactis ATCC 19435	good / very good

Arginine Broth acc. to SCHUBERT

Arginine Brilliant-green Glucose Peptone Broth (ABGP Medium)

Enrichment broth for chlorine damaged Pseudomonas aeruginosa within the testing of swimming pool water acc. to bathingwater commission of "Umweltbundesamt" (German Authority for the Environment).

Mode of Action

The rich nutrient base of this medium allows best growth conditions.

Brilliant-green inhibits the accompanying Gram-positive flora. The concentration of brilliant-green has no toxic effect on preinjured Pseudomonas aeruginosa.

A colour change from grey-green to blue-violet indicates the presence of Ps. aeruginosa allowing a presumptive information about the presence of Ps. aeruginosa.

The indicator system is based on the strong alcalisation of Arginine in the presence of Ps. aeruginosa. In combination with bromothymolblue and cresolred the colour changes to blue-violet.

Typical Composition (g/litre)

Peptone from casein 17.0; peptone from soymeal 3.0; L-argininemonohydrochloride 10.0; bromothymolblue 0.015; cresolred 0.02; brilliant-green 0.00038; D(+)glucose 0.5; sodium chloride 5.0.

Preparation

Suspend 35.5 g in 1 litre demin. water, dispense into test tubes and autoclave (15 min at 121 °C).

pH: 7.0 ± 0.2 at 25 °C.

FOR ENVIRONMENTAL WATER SAMPLES ONLY:

For the inhibition of the accompanying flora it is recommended to add 1 ml of nalidixic acid solution (dissolve 5 mg nalidixic acid in 1 ml demin. water) to the medium at a temperature of 45-50 °C. Homogenize by gently shaking.

(Do **not add** nalidixic acid solution to chlorinated water sample!)

The prepared broth is clear and grey-green.

Experimental Procedure and Evaluation

Membrane filter method:

100 ml water sample is filtered through a membrane filter which is then immersed into 15-20 ml of Arginine Broth.

Direct Enrichment:

Suspend 100 ml water sample into 100 ml of double strength Arginine Broth.

Incubation: 48 h aerobically at 35 °C.

A colour change to violet is an indication for the presence of Pseudomonas aeruginosa.

For confirmation streak onto selective agars, e.g. methods acc. to DIN 38411, part 8.

Literature

SCHUBERT, R.: The use of Arginine Brilliant Green Glucose Peptone Broth (ABGP Medium) as a Primary Culture Medium for Pseudomonas aeruginosa. - **Zbl. Bakt. Hyg. B 187**; 266-268 (1989).

Mitteilung der Badewasserkommission des Umweltbundesamtes: Hygienische Überwachung öffentlicher und gewerblicher Bäder durch die Gesundheitsämter. - **Bundesgesundheitsbaltt 4/96**.

Ordering Information

Product	Merck Cat. No.	Pack size
Arginine Broth acc. to SCHUBERT	1.13892.0500	500 g
DEV ENDO Agar	1.10684.0500	500 g
MacCONKEY Agar	1.05465.0500	500 g
Neßler's reagent	1.09029.0100	100 ml
Pseudomonas Agar F, Base	1.10989.0500	500 g
Pseudomonas Agar P	1.10988.0500	500 g

1 + 2 Pseudomonas aeruginosa
3 Negative control
4 Enterococcus faecalis
5 E. coli

Quality control

Test strains	Growth	Colour change to violet
Pseudomonas aeruginosa ATCC 27853	good / very good	+
Pseudomonas aeruginosa ATCC 9027	good / very good	+
Pseudomonas stutzeri ATCC 17832	none	-
Aeromonas hydrophila ATCC 7966	good / very good	+
Enterococcus faecalis ATCC 19433	none	-
Escherichia coli ATCC 25922	good / very good	+ / - (yellow after 24h)

ww

Azide Dextrose Broth

Used as a preliminary test for enterococci and also for their selective enrichment.

See also Bromocresol-purple Azide Broth.

Mode of Action

The concentration of sodium azide present in this medium largely inhibits the growth of the accompanying Gram-negative microbial flora, while sparing the enterococci.

The use of sodium azide as a selective inhibitor for Gram-negative bacteria was reported in the studies of EDWARDS (1933, 1938) and HARTMANN (1936) on the isolation of Str. agalactiae. MALLMANN (1940) and SNYDER and LICHSTEIN (1940) later showed that sodium azide can also be used for the isolation of enterococci from water.

The presence of enterococci (Enterococcus faecalis, S. durans, S.bovis and S.equinus) serves as an indicator for faecal contamination, particularly when this took place a long time ago and the less resistant coliform bacteria, including E. coli, may be already dead when the analysis is carried out.

Typical Composition (g/litre)

Peptone from casein 15.0; meat extract 4.5; D(+)glucose 7.5; sodium chloride 7.5; sodium azide 0.2.

Preparation

Suspend 35 g or 70 g/litre, dispense into suitable vessels, autoclave (15 min at 121 °C). **Do not overheat**.

pH: 7.2 ± 0.2 at 25 °C.

The prepared broth is clear and yellowish-brown.

Experimental Procedure and Evaluation

Small sample volumes (up to 1 ml) can be added to the normal strength broth. Larger volumes (10 ml or more) should be diluted with an equal volume of the double-strength broth.

Incubation 24-48 hours at 35 °C aerobically.

If the broth becomes turbid due to microbial growth it is likely that enterococci are present. The culture should then be inoculated into Bromocresol-purple Azide Broth. If this broth does not become turbid enterococci are not present.

Literature

American Public Health Association, American Water Works Association and Water Pollution Control Federation: Standard Methods for the Examination of Water and Wastewater, 20th ed., Washington, 1998.

EDWARDS, S.J.: Studies on bovine mastitis. IX. A selective medium for the diagnosis of Streptococcus mastitis. - **J. Comp. Path. Therap. 46**; 211-217 (1933).

EDWARDS, S.J.: The diagnosis of Streptococcus mastitis by cultural methods. - **J. Comp. Path Therap. 51**; 250-263 (1938).

LITSKY, W., MALLMANN, W.L., a. FIFIELD, C.W.: A new medium for the detection of enterococci in water. - **Amer. J. Publ. Hlth.**, 43; 873-879 (1953).

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Ordering Information

Product	Merck Cat. No.	Pack size
Azide Dextrose Broth	1.01590.0500	500 g
Bromocresol-purple Azide Broth	1.03032.0500	500 g

100 ml sample into 100 ml of double-strengh Azide Dextroxe Broth

Quality control

Test strains	Growth
Enterococcus faecalis ATCC 11700	good / very good
Enterococcus faecalis ATCC 19433	good / very good
Enterococcus hirae ATCC 8043	good / very good
Streptococcus bovis DSMZ 20065	fair / very good
Staphylococcus aureus ATCC 25923	none / poor
Escherichia coli ATCC 25922	none / poor
Pseudomonas aeruginosa ATCC 27853	none / poor

Geobacillus stearothermophilus Spore Suspension

For the antibiotic sulfonamide residue test according to KUNDRAT.

The Geobacillus stearothermophilus spore suspension is used in conjunction with the test agar for performing the antibiotics sulfonamide residue test according to KUNDRAT, Cat. No. 1.10662. The test detects antimicrobial residues such as antibiotics, sulfonamides and other chemotherapeutics in meat and other foodstuffs of animal origin. It is a routine qualitative procedure.

Mode of Action

The test is based on agar diffusion, using spores of Geobacillus stearothermophilus as test organisms. Antibiotic and sulfonamide residues inhibit the growth of the test organism. This inhibition of growth is indicated by the formation of inhibition zones. These zones remain purple in colour whilst the rest of the nutrient medium turns yellow. Cleaning agents, disinfectants and preservatives do not influence the test.

Instruments required

Autoclave or steam bath and incubator.

Ancillary items required

Petridishes or other nutrient vessels equipped with lids.

Filter paper discs of 6mm diameter and capable of absorbing double their weight of water.

Reagents

Geobacillus stearothermophilus spore suspension, adjusted to a concentration of 10^8 KBE/ml (stray range: $7 \cdot 10^7$ to $3 \cdot 10^8$ KBE/ml).

Test agar for the antibiotics residue test accoring to KUNDRAT, Cat. No.1.10662.

Composition	(g/l)
Peptone	17.0
Sodium chloride	3.0
D(+)glucose	3.0
Saccharose	2.0
Starch	3.0
Gelatine	2.5
Bromocresol purple	0.016
Agar-agar	10.0

Preparing the ready-to-use agar

Suspend 8.0g of the nutrient powder in 200ml of freshly distilled or completely demineralized water and allow to stand for 15minutes. Boil in a water bath until completely dissolved and the autoclave for 15minutes at 121°C. Allow to cool to under 60°C and add 2ml of Geobacillus stearothermophilus spore suspension (the contents of one ampoule; shake before opening). Cast the mixture in Petridishes (15ml per dish).

The pH of the ready-to-use solution at 25°C: 6.8 ± 0.2.

Storage of the ready-to-use test agar

The ready-to-use test agar can be stored in air-tight Petridishes (sealed with adhesive tape) in a refrigerator (+2 to +8°C) for up to 3 months.

Preincubated test agar (135 min. at 65°C) can be kept under the same conditions for 1 month. It should additonally be placed in a plastic bag.

Test procedure

Wet the filter paper discs with the sample fluid or place them on sections of organ (kidney, liver) or muscle before pressing them gently onto the surface of the test agar. Up to six such discs can be used per Petridish.

Two methods ca be used to carry out the test:

1. 45-minute incubation rapid test

   Preincubate the test agar for 135 min. at 65°C. Once the discs have been added, incubate again for 45 min. at 65°C without prediffusion.

2. 3-hour incubation

   Place the discs on the non-incubated test agar and incubate for 3hours at 65°C without prediffusion.

Evaluation

With the rapid test, the formation of an inhibition zone may be observed after 15-25 min. incubation period. The zones become more learly defined on completion of the 45-minute incubation period due to the colour change that takes place. The presence of an inhibition zone should be taken as a positive result.

In the 3-hour method, only those inhibition zones with a diameter greater than 10mm should be regarded as being positive.

Should the formation of inhibiton zones be unclear after the 45-min. or 3-hour incubation period, the period can be prolonged.

Storage of Geobacillus stearothermophilus spore suspension

The suspension should be kept in a refrigerator at +2 to +8°C. At room temperature (up to +25°C), the suspension may be kept for 1-2days only.

Shelf life

If kept in a refrigerator according to instructions, the test can be stored until the expiry date indicated. If used after this date, the spores may begin to lose their activity.

Literature

Kundrat, W.: Methoden zur Bestimmung von Antibiotika-Rückständen in tierischen Produkten. - Zeitschrift f. anal. Chemie, **243**; 624 (1968).

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Forschner, E.: Rationalisierungsmöglichkeiten beim Nachweis von Hemmstoffen in Milch im Agardiffusionsverfahren. - Archiv. f. Lebensmittelhygiene, **5**; 101-104 (1972).

Ordering Information

Product	Merck Cat. No.	Pack contents
Geobacillus stearothermophilus Spore Suspension	1.11499.0001	5 x 2 ml ampoules

Bacillus cereus Selective Supplement

Additive for the preparation of Cereus Selective Agar Base acc. to MOSSEL, Merck Cat. No. 1.05267.0500.

Mode of Action

Bacillus cereus Selective Supplement contains Polymyxin B sulfate in lyophilized form.

It suppresses the growth of accompanying bacterial flora during culturing Bacillus cereus.

Composition (per vial)

Polymyxin B sulfate 50,000 IU.

Experimental Procedure

The lyophilisate is dissolved in the original vial by adding 1 ml of sterile, distilled water.

In the preparation of Cereus Selective Agar, the dissolved content of one vial is evenly mixed together with 50 ml sterile egg-yolk emulsion into 450 ml of sterile, still liquid medium cooled to 45-50 °C.

Ordering Information

Product	Merck Cat. No.	Pack size
Bacillus cereus Selective Supplement	1.09875.0001	1 x 16 vials

Bacillus Subtilis (BGA) Spore Suspension

For the inhibitor test.

Bacillus subtilis (BGA) spore suspension is used with the culture media test agar pH 6.0 for the inhibitor test Cat. No. 1.10663. and test agar pH 8.0 for the inhibitor test Cat. No. 1.10664. for the detection of antimicrobial inhibitors in meat by routine methods.

Mode of Action

The test is conducted as an agar diffusion test. The spores of bacillus subtilis (BGA) are used as the test organisms. Inhibitors inhibit the growth of the test bacteria. The inhibition of growth is indicated by inhibition zones.

Equipment

Autoclave or pressure cooker, incubation cabinet.

Auxilliaries

Petridish or other dish with lid for nutrient media.

Reagents

Bacillus subtilis (BGA) spore suspension, adjusted to a content of 10^7 CFU/ml (range: 8 x 10^6 to 5 x 10^7 CFU/mg).

Test agar pH 6.0 for the inhibitor test Cat. No. 1.10663.

Composition	(g per litre)
Peptone from casein	3.45
Peptone from meat	3.45
Sodium chloride	5.1
Agar agar	13.0

Test agar pH 8.0 for the inhibitor test Cat. No. 1.10664.

Composition	(g per litre)
Peptone from casein	3.45
Peptone from meat	3.45
Sodium chloride	5.1
Phosphate buffer	2.4
Agar agar	13.0

Preparation of the Ready-to-use Test Agar

Suspend 25 g/l (test agar pH 6.0) or 27.5g/litre (test agar pH8.0) in freshly distilled or fully demineralized water. Then boil in a pressure cooker until completely dissolved. The sterilization is carried out in an autoclave (15 minutes at +121°C).

Cool to +50 °C, then add 1ml bacillus subtilis (BGA) spore suspension per litre of nutrient medium and shake. Pour 15ml of the nutrient medium into each Petridish.

Storage of the Ready-to-use Test Agar

The ready-to-use test agar can be sealed into Petridishes with airtight adhesive tape and stored in a refrigerator (+2 to +8 °C) for up to 2weeks. Additional packaging in plastic bags is recommended.

Carrying out the Test

For instructions on taking and sending samples as well as conducting the test, refer to the regulations on the inspection of meat products.

Stamp out cylindrical disks of tissue with a diameter of 8mm and a thickness of 2mm under clean conditions avoiding contamination and place one on a plat of pH6.0 and one on a plate of pH8.0. As a control, place a test strip with 0.01IU penicillin G-sodium on a pH6.0 plate and one test strip with 0.5µg streptomycin on a pH8.0 plate.

Incubation: 18 to 24 hours at +30°C.

Evaluation

Measure the inhibition zone between the edge of the piece of tissue and the limit of growth. Complete inhibition of growth with an inhibition zone of at least 2mm can be regarded as a positive result, an inhibition zone of 1 to 2mm as a dubious result, if the parallel controls have inhibition zones of approx. 6mm.

Storage of Bacillus Subtilis (BGA) Spore Suspension

Storage at +2 to +8 °C in a refrigerator is recommended. Storage at room temperature (up to +25 °C) is only possible for 1 to 2days, otherwise the stability is adversely affected.

Stability

Only with proper storage in a refrigerator the stability can be guaranteed up to the expiry date given. Thereafter, the activity of the spores must be expected to begin to decline.

Literature

Levetzow, R.: Untersuchungen auf Hemmstoffe im Rahmen der Bakteriologischen Fleischuntersuchung (BU). - **Bundesgesundheitsblatt**, 1971; **14**; **15/16**, 211-213.

Ordering Information

Product	Merck Cat. No.	Pack contents
Bacillus Subtilis (BGA) Spore Suspension	1.10649.0001	15 x 2ml ampoules

Bactident® Aminopeptidase

For the detection of L-alanine aminopeptidase in microorganisms

Mode of Action

L-alanine aminopeptidase is an enzyme which is localized in the cell envelope of bacteria and which is found in relevant activities almost exclusively in Gram-negative microorganisms.

This enzyme splits off the amino acid L-alanine from various substrates.

In the case of these test strips, the substrate L-alanine-4-nitroanilide is split into 4-nitroaniline and the amino acid L-alanine in the presence of alanine aminopeptidase. The presence of L-alanine aminopeptidase, is indicated by the yellow colouration of the 4-nitroaniline.

The results of the investigations performed so far indicate that there is a very good correlation between the amino-peptidase reaction and the Gram-behaviour of the microorganisms.

Typcial Composition

The reaction zone of a test strip contains:

L-alanine-4-nitroanilide 0.5µmol; buffering agents.

Preparation

Suspend a thickly grown individual colony (about 2 mm Ø) in 0.2ml of distilled water to give an opalescent mixture.

Note:
Only bacterial colonies without strong intrinsic colourations should be used for the aminopeptidase test. We recommend that a control test with an aminopeptidase-positive bacterium (e.g. E.coli) and an aminopeptidase-negative bacterium (e.g. Staphylococcus aureus) should always be carried out at the same time as the main test.

Stability

See expiring date.

Only remove the amount of strips needed at the time! Do not touch the reaction zone of the test strips. Close receptacle firmly immediately after use. Please store at the specified temperature.

Safe Removal

The test strip is to be removed safety after use like bacteria containing material. This may be done by burning, autoclaving or by placing into a 5 to 6% disinfectant solution - for at least 6 hours.

Experimental Procedure

1. Using an inoculation loop, remove an individual, thickly-grown colony from the nutrient medium.
2. In a small test tube, suspend the bacterial mass in 0.2ml of distilled water.
3. Insert the aminopeptidase test strip into the test tube such that the reaction zone is completely immersed in the bacteria suspension.
4. Incubate the test tube in a water bath (or incubation cupboard) for 10 to a maximum of 30 minutes* at 37°C.
5. Read off the reaction by comparison with the colour scale.

Note

* A clear yellow colouration of the bacteria suspension can be seen after only 10 minutes in the case of most amino-peptidase-positive microorganisms; if no yellow colouration appears within this time, the incubation should be extended to a maximum of 30 minutes so that the weakly aminopeptidase-positive strains can be recognized or the absence of Gram-negative microorganisms can be confirmed (see table for exceptions).

Aminopeptidase-positive Strains*

all Gram-negative microorganisms
The suspension of bacteria turns yellow if L-alanine amino-peptidase-positive organisms are present.

Exceptions: Bacteroides vulgatus, Bacteroides fragilis, Camphylobacter species, Veillonella parvula

Aminopeptidase-negative Strains*

all Gram-positive microoganisms

* acc. to the results of investigarions performed so far.

Ordering Information

Product	Merck Cat. No.	Pack contents
Bactident® Amino-peptidase	1.13301.0001	50 test strips

Bactident® Catalase

Test reagent for detecfting the enzyme catalase.

Mode of Action

Catalase is an enzyme which is present in all cells with aerobic metabolisms. It contains iron protoporphyrin (haemin) as the coenzyme. Catalase cleaves toxic hydrogen peroxide, which metabolic processes produce, into hydrogen and peroxide.

The presence or absence of catalase activity is a taxonomic property of microorganisms and can be used for their differentiation or identification.

Typical Composition

3% aqueous solution of hydrogen peroxide.

Application

Part of the colony to be examined is picked up with a platinum loop and placed on a dry glass slide. A drop of catalase reagent is placed on the bacteria. The catalase drop can be placed directly on colonies on solid culture media (apart from blood culture media).

Positive reaction:
Immediate gas formation (oxygen) on the colony or bacteria mass.

Negative reaction:
No gas development.

Reaction	Microorganisms
Catalase negative	Anaerobes Aerotolerant anaerobes Lactobacetiaceae Streptococci etc.
Catalase positive	Aerobes Propioni bacteria Enterobacetriaceae Staphylococci

Ordering Information

Product	Merck Cat. No.	Pack contents
Bactident® Catalase	1.11351.0001	30 ml for about 300 tests

Bactident® Coagulase

For the detection of the enzyme coagulase developed by staphylococcus aureus.

Mode of Action

Coagulase is an enzyme with the ability to coagulate plasma. Staphylococcus aureus forms two types of coagulase. The free coagulase is an extracellular enzyme, the bound coagulase is localized on the surface of the cell wall. Both enzymes are detected in the tube test. With the slide test, only the bound coagulase can be detected.

Method

An overnight broth culture of staph. aureus is incubated with rehydrated EDTA-rabbit plasma. The coagulase test is positive, if more than three quarters of the tube contents forms a coherent clot.

Stability

See expiry date.

Bactident® Coagulase is stable for 5 days in the dissolved (rehydrated) condition at +2°C to +8°C. At -20°C it is stable for up to 30 days.

Storage

Store cool, dry and tightly closed at +2°C to +8°C. Store dissolved plasma at +2°C to +8 °C or deepfrozen at -20°C. Do not refreeze once defrozen.

Safe Disposal

The contents of the tube include bacteria and must be disposed of safely. This can be done by autoclaving or placement in a 5-6% solution of disinfectant for at least 6hours.

Experimental Procedure

a. Conduct the coagulase test on 5 typical and/or 5 a-typical colonies on BAIRD-PARKER agar (Merck Cat. No. 1.05406.) or 5 suspect colonies from other culture media (CHAPMAN agar, Merck Cat. No. 1.05469., VOGEL-JOHNSON agar, Merck Cat. No. 1.05405., Blood agar (base), Merck Cat. No. 1.10886.).

b. Transfer each of the selected colonies with a sterile inoculation loop to separate culture tubes containing brain-heart broth (Merck Cat. No. 1.10493.) and incubate at 37°C for 20-24 hours.

c. Dissolve the freeze-dried EDTA-rabbit plasma in 3ml of distilled or demineralized water.

d. Pipette 0.3ml of the rehydrated Bactident® Coagulase into a sterile culture tube using a sterile pipette.

e. Carefully mix 0.1ml of the brain-heart broth culture 1/2 an inoculation loop of colony material from the CHAPMAN blood or BAIRD-PARKER agar with the 0.3ml of plasma and incubate in a water bath at 37 °C. (Colony material directly from VOGEL-JOHNSON or mannitol-sodium chloride-phenol red agar is not suitable for the test. A brain-heart broth culture is required first.).

f. Every hour, check the tube contents for coagulation by gently tipping to the side (not by shaking).

g. The coagulase test is positive, if more than three quarters of the tube contents has formed a coherent clot.

If the test is negative after 4-6 hours, continue incubating the tube and make a final assessment after 24hours. For the negative control, prepare a brain-heart broth, but do not inoculate. There must be no sign of clotting.

For the positive control, conduct the test with a coagulase-positive strain of staphylococcus.

negative	no coagulation
1 + positive	a few small separate clots
2 + positive	a few small joined clots
3 + positive	large extensively coagulated clots
4 + positive	complete coagulation, contents do not shift when tube is inverted

Notes

The slide test in which a colony is mixed with rabbit plasma on a microscope slide (clumping factor), only detects the bound coagulase and can at best only service as a screening test. False-positive reactions and autoagglutination can occur.

Ordering Information

Product	Merck Cat. No.	Pack contents
Bactident® Coagulase	1.13306.0001	6 vials each containing 3ml of lyophilized rabbit plasma with EDTA

Bactident® E. coli

Test kit for the rapid identification of E. coli.

β-D-Glucuronidase activity is a specific marker for E.coli as fas as the Enterobacteriaceae are concerned; it can otherwise only be detected in a few Salmonella and Shigella species. 94% of all E.coli strains possess the enzyme (FENG and HARTMANN 1982, HANSEN and YOURASSOWSKY 1984). Tryptophanase activity (i.e. the ability to form indole from tryptophan) is present in 99% of all E.coli strains. Detection of both enzymes is a reliable indicator for the presence of E.coli.

Mode of Action

The test kit contains the strips whose reaction zones are impregnated with 4-methylumbelliferyl-β-D-glucuronide (MUG). β-D-Glucuronidase cleaves this substrate form 4-methylumbelliferone with fluorescens light blue when excited with long-wavelength UV light (about 366 nm) and thus indicates that the enzyme is present.

Indole formation is indicated, if the bacterial suspension turns red on addition of KOVÁCS' reagent (see KOVÁCS' Indole Reagent, Merck, Cat. No. 109293.).

Typical Composition

50 test strips, 50 reaction cuvettes; 1 tray for holding the reaction cuvettes; 1 dropper bottle filled with KOVÁCS' reagent.

Experimental Procedure and Evaluation

Remove an isolated colony from the culture medium with a loop, suspend thoroughly in a reaction cuvette containing 200µl water and place a test strip in the suspension. Incubate for 30-120 minutes at 37°C and then evaluate with a UV lamp (e.g. UV lamp, Merck, Cat. No. 1.13203.). Subsequently add one drop of KOVÁCS' reagent to the suspension and leave the react for 1-2 minutes.

If the bacterial suspension displays light blue fluorescence in UV light and shows a red ring after addition of KOVÁCS' reagent, it is positive for E.coli.

Literature

FENG, P.C.S., a. HARTMANN, F.P.: Fluorogenic assay for immediate confirmation of E.coli. **- Appl. Environm. Microbiol. 43**; 1320-1329 (1982).

GEISS, H.K., u. ZAHRAN, M.: Schnellidentifizierung von E.coli durch Enzymnachweis. **- Lab. med., 11**; 251-252 (1987).

GEISS, H.K., RIFFLER-KLEIS, U., a. STOBER, W.: Rapid Identification of E.coli by Detection of β-Glucuronidase. - 5th Int. Symp. of Rapid Methods and Automation in Microbiol. and Immunol. Florenz, Nov. 1987.

GLAESER, H.: Differenzierung coliformer Keime aus Weichkäse - Methoden und Ziele. **- dmz, 27** ; 870-873 (1987).

HANSEN, W., a. YOURASSOWSKY, E.: Detection of β-Glucuronidase in Lactose-Fermenting Membres of the Family Enterobacteriaceae and its Presence in Bacterial Urine Cultures. **- J. Clin. Microbiol., 20**; 1177-1179 (1984).

HOFMANN, O., u. RAGER, K.TH.: Der Bactident®-Test in der Praxis - dargestellt am Beispiel der Münchener Wasserversorgung. **- gwf Wasser-Abwasser, 129 (1)**; 19-21 (1988).

Ordering Information

Product	Merck Cat. No.	Pack size
Bactident® E. coli	1.13303.0001	1 x 50 tests

Bactident® Indole

KOVÀCS' Indole Reagent in a practical dropper bottle.
Product information: see under Merck, Cat. No. 1.09293., KOVÁCS' Indole Reagent.

Test strip with a reactive zone for detecting cytochrome oxidase in microorganisms.

Mode of Action

Cytochrome oxidase is a very widespread enzyme belonging to the group of iron porphyrins. It oxidizes reduced cytochrome c and is itself converted to its reduced inactive form. The reduced cytochrome oxidase is reconverted to its oxidized active form by transfer of electrons to molecular oxygen.

In the presence of molecular oxygen the electrons can be removed by the cytochrome oxidase/cytochrome c system from a number of organic compounds, e.g. the so-called Nadi reagent (naphthol + dimethylparaphenylenediamine) with formation of the condensation molecule indophenol blue.

This reaction is utilized for the classification and identification of bacteria.

Typical Composition

N,N-Dimethyl-1,4-phenylenediammonium chloride; naphthol-(1).

Experimental Procedure and Evaluation

Tests are performed using individual colonies or material taken from a pure colony with a loop. Instead of utilizing bacterial mass, the reaction can also be performed with a dense bacterial suspension. Remove a single isolated, well-developed colony from the culture media with a loop. Apply the colony to the reactive zone and distribute with the aid of the loop. After 20-60seconds compare the test strip with the colour scale provided.

IF cytochrome oxidase-positive bacteria are present the reactive zone exhibits a blue to purple colour.

Ordering Information

Product	Merck Cat. No.	Pack size
Bactident® Indole	1.11350.0001	1 x 30 ml

Bactident® Oxidase

For the testing of cytochrome oxidase in microorganisms.

IVD *in vitro diagnosticum –*
For professional use only

See also General Instruction of Use
Warnings and precautions see ChemDAT®
(www.chemdat.info)

Mode of Action

The cytochrome oxidase is an enzyme of the iron porphyrine group which is very widely distributed in nature. It oxidizes the reduced cytochrome c and is thus transformed itself into the reduced and inactive form. Through transfer of the electrons to molecular oxygen the reduced cytochrome oxidase is transformed again into the active form.

In the presence of molecular oxygen the cytochrome oxidase/cytochrome c-system can reduce a whole series of organic substances, among them the socalled NaDi reagent (1-naphthol + dimethylparaphenylene diamine) with formation of the condensation molecule indophenol blue.

This reaction is used for the classification and identification of bacteria.

Typical Composition

The reaction zone of a test-strip contains:

N,N-dimethyl-1,4-phenylene diammonium chloride 0.1µmol; 1-naphthol 1.0µmol.

Application

The separate colonies grown on a culture medium or, in the case of pure cultures, an inoculation loop full are being tested. Instead with bacterial mass the reaction may also be performed with a dense bacterial suspension.

See also General Instruction of Use.

Stability

See expiry date.

Only remove the amount of strips needed at the time!

Do not touch the reaction zones of the test strips.

Close receptacle firmly immediately after use. The strips with deep brown coloured reaction zone are unusable. Please store at the specified temperature.

Storage

Store tightly closed in a cool dry place at +2°C to +8°C.

Safe removal

The test strip is to be removed safety after use like bacteria containing material. This may be done by burning, autoclaving or by placing into a 5 to 6% desinfectant solution - for at least 6 hours.

Experimental Procedure

With an inoculating loop take a separate, well-grown colony from the culture medium.

Apply the colony to the reaction zone and spread with the inoculating loop.

After approx. 20 to 60 seconds compare with the colour scale.

Evaluation

In the case of cytochrome oxidase-positive germs the reaction zone is coloured blue to blue-violet.

Medically important oxidase-**positive** microorganisms

Neisseria (all species)	Actinobacillus lignieresii
Aeromonas spp.	Actinobacillus equuli
Pasteurella spp.	Bordetella pertussis
Vibrio spp.	Bac. anthracis
Cordiobacterium hominis	Bac. subtiliis
Pseudomonas spp.	Brucella spp.
Flavobacterium spp.	Chromobacterium spp.
Alcaligenes spp.	Eikenella corrodens
Moraxella spp.	Plesionmonas spp.
Campylobacter spp.	Branhamella catarrhalis
Micrococcus spp.	

Oxidase-**negative** microorganisms

Staphylococcus spp.	Pseudomonas mallei
Streptococcus spp.	Pseudomonas maltophilia
Gemella haemolysans	Bordetella parapertussis
Peptococcus spp.	Actinobacillus
Peptostreptococcus spp.	Actinomycetem-comitans
Leuconostoc spp.	Anaerobier (all)
Corynebacterium spp.	Haemophilus spp.
Listeria spp.	Pasteurella haemolytica
Lactobacillus spp.	Type T
Bacillus spp.	Streptobacillus
Enterobacteriaceae (all kinds)	Mycoplasma spp.
Acinetobacter spp.	Acholeplasma spp.

Bactident® Oxidase

Note:

It is always recommended to carry out a control test with a negative culture (e.g. E.coli), with a weakly positive culture (e.g. Pasteurella) and with a strongly positive culture (e.g. Pseudomonas for Aeromonas). The most suitable cultures for this test are those from culture media without dyes, indicators or inhibitors. Should the bacteria culture itself have a colour, this must be taken into consideration in the assessment of the tests.

Bacterial colonies taken from media with pH values below 5.5 (e.g. after the metabolism of carbohydrates with subsequent acidification of the culture medium) can give a false **negative** oxidase reaction. In such cases, the mciroorganisms should be subjected to an intermediate passage on a medium on which the bacteria concerned cannot reduce the pH value below 6.0.

Ordering Information

Product	Merck Cat. No.	Pack contents
Bactident® Oxidase	1.13300.0001	50 test strips

Bactident® Staph plus

Latex agglutination test of *Staphylococcus aureus* from culture for laboratory use only.

Contents

- Bactident® latex reagent (white cap):
  latex particles coated with rabbit proteins suspended in a buffer containing a preservative.
- Bactident® Positive Control Reagent (red cap):
  a formulation of non-viable *S. aureus* in a buffer containing a preservative.
- Bactident® Negative Control Reagent (blue cap):
  a formulation of non-viable *S. epidermidis* in a preservative.
- 10 disposable, white test cards with 6 test-ovals.
- Insert sheet with instructions for use.
- Sterile wooden sticks, Ø 2 mm **(not included)**.

Intended Use

Bactident® Staph *plus* is a rapid, colour enhanced lated agglutination slide test to detect coagulase and/or Protein-A characteristics associated with *Staphylococcus aureus* colonies obtained from culture.The formulation of the latex detection reagent will react with either or both of these two characteristics.

Summary

Staphylococcus aureus has been shown to be a pathogenic bacterial species. Since it is an organism commonly found on the skin, nasal passages and mucous membranes, an injury of these sites provides an opportunity for these agent to produce an infection. *S. aureus* is responsible for most superficial suppurative infections and food poisonings. It is also a cause of nosocomial infection.

The coagulase and Protein-A characteristics associated with *S. aureus* allows for the identification of at least 98% of this species. Indeed, coagulase-negative *Staphylococcus* species induce infection as well. Coagulase can be either bound (clumping factor) to the staphylococci or released as a free enzyme. Coagulase converts fibrinogen to form a clot when EDTA-plasma is added to coagulase-positive *S. aureus*. Differential medium has been described for growth of coagulase positive staphylococci. In addition, vaious mediums have been described for other individual properties of pathogenic staphylococci. Most of the above identified culture efforts require many hours of testing and evaluation before the results become available. Independent from coagulase activity is Protein-A substance. Protein-A is a constituent of *S. aureus* cell wall. It combines with the Fc portion of most IgG immunoglobulins and serves as another marker. In contrast to lengthy culture procedures, the speed, convenience and accuracy of Bactident® Staph *plus* provides for an appropriate alternative test. Rapid lated agglutination tests have been shown to be as reliable as the tube coagulase system (Bactident® Coagulase, Merck Cat. no. 1.13306.) in most cases. **Methicillin-resistant (MRSA) or sensitive** *S. aureus* **did not interfere with the detection of coagulase-positive or negative staphylococci. Bactident® Staph** *plus* **has a relative sensitivity of 100% with a relative specificity of 99%.**

Principle

The latex particles used in Bactident® *plus* reagent are sensitized with specific concentrations of rabbit plasma proteins. When coagulase and/or Protein-A is provided by the culture specimen at detectable levels, they will interact with the sensitized particles to produce visible red agglutionation in a blue background. This is a positive result.

Experimental Procedure

Ensure that the reagents have reached room temperature before use! It is advised to perform a catalase-test (e.g. Bactident® Catalase, Merck Cat. no. 1.11351.) of suspect colonies and to investigate morphology and gram staining prior to the test:

1. Resuspend the latex reagent, the positive and the negative control by repeated but gentle inversions.
2. Identify ovals for the positive, negative and the specimen-test.
3. Squeeze the vial to deliver a drop of reagent into the appropriate oval. Place and drop of the latex reagent for each specimen to be tested in a seperate oval on the test-card.
4. Pick a fresh colony (colonies older than 45h can give non-interpretable results, or a very weak agglutination pattern) e.g. from Baird-Parker Agar (Merck Cat. no. 1.05406.), Chapman Agar (Merck Cat. no. 1.05469.) or Blood Agar (Merck Cat. no. 1.10886.) with a wooden stick or loop.
5. Mix thoroughly for 10 seconds and blend the colony into the latex reagent by slightly rubbing the surface of the test-card with the wooden stick or loop to the inside limits of the oval. Disgard the stick or burn the loop.
6. For 20 seconds, gently hard-rock the card to agitate the combination. Do not allow the combinations to spill over into adjacent ovals!
7. Clumping of the latex should be instantaneous with mot *S. aureus* strains or will progressively increase during the rocking of the card within its 20 seconds period. Record the results.
8. Dispose of the card into a disinfectant of for incineration.

Interpretation of Results

A **positive result** is any indication of a red agglutination in a slight to significant blue background within 30 seconds after the initial mixing of the specimen and the detection latex reagent. When agglutination is observed, coagulase and/or Protein-A was presented by the specimen and presumend to be *S. aureus*. There are not cross reactions with *S. saprophyticus* or *S. haemolyticus*.

A **negative result** has occured, when no agglutination or very moderate stringiness of the latex reagent is observed within the 30 seconds. A homogeneous background of purple colour will persist in the particular oval.

Bactident® Staph plus

Good Laboratory Practises to Follow

1. Use the test directions as provided.
2. Allow reagents to achieve room temperature before using.
3. Re-suspend the reagents before dispensing them into the ovals.
4. do not re-use an oval on the card.
5. Use a fresh stick (or loop) to deliver each specimen.
6. Do not allow the tip of the latex vial to touch a specimen.
7. Following appropriate microbiological procedures in handling and disposing of the material used in the performance of the test.
8. Replace the proper caps on their respective vials.
9. Do not interchange and use reagents among different lot numbers.

Stability and Storage

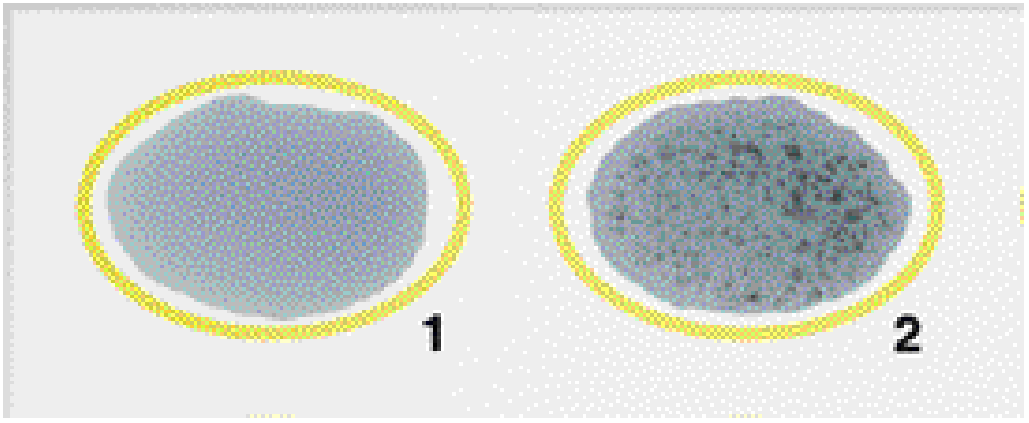
Store tightly closed in a cool dry place stored at +2 °C - +8°C.

DO NOT FREEZE ANY REAGENT!

Stability: see expiry date on the pack.

Ordering Information

Product	Merck Cat. No.	Pack size
Bactident® Staph *plus*	1.13316.0001	50 test strips



Detail negative result **Detail positive result**

BAIRD-PARKER Agar
(Staphylococcus Selective Agar Base acc. to BAIRD-PARKER)

For the isolation and enumeration of Staphylocsoccus aureus in foods and pharmaceutical materials according to BAIRD-PARKER (1962).

This culture medium complies with the recommendations of the United States Pharmacopeia XXVI (2003), the European Pharmacopeia II, the International Organization for Standardization (ISO) (1977, 1984), the International Dairy Federation (Internationaler Milchwirtschaftsverband) (1978) and the DIN Norms 10163 and 10178.

Mode of Action

This medium contains lithium chloride and tellurite to inhibit the growth of accompanying microbial flora, whereas pyruvate and glycine selectively stimulate the growth of staphylococci.

Staphylococcus colonies show two characteristic features when grown in this opaque medium (opaque, because of its egg-yolk content)

a. characteristic zones and rings are formed as a result of lipolysis and proteolysis,
b. reduction of tellurite to tellurium produces a black colouration.

The egg-yolk reaction and tellurite reduction are usually found to occur together with a positive coagulase reaction and can thus serve as an index for the latter.

STADHOUDERS et al. (1976) recommend that egg-yolk should be replaced with blood plasma, if coagulase-positive staphylococci are to be detected directly.

SMITH and BAIRD-PARKER (1964) recommend the addition of sulfamethazine to suppress the growth and swarming of Proteus species.

Typical Composition (g/litre)

Peptone from casein 10.0; meat extract 5.0; yeast extract 1.0; sodium pyruvate 10.0; glycine 12.0; lithium chloride 5.0; agar-agar 15.0.

Also to be added:

Egg-yolk tellurite emulsion 50ml; if required, sulphamethazine 0.05 g/l.

Preparation

Suspend 58 g in 0.95 litre, autoclave (15 min at 121 °C). Cool to 45-50 °C, mix in 50 ml Egg-yolk Tellurite Emulsion and, if required, 50 mg sulfamethazine/litre. Pour plates.

pH: 6.8 ± 0.2 at 25 °C.

The plates are opalescent and yellowish-brown in colour.

The ready-to-use culture medium can be stored in the refrigerator (approx. 4 °C) for up to 1 month.

Experimental Procedure and Evaluation

Dilute the sample material and spread thinly on the surface of the culture medium.

Incubation: 24-48 hours at 35 °C aerobically.

Appearance of Colonies	Microorganisms
Black, shiny, convex colonies 1-5 mm in diameter with a narrow, white edge surrounded by a clear zone 2-5mm wide. Opaque rings within the clear zones only appear after 48hours of incubation	Staphylococcus aureus
Black, shiny, irregular shape. Opaque zone develop around the colonies after 24hours.	Staphylococcus epidermis
Growth sometimes: Very small, brown to black, nor clear zones.	Micrococci
Dark brown, dull, clear zones sometimes appear after 48hours.	Bacillus species
White, no clear zones	Yeasts

Literature

BAIRD-PARKER, A.C.: An improved diagnostic and selective medium for isolating coagulase positive Staphylococci. - J**. Appl. Bact., 25**; 12-19 (1962).

DIN Deutsches Institut für Normung e.V.: Nachweis Koagulase-positiver Staphylokokken. Referenzverfahren für Milchpulver. - **DIN 10178**.

DIN Deutsches Institut für Normung e.V.: Nachweis Koagulase-positiver Staphylokokken. Referenzverfahren für Milchpulver. - **DIN 10163**.

European Pharmacopeia II, Chapter VII, 10.

Internationaler Milchwirtschaftsverband; Nachweis Koagulase-positiver Staphylokokken (Referenzmethode). - **Internationaler Standard 60 A** (1978).

NISKANEN, A., a. AALTO, M.: Comparison of selective media for coagulase-positive enterotoxigenic Staphylococcus aureus.- **Appl. Envir. Microbiol., 35**; 1233-1236 (1978).

SMITH, B.A., a. BAIRD-PARKER, A.C.: The use of sulfamethazine for inhibiting Proteus spp. on Baird-Parker's isolation medium for Staphylococcus aureus. - J**. Appl. Bact., 27**; 78-82 (1964).

STADHOUDERS, J., HASSINGS, F., a. VAN AALSTEN-VAN MAREN, N.O.: A pour-plate method for the detection and enumeration of coagulase-positive Staphylococcus aureus in the BAIRD-PARKER Medium without egg-yolk. - **Netz. Milk Diary J., 30**; 222-229 (1976).

United States Pharmacopeia XXVI, Chapter "Microbial limit Tests", 2003.

ISO/FDIS: Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of coagulase-positive staphylococci (Staphylococcus aureus and other species) - Part 1: Technique using Baird-Parker agar medium. **ISO 6888-1** (2003).

BAIRD-PARKER Agar
(Staphylococcus Selective Agar Base acc. to BAIRD-PARKER)

Ordering Information

Product	Merck Cat. No.	Pack size
BAIRD-PARKER Agar (Staphylococcus Selective Agar Base acc. to BAIRD-PARKER)	1.05406.0500	500 g
Bactident® Catalase	1.11351.0001	1 x 30 ml
Egg-yolk tellurite Emulsion	1.03785.0001	10 x 50 ml

Staphylococcus aureus ATCC 25923

Quality control (spiral plating method)

Test strains	Inoculum (cfu/ml	Recovery rate (%)	Black colonies	Clear zones round the colonies
Staphylococcus aureus ATCC 25923	10^3-10^5	≥ 70	+	+
Staphylococcus aureus ATCC 6538	10^3-10^5	≥ 70	+	+
Staphylococcus epidermidis NCTC 11047	10^3-10^5	Not limited!	+ / -	-
Enterococcus hirae ATCC 8043	10^3-10^5	Not limited!	+ / -	-
Bacillus subtilis ATCC 6051	> 10^5	≤ 0.01		
Escherichia coli ATCC 8739	> 10^5	≤ 0.01		
Proteus mirabilis ATCC 29906	10^3-10^5	Not limited!	brown-black	-
Pseudomonas aeruginosa ATCC 9027	> 10^5	≤ 0.01		
Salmonella typhimurium ATCC 14028	> 10^5	≤ 0.01		

BAT Medium

Medium for the detection of Alicyclobacillus in Fruit Juices.

Alicyclobacilli are aerobe, gram-positive spore forming bacteria, whose optimum of growth is at low pH value and increased temperatures. Alicyclobacilli are spoilage organisms especially effecting the quality of fruit juices. (CERNY et al. 1984, BAUMGART und MENJE 2000).

The Medium complies with First Standard IFU-Method on the Detection of Alicyclobacillus in Fruit Juices (2003).

Mode of Action

The BAT Medium supports the growth of Alicyclobacilli.The low pH-value in combination with the high incubation temperature inhibit the contaiminating flora in growth.

Typical Composition (g/litre)

Yeast extract 2.0; D(+)glucose 5.0; Calcium chloride 0.25; Magnesium sulfate 0.5; Ammonium sulfate 0.2; Potassium-dihydrogenphosphate 3.0; Zinc sulfate 0.00018; Copper sulfate 0.00016; Manganese sulfate 0.00015; Cobalt-chloride 0.00018; Boric acid 0.00010; Sodium molybdate 0.00030; Agar-Agar 18.0.

Preparation

Dissolve 14.5 g in 500 ml of demin. water and heat to boiling until completely dissolved.

Note: he medium has a spontaneous pH of 5.3 ± 0.2 in order to maintain the gel strength during autoclavation. Adjustment of the pH to 4.0 ± 0.2 is made after the autoclavation.

Autoclave (15 min. at 121°C).

Cool to 45-50 °C. Adjust the pH to 4.0 ± 0.2 by adding 1 N H2SO4. Mix well and pour into sterile Petridishes.

pH: 4.0 ± 0.2 at 25 °C.

The prepared medium is clear and yellowish.

The prepared plates can be stored for up to 2 weeks at 2-8°C.

Keep protected from light and drying.

Application and Interpretation

Inoculate the medium by spreading 0.1 ml on the surface.

Membranefilter technique can be used with samples being filterable.

Incubation for 3-5 days at 45 ± 1.0 °C.

Count all colonies growing on the BAT Medium as suspicious Alicyclobacilli.

Confirm the suspicious colonies by further testing.

Literature

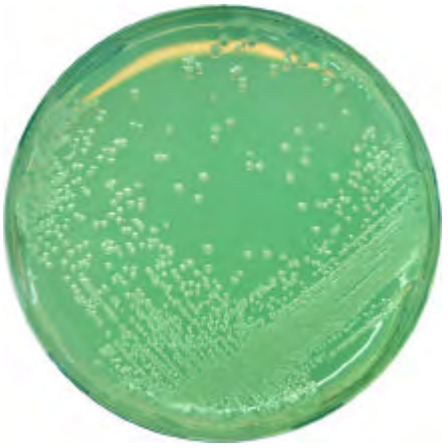
CERNY, G., W. HENNLICH und K. PORALLA. Fruchsaftverderb durch Bacillen: Isolierung und Charakterisierung des Verderbserregers. – Z **Lebens Unters Forsch 179**; 224 – 227 (1984).

BAUMGART, J. and S. MENJE. The Impact of Alicyclobacillus acidoterrestris on the Quality of Juices and Soft Drinks. **FRUIT PROCESSING 7**; 251 – 254 (2000).

IFU Working Group Microbiology. First Standard IFU-Method on the Detection of Alicyclobacillus in Fruit Juices. April 2003.

Ordering Information

Product	Merck Cat. No.	Pack size
BAT Medium	1.07994.0500	500 g



Alicyclobacillus acidoterrestris DSMZ 2498

Quality control

Test strains	Growth
Alicyclobacillus acidocaldarius DSMZ 446	good
Alicyclobacillus acidoterrestris DSMZ 2498	good
Alicyclobacillus cycloheptanicus DSMZ 4006	good
Alicyclobacillus hesperidium DSMZ 12766	good
Staphylococcus aureus ATCC 25923	none
Escherichia coli ATCC 25922	none

Bile Aesculin Azide Agar

For the detection and enumeration of intestinal enterococci (faecal streptococci) acc. to ISO 7899-2

Mode of Action

The presence of intestinal enterococci, also termed faecal streptococci, serves as an indicator for faecal contamination, particularly when the contamination took place a long time ago and the less resistant coliform bacteria, including Escherichia coli, may be already dead when the analysis is carried out.

Bile salt aesculin azide agar is employed acc. to ISO 7899-2 as a confirmation and enumeration medium for typical isolate on the primary isolation Membrane Enterococcus Selective Agar acc. to Slanetz and Bartley (Cat. no. 1.05262.0500 or 1.05289.0500).

Enterococci and some species of the genus Streptococcus namely S. bovis and S. equines can reproduce normally in this medium.

Esculin hydrolysis and bile tolerances are regarded as reliable characteristics of enterococci (FACKLAM 1971, 1973).

Intestinal Enterococci hydrolyse the glycoside esculin to give dextrose and esculetin. Esculetin forms an olive green to black complex with iron(III) ions.

Enterococci are bile tolerant. Bile salts inhibit the growth of numerous accompanying bacteria. The concentration of sodium azide present in this medium largely inhibits the growth of the accompanying Gram-negative microbial flora, while sparing the enterococci.

The use of sodium azide as a selective inhibitor for Gram-negative bacteria was reported in the studies of EDWARDS (1933, 1938) and HARTMANN (1936) on the isolation of Str. agalactiae. MALLMANN (1940) and SNYDER and LICHSTEIN (1940) later showed that sodium azide can also be used for the isolation of enterococci from water.

Typical Composition (g/litre)

Peptone from Casein 17.0; peptone 3.0; yeast extract 5.0; sodium chloride 5.5; aesculin 1.0; ammonium iron(III) citrate 0.5; ox bile 10.0; sodium azide 0.15; agar-agar 13.0:

Preparation

Suspend 54.65 g in 1 litre water and dissolve by boiling. Sterilise for 15 min. at 121 °C. After cooling to 45-50 °C pour into Petridishes to a depth of 3 mm to 5 mm and allow to solidify.

pH: 7.1 ± 0.2 at 25 °C.

The plates are clear and yellow.

Poured plates can be stored at +2 - +8°C for up to 2 weeks.

Experimental Procedure and Evaluation

For the confirmation typical red, maroon or pink coloured colonies on membrane filter Enterococcus selective agar acc. to Slanetz and Bartley (Cat. no. 1.05262.0500 or 1.05289.0500) are transferred, with sterile forceps without inverting the filter onto a plate of bile salt aesculin azide agar which has been pre-heated at 44°C. After the inoculation plates are incubated at 44 ± 0.5°C for 2h.

Regard all typical colonies showing a tan to black colouration in the surrounding medium as giving a positive reaction and count as intestinal enterococci.

Literature

ISO INTERNATIONAL STANDARDISATION ORGANISATION WATER QUALITY DETECTION AND ENUMERATION OF INTESTINAL ENTEROCOCCI PART 2 MEMBRANE FILTRATION **ISO 7899**-2 2000.

EDWARDS, S.J.: Studies on bovine mastitis. IX. A selective medium for the diagnosis of Streptococcus mastitis. - **J. Comp. Path. Therap. 46**; 211-217 (1933).

EDWARDS, S.J.: The diagnosis of Streptococcus mastitis by cultural methods. - J**. Comp. Path Therap. 51** ; 250-263 (1938).

FACKLAM, R.R., a MOODY, M.: Presumptive identification of group D streptococci: the bile-esculin test. - **Appl. Microbiol., 20**; 245-250 (1970).

FACKLAM, R.R.: Recognification of group D strptococcal species of human origin by biochemical and physiological test. - **Appl. Microbiol., 23**; 1131-1139 (1972).

FACKLAM, R.R.: Comparison of several laboratory media for presumptive identification of enterococci and group D streptococci. - **Appl. Microbiol., 26**; 138-145 (1973).

HARTMANN, G.: Ein Beitrag zur Reinzüchtung von Mastitisstreptokokken aus verunreinigtem Material. - **Milchw. Forsch., 18**; 116-122 (1936).

LITSKY, W., MALLMANN, W.L., a. FIFIELD, C.W.: A new medium for the detection of enterococci in water. - **Amer. J. Publ. Hlth., 43**; 873-879 (1953).

MALLMANN, W.L.: A new yardstick for measuring sewage pollution. - **Sewage Works J., 12**; 875-878 (1940).

SNYDER, M.L., a. LICHSTEIN, H.C.: Sodium azide as an inhibiting substance for Gram-negative bacteria. - **J. Infect. Dis., 67**; 113-115 (1940).

Verordnung über Trinkwasser und über Wasser für Lebensmittelbetriebe (Trinkwasserverordnung) vom 22. Mai 1986. - **Bundesgesetzblatt**, Teil I, 760-773 (1986).

SWAN, A.: The use of bile-esculin medium and of Maxted's technique of LANCEFIELD grouping in the identification of enterococci (Group D streptococci). - **J. Clin. Pathol., 7**; 160-163 (1954).

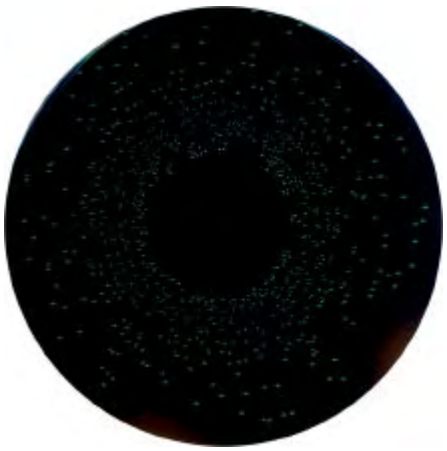
Ordering Information

Product	Merck Cat. No.	Pack size
Bile Aesuclin Azide Agar	1.00072.0500	500 g

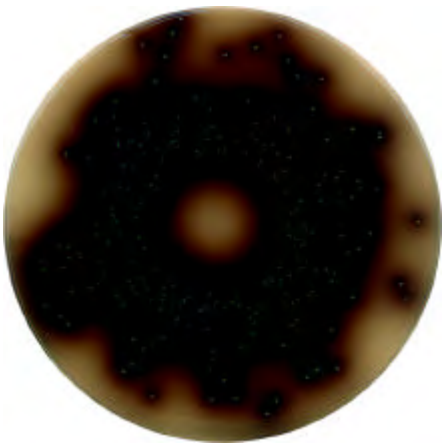
Bile Aesculin Azide Agar

Quality control

Test strains	Recovery rate (%)	Colony colour
Enterococcus faecium ATCC 882	≥ 60	Black
Enterococcus faecalis ATCC 19433	≥ 70	Black
Enterococcus durans ATCC 6056	≥ 50	Black
Enterococcus hirae ATCC 8043	≥ 60	Black
Listeria monocytogenes ATCC 19118	≤ 0.01	Colourless
Staphylococcus aureus ATCC 25923	≤ 0.01	Colourless
Escherichia coli ATCC 25922	≤ 0.01	Colourless



Enterococcus faecalis
ATCC 19433



Enterococcus hirae
ATCC 8043

Bismuth Sulfite Agar acc. to WILSON-BLAIR

Selective agar introduced by WILSON and BLAIR (1927, 1931) for the isolation and differentiation of Salmonella typhi and other salmonellae from clinical specimens, e.g. feces.

IVD *in vitro diagnosticum – For professional use only*

See also General Instruction of Use
Warnings and precautions see ChemDAT®
(www.chemdat.info)

Principle

Microbiological method

Mode of Action

Brilliant green and bismuth largely inhibit the accompanying bacterial flora. Colonies of H_2-S-positive salmonellae exhibit blackening due to the formation of iron sulfide. Reduction of bismuth ions to metallic bismuth produces a metallic lustre around the colonies (McCOY 1962).

Typical Composition (g/litre)

Meat extract 5.0; peptone from meat 10.0; D(+)glucose 5.0; di-sodium hydrogen phosphate 4.0; iron(III) sulfate 0.3; brilliant green 0.025; bismuth sulfite indicator 8.0; agar-agar 15.0.

Preparation and Storage

Usable up to the expiry date when stored dry and tightly closed at +15 to +25° C. Protect from light.After first opening of the bottle the content can be used up to the expiry date when stored dry and tightly closed at +15 to +25°C.

Suspend 47.5 g/litre, mix the resulting precipitate to give a uniform suspension, pour plates to give thick layers (25 ml).

- **Do not autoclave.**

pH: 7.6 ± 0.2 at 25 °C.

The prepared medium is turbid and green in colour.

- **The freshly prepared medium is strongly inhibitory and is thus especially suitable for heavily contaminated samples. The metallic lustre of the colonies usually only appears after 48 hours on incubation. After 4 days storage at 4 °C the inhibitory action of the medium is not as strong and it should then be used for less heavily contaminated specimens; in this case the metallic lustre appears after a shorter period of incubation.**

Specimen

e.g. Stool.Clinical specimen collection, handling and processing, see general instructions of use.

Experimental Procedure and Evaluation

Inoculate by thinly spreading the sample or material from an enriched culture on the surface of the medium.

Incubation: up to about 48 hours at 35°C aerobically.

Salmonella colonies often display blackening after 18 hours of incubation, the metallic sheen appears several hours later depending on the age of the medium.

Appearance of Colonies	Microorganisms
Black centre, light edges surrounded by a black precipitate with metallic sheen (so-called rabbit's or fish-eye)	Salmonella with the exception of S. parathyphi A. and S. pullorum
Small, green to brown, sometimes mucoid	Coliform bacteria, Serratia, Proteus and others

Literature

McCOY, J.H.: The isolation of Salmonellae. – J**. Appl. Bact., 25**; 213-224 (1962).

WILSON, W.J., a BLAIR, E.M. McV.: Use of glucose bismuth sulfite iron medium for the isolation of Bacillus typhosus and Bacillus proteus. – J**. Hyg., 26**; 374-391 (1927).

WILSON, W.J., a. BLAIR, E.M. McV.: Further experience of the bismuth sulfite media in the isolation of Bacillus typhosus and Bacillus paratyphosus B from faeces, sewage and water. – **J. Hyg. 31**; 138-161 (1931).

Ordering Information

Product	Merck Cat. No.	Pack size
Bismuth Sulfite Agar acc. to WILSON-BLAIR	1.05418.0500	500 g
Bismuth Sulfite Agar acc. to WILSON-BLAIR	1.05418.5000	5 kg

Bismuth Sulfite Agar acc. to WILSON-BLAIR

Quality control

Test strains	Growth	Black centre	Metallic sheen
Salmonella typhimurium ATCC 14028	good / very good	+	+
Salmonella choleraesius ATCC 13312	good / very good	+	+
Salmonella enteritidis NCTC 5188	good / very good	+	+
Salmonella arizonae ATCC 13314	good / very good	+	+
Salmonella aboni NCTC 6017	good / very good	+	+
Escherichia coli ATCC 25922	poor / fair	-	-
Proteus mirabilis ATCC 29906	good / very good	±	-
Shigella sonnei ATCC 11060	none		
Staphylococcus aureus ATCC 25923	none		
Bacillus cereus ATCC 11778	none		

M
MPF

Blood Agar Base

For preparing blood plates and boiled blood (chocolate) plates used for the isolation and cultivation of various fastidious microorganisms, especially of pathogenic species, and for establishing their forms of haemolysis.

IVD *in vitro diagnosticum –*
For professional use only

See also General Instruction of Use
Warnings and precautions see ChemDAT®
(www.chemdat.info)

This culture medium can be used without blood e.g. for setting up blood cultures (UPDYKE 1970) and as a base for preparing special culture media

The medium complies with the recommendations of APHA (1992) for the examination of foodstuffs.

Principle

Microbiological method

Mode of Action

This culture medium represents a rich nutrient base, which provides optimal growth conditions for all relevant microorganisms. The pH value of 6.8 stabilizes the red blood corpuscles and favours the formation of clear haemolysis zones (NORTON 1932). Fresh, defibrinated sheep blood is most suitable for determining haemolysis forms. Boiled blood agar ("chocolate agar") is an extremely rich culture medium and can be prepared by heating after the blood has been added.

If the culture medium base is to be used without blood, the pH should, however, be adjusted to 7.2 to 7.4 since most bacterial colonies appear somewhat earlier and grow better in a slightly alkaline medium.

TARSHIS and FRISH (1951) recommended addition of 1% glycerol and 25 % human blood when isolating tubercle bacilli from sputum, since recognizable mycobacteria colonies grow from even minimal amounts of sample material.

HOSTY et al. (1953) reported, however, that 0.1 % glycerol and 2.5 % human blood together with 100 IU/mol of penicillin as a selective agent are sufficient. According to SONDAG et al. (1977) and BLACK a. VAN BUSKIRK (1973), addition of 5 mg/l gentamicin (e.g. 0.1 ml gentamicin solution) to blood agar permits selective cultivation of Streptococcus pneumoniae and other Streptococci as well as bacterioides, Clostridium and yeasts. For the selective cultivation of Aeromonas MISHRA et al. (1987) recommend an ampicillin sheep blood agar (ASBA 30).

Typical Composition (g/litre)

Nutrient substrate (heart extract and peptones) 20.0; sodium chloride 5.0: agar-agar 15.0.

Also to be added:

Blood 50-80 ml.

Preparation and Storage

Usable up to the expiry date when stored dry and tightly closed at +15 to +25 °C. Protect from light.After first opening of the bottle the content can be used up to the expiry date when stored dry and tightly closed at +15 to +25 °C.

Suspend 40 g/litre, autoclave (15 min at 121 °C), cool to 45-50°C, add 5-8 % defibrinated blood, mix.

pH: 6.8 ± 0.2 at 25 °C.

Before adding blood, the prepared medium is clear and yellowish-brown, then blood coloured and not haemolytic.

- **Poured blood plates can be stored for a maximum of 3months in the refrigerator.**
  Preparation of boiled blood agar: after adding the blood, heat the culture medium for about 10 minutes at approx. 80 °C with frequent swirling until it turns brownish (chocolate colour).

Specimen

e.g. Secretions of respiratory tract, sputum.Clinical specimen collection, handling and processing, see general instructions of use.

Experimental Procedure and Evaluation

Inoculate the surface of the plates.

Incubation: under optimal conditions usually 24 hours at 35 °C aerobically (Cl. perfringens anaerobically).

Check the plates for kind of hemolysis.

Literature

American Public Health Association: Compendium of Methods for the Microbiological Examination of Foods. 3rd ed., 1992.

BLACK, W.A. a. VAN BUSKIRK, F.: Gentamicin blood agar used as a general-purpose selective medium. – **Appl. Microbiol., 25** ; 905-907 (1973).HOSTY, FREEMAN a. IRWIN: **Publ. Hlth. Lab., 11**; 143 (1953).

MISHRA, S., NAIR, G.B., BHADRA, R.K., SIKDER, S.N., a. PAL, S.C.: Comparison of selective media for primary isolation of Aeromonas species from human and animal faeces. – **J. Clin. Microbiol., 25**; 2040-2043 (1987).

NORTON, J.F.: Bacteriology of pus. – **J. Lab. Clin. Med., 17**; 558-565 (1932).

SONDAG, J.E., MORGENS, R.K., HOPPE, J.E., a. MARR, J.J.: Detection of pneumococci in respiratory secretions: clinical evaluation of gentamicin blood agar. – **J. Clin. Microbiol. 5**; 397-400 (1977).

TARSHIS, M.S., a. FRISCH, A.W.: Blood media for the cultivation of Mycobacterium tuberculosis. – **Amer. J. Clin. Pathol. 21**; 101-113 (1951).
UPDYKE, E.L.: Pneumococcal Infections – in **Diagnostic Procedures for Bacterial, Mycotic and Parasitic Infections, 5th Edition**, APHA New York 1970.

Blood Agar Base

Ordering Information

Product	Merck Cat. No.	Pack size
Blood Agar Base	1.10886.0500	500 g
Anaerobic jar	1.16387.0001	1 ea
Anaeroclip®	1.14226.0001	1 x 25
Anaerocult® A	1.13829.0001	1 x 10
Anaerocult® A mini	1.01611.0001	1 x 25
Anaerocult® P	1.13807.0001	1 x 25
Anaerotest®	1.15112.0001	1 x 50
Gentamicin solution	1.11977.0001	10 ml
Glycerol (about 87 %)	1.04094.0500	500 ml
Plate basket	1.07040.0001	1ea
Blood		
Ampicillin mono-sodium salt	CN Biosciences	
Penicillin G potassium salt	CN Biosciences	

Quality control

Test strains	Inoculum(cfu/ml)	Recovery rate (%)	Hemolysis	Bacitracin test
Staphylococcus aureus ATCC 25923	10^3-10^5	≥ 70	β	
Streptococcus pyogenes ATCC 12344	10^3-10^5	≥ 70	β	+
Streptococcus agalactiae ATCC 13813	10^3-10^5	≥ 70	-	
Streptococcus pneumoniae ATCC 6301	10^3-10^5	≥ 70	α	-
Listeria monocytogenes ATCC 19118	10^3-10^5	≥ 70	-	
Bacillus cereus ATCC 11778	10^3-10^5	≥ 70	β	
Clostridium perfringens ATCC 13124	10^3-10^5	≥ 70 (anaerobic incubation)	β	

Blood Agar Base No. 2

For the isolation and cultivation of various fastidious microorganisms, especially of pathogenic species, and for establishing their forms of haemolysis

IVD *in vitro diagnosticum –*
For professional use only

See also General Instruction of Use
Warnings and precautions see ChemDAT®
(www.chemdat.info)

Principle

Microbiological method

Typical Composition (g/litre)

Nutrient substrate (yeast extract, peptone, liver-hydrolysate) 23.0; sodium chloride 5.0; agar-agar 12.0.

Preparation and Storage

Usable up to the expiry date when stored dry and tightly closed at +15 to +25°C. Protect from light.After first opening of the bottle the content can be used up to the expiry date when stored dry and tightly closed at +15 to +25°C.

Suspend 40 g in 1 litre of demin. water and autoclave (15 min at 121°C). Cool to 45-50°C, add 5-8 % of sterile defibrinated blood without bubbles (ensure adequate aeration of the blood). Mix gently and pour into plates.

pH: 7.4 ± 0.2 at 25 °C.

Before adding blood the prepared medium is clear and yellowish-brown, afterwards blood-coloured and non-hemolytic.

Specimen

e.g. Throat swabs, sputum, genital swabs.Clinical specimen collection, handling and processing, see general instructions of use.

Experimental Procedure

Inoculate the plates.

Incubation: under optimal conditions usually 24 hours at 35°C aerobically (Cl. perfringens anaerobically).

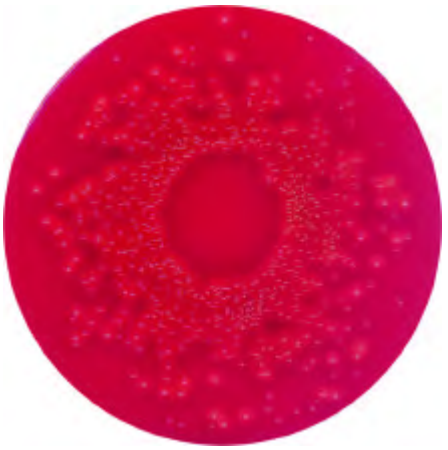
Investigate hemolytic reactions.

Literature

WATERWORTH, P.M.: Brit. J. Exp. Pathol., 36(2); 186-194 (1955).

Ordering Information

Product	Merck Cat. No.	Pack size
Blood Agar Base No. 2	1.10328.0500	500 g
Blood Agar Base No. 2	1.10328.5000	5 kg
Merckoplate® Blood Agar	1.13414.0001	20 plates
Merckoplate® Blood Agar	1.13421.0001	480 plates
Blood		



Streptococcus pyogenes
ATCC 19615

Bacillus cereus
ATCC 11778

Blood Agar Base No. 2

Quality control

Test strains	Inoculum cfu/ml	Recovery rate (%)	Hemolysis	Bacitracin test
Staphylococcus aureus ATCC 25923	10^3-10^5	≥ 70	β	-
Streptococcus pyogenes ATCC 19615	10^3-10^5	≥ 70	β	+
Streptococcus pneumoniae ATCC 6305	10^3-10^5	≥ 70	α	-
Streptococcus agalactiae ATCC 13813	10^3-10^5	≥ 70	-	-
Listeria monocytogens ATCC 19118	10^3-10^5	≥ 70	-	
Bacillus cereus ATCC 11778	10^3-10^5	≥ 70	β	
Clostridium perfringens ATCC 13124	10^3-10^5	≥ 70	β	

Bolton Selective Enrichment Broth (Base)

Medium for the selective enrichment of Campylobacter from foods.

Mode of Action

Bolton Selective Enrichment Broth contains nutrients to aid resuscitation of sublethally damaged cells of Campylobacter. By this microaerophilic incubation is not needed. Addition of Bolton Broth Selective Supplement inhibits the accompanying grampositive and gramnegative bacteria as well as yeasts and moulds.

Typical Composition (g/litre)

Peptone from Meat 10,0; Lactalbumin hydrolysate 5,0; Yeast Extract 5,0; Sodium chloride 5,0; α-ketoglutaric acid 1,0; Sodium pyruvate 0,5; Sodium metabisulphite 0,5; Sodium carbonate 0,6; Haemin 0,01

Preparation

Dissolve 13,8 g in 500 ml of demin water.

Autoclave (15 min. at 121°C).

Cool to 45 - 50°C. Aseptically add 25 ml lysed horse blood and the content of 1 vial of Bolton Broth Selective Supplement. Mix well and distribute the broth into sterile screw top containers. After the adition of the sample the space between screw top and broth should be approx. 2cm.

pH: 7.4 ± 0.2 at 25 °C.

The ready-to-use broth in the container is dark red to black.

Experimental Procedure and Evaluation

Mix 25 g of food sample in 225 ml Bolton Selective Enrichment Broth and incubate for 4 hours at 37 °C. Afterwards continue incubation for 14-44 hours at 41,5 °C.

Subculture after 18 resp. 48 hours on Campylobacter Blood Free Selective Agar (modified CCDA, Merck Cat. No. 1.00070.0500).

Direct screening for Campylobacter jejuni and coli using Singlepath® Campylobacter, Merck Cat. No. 1.04143 is possible.

Literature

HUNT, J.M.: Campylobacter, **F.D.A. Bacteriological Analytical Manual, 8th Edition (Revision A) 7.01-7.27, AOAC, Arlington Va**, (1998)

BOLTON, F.J.: Personal communication, (1995)

Ordering Information

Product	Merck Cat. No.	Pack size
Bolton Selective Enrichment Broth (Base)	1.00068.0500	500 g
Bolton Broth Selective Supplement	1.00079.0001	1 x 16 vials
Campylobacter Blood Free Selective Agar (Base)	1.00070.0500	500 g
Singlepath® Campylobacter	1.04143.0001	20 tests

Quality control

Test strains	Growth after 48 hours
Campylobacter jejuni ATCC 33291	> 10^6 CFU/ml
Campylobacter jejuni ATCC 29428	> 10^6 CFU/ml
Campylobacter coli ATCC 33559	> 10^6 CFU/ml
E. coli ATCC 25922	-
Saccharomyces cererisiae ATCC 9763	-

Bolton Broth Selective Supplement

Additive for the preparation of Bolton Selective Enrichment Broth for the enrichment of Campylobacter from foods.

Mode of Action

Bolton Broth Selective Supplement is a mixture of four different antibiotics in lyophilized form acc. to ISO 10272-1.

Vancomycin, Cefoperazone und Trimethoprim inhibit the growth of grampositive and gramnegative bacteria. Amphotericin B 5 mg largely reduces the growth of Yeasts and Moulds.

Composition (per vial)

Vancomycin 10 mg; Cefoperazone 10 mg;

Trimethoprim 10 mg; AmphotericinB 5mg

Preparation

The lyophilisate is to dissolve in the original vial by adding of 5 ml of a 50:50 mixture of sterile distilled water and ethanol. Mix gently.

Take care for completely dissolving!

Add contents of a vial (5 ml) aseptically to 500 ml of sterile Bolton Selective Enrichment Broth (Base) cooled to 45-50 °C. Mix well.

Ordering Information

Product	Merck Cat. No.	Pack size
Bolton Broth Selective Supplement	1.00079.0001	1 x 16 vials
Bolton Selective Enrichment Broth	1.00068.0500	500 g

BPL Agar
(Brilliant-green Phenol-red Lactose Agar acc. to KAUFFMANN)

Selective agar proposed by KAUFFMANN (1935) for the identification and isolation of Salmonella with the exception of S. typhosa in faeces, urine, meat, milk and other materials.

IVD *in vitro diagnosticum –*
For professional use only

See also General Instruction of Use
Warnings and precautions see ChemDAT®
(www.chemdat.info)

This culture medium complies with the Fleischbeschaugesetz (German Meat Inspection Law) and the Einfuhruntersuchungs-Verordnung (German Regulations for the Examination of Imported Goods).

Principle
Microbiological method

Mode of Action
This culture medium contains lactose, whose degradation to acid is indicated by the pH indicator phenol red, which changes its colour to yellow. The indicator exhibits a deep red colour in the alkaline range. The growth of the accompanying Gram-positive microbial flora, Salmonella typhi and Shigella is largely inhibited by brilliant green. ADAM (1966) recommended that 0.2 % sodium deoxychlolate should be added to the culture medium to inhibit the swarming of Proteus colonies.

Typical Composition (g/litre)
Peptone from meat 7.0; sodium chloride 5.0; lactose 15.0; phenol red 0.04; brilliant green 0.005; agar-agar 13.0.

Preparation and Storage
Usable up to the expiry date when stored dry and tightly closed at +15 to +25° C. Protect from light.After first opening of the bottle the content can be used up to the expiry date when stored dry and tightly closed at +15 to +25° C.

Suspend 40 g/litre, autoclave (15 min at 121 °C), pour plates.

pH: 6.5 ± 0.2 at 25 °C.

The plates are clear and red-brown/greenish.

Specimen
e.g. Stool, urine .Clinical specimen collection, handling and processing, see general instructions of use.

Experimental Procedure and Evaluation
Inoculate the surface of the culture medium massively using either the sample material itself or material taken from an enrichment culture.

Incubation: 18-24 hours at 35 °C aerobically.

Tests with less inhibitory culture media such as SS Agar, LEIFSON Agar, ENDO Agar or GASSNER Agar should also be carried out.

Appearance of Colonies	Microorganisms
Pale pink, translucent, surrounded by a red zone	Lactose-negative: Salmonella, occasionally Proteus and Citrobacter
Yellow-green, opaque, surrounded by a yellow-green zone	Lactose-positive: If growth with a good E. coli, Enterobacter, Klebsiella. All others are largely inhibited.

Literature

ADAM, D.: Zusatz von Natriumdesoxycholat zum Brilliantgrün-Phenolrot-Agar nach Kristensen-Kauffmann zur Hemmung des Schwärmvermögens von Proteuskeimen. - Ärztl. Lab. 12; 245-246 (1966).

Deutsches Fleischbeschaugesetz: Ausführungsbestimmungen über die Untersuchung von gesundheitspolizeilicher Behandlung der Schlachttiere und des Fleisches bei Schlachtungen im Inland. Anlage 1 zu § 20 Abs. 4: Vorschriften über die bakteriologische Fleischuntersuchung.

Verordnung über die Untersuchung des in das Zollgebiet eingehenden Fleisches (Einfuhruntersuchungs-Verordnung). Anlage 1 zu § 20 Abs. 1: Untersuchungsverfahren.

KAUFFMANN, F.: Weitere Erfahrungen mit dem kombinierten Anreicherungsverfahren für Salmonellabacillen. - Z. Hyg. Infekt. Kr., 177; 26-32 (1935).

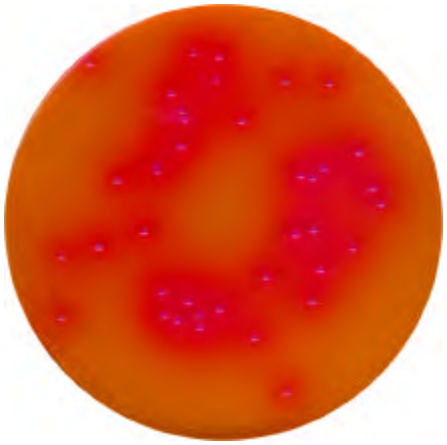
Ordering Information

Product	Merck Cat. No.	Pack size
BPL Agar (Brilliant-green Phenol-red Lactose Agar acc. to KAUFFMANN)	1.07236.0500	500 g
ENDO Agar	1.04044.0500	500 g
GASSNER Agar	1.01282.0500	500 g
LEIFSON Agar (Deoxycholate Citrate Agar acc. to LEIFSON, modified)	1.02896.5000	5 kg
Salmonella-Shigella Agar	1.07667.0500	500 g
Sodium deoxycholate	1.06504.0100	100 g

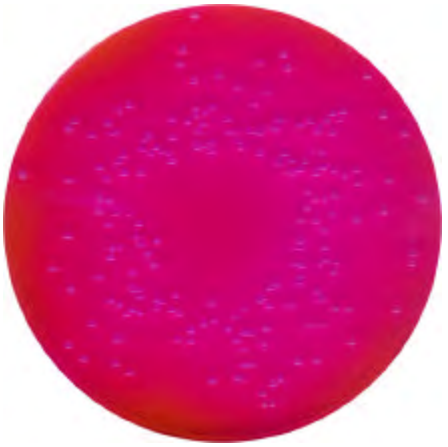
BPL Agar
(Brilliant-green Phenol-red Lactose Agar acc. to KAUFFMANN)

Quality control (spiral plating method)

Test strains	Inoculum(cfu/ml)	Recovery rate (%)	Colony colour	Culture medium
Salmonella typhimurium ATCC 14028	10^3-10^5	≥ 40	pink	red
Salmonella choleraesius ATCC 13312	10^3-10^5	≥ 40	pink	red
Salmonella enteritidis NCTC 5188	10^3-10^5	≥ 40	pink	red
Escherichia coli ATCC 25922	10^3-10^5	≥ 40	yellow-green	yellow
Proteus vulgaris ATCC 13315	10^3-10^5	not limited	pink	green
Staphylococcus aureus ATCC 25923	$>10^5$	≥ 0.01		
Enterococcus faecalis ATCC 33186	$>10^5$	≥ 0.01		
Bacillus subtilis ATCC 6633	$>10^5$	≥ 0.01		



Salmonella enteritidis NCTC 5188



Salmonella typhimurium ATCC 14028

BPLS Agar
(Brilliant-green Phenol-red Lactose Sucrose Agar)

Selective culture medium for the isolation of Salmonella with the exception of S. typhosa and Shigella from pathological material, faeces, urine, foodstuffs etc.

IVD *in vitro diagnosticum –*
For professional use only

See also General Instruction of Use
Warnings and precautions see ChemDAT®
(www.chemdat.info)

Principle
Microbiological method

Mode of Action
This culture medium contains lactose, whose degradation to acid is indicated by the pH indicator phenol red, which changes its colour to yellow. The indicator exhibits a deep red colour in the alkaline range. The growth of the accompanying Gram-positive microbial flora, Salmonella typhi and Shigella is largely inhibited by brilliant green. The growth of Salmonella is, however, improved by the richer nutrient base. Increased growth of accompanying microorganisms is considerably prevented by raising the concentration of brilliant green. Salmonellae are not able to ferment either lactose or sucrose. Thus in contrast to BPL agar, the sucrose contained in this medium allows identification of accompanying, weakly lactose-positive or lactose-negative, but sucrose-positive microorganisms.

Typical Composition (g/litre)
Peptone from meat 5.0; peptone from casein 5.0; meat extract 5.0; sodium chloride 3.0; di-sodium hydrogen phosphate 2.0; lactose 10.0; sucrose 10.0; phenol red 0.08; brilliant green 0.0125; agar-agar 12.0.

Preparation and Storage
Usable up to the expiry date when stored dry and tightly closed at +15 to +25°C. Protect from light.

After first opening of the bottle the content can be used up to the expiry date when stored dry and tightly closed at +15 to +25°C.

Suspend 57 g/litre, autoclave (15 min at 121°C), pour plates.

pH: 6.9 ± 0.2 at 25 °C.

The plates are clear and red.

Specimen
e.g. Stool, urine .Clinical specimen collection, handling and processing, see general instructions of use.

Experimental Procedure and Evaluation
Inoculate the plates with the sample material itself or material taken from an enriched culture. Tests should also be performed with less inhibitory culture media.

Incubation: 24 hours at 35 °C aerobically.

Appearance of Colonies	Microorganisms
Pink surrounded by a red zone	Lactose- and sucrose-negative: Salmonella and others
Yellow-green surrounded by a yellow-green zone	Lactose- or sucrose-positive: E.coli, Citrobacter, Proteus vulgaris, Klebsiella and others. Occasionally complete inhibition of growth.

Ordering Information

Product	Merck Cat. No.	Pack size
BPLS Agar (Brilliant-green Phenol-red Lactose Sucrose Agar)	1.07237.0500	500 g
Merckoplate ® BPLS Agar	1.15164.0001	1 x 20 plates

BPLS Agar
(Brilliant-green Phenol-red Lactose Sucrose Agar)

Quality control (spiral plating method)

Test strains	Inoculum(cfu/ml)	Recovery rate (%)	Colony colour	Culture medium
Salmonella typhimurium ATCC 14028	10^3-10^5	≥ 70	pink	red
Salmonella choleraesius ATCC 13312	10^3-10^5	≥ 70	pink	red
Salmonella enteritidis NCTC 5188	10^3-10^5	≥ 70	pink	red
Escherichia coli ATCC 25922	10^3-10^5	≥ 70	yellow	yellow
Proteus vulgaris ATCC 13315	10^3-10^5	≥ 70	yellow	yellow
Staphylococcus aureus ATCC 25923	10^3-10^5	not limited	yellow	yellow
Enterococcus faecalis ATCC 33186	10^3-10^5	not limited	yellow	yellow
Bacillus subtilis ATCC 6633	10^3-10^5	not limited	orange / yellow	yellow

Escherichia coli ATCC 25922

Salmonella typhimurium ATCC 14028

BPLS Agar, mod.
(Brilliant-green Phenol-red Lactose Sucrose Agar, modified)

Selective agar for the isolation of salmonellae (with the exception of S. typhosa) from meat, meat products and other foodstuffs.

The culture medium complies with the recommendations of the ISO (1993) and the DIN Norms 10160 and 10181. Its composition corresponds with that of the modification of the brilliant-green agar acc. to KAUFFMANN (1935), developed by a research group in Utrecht (Netherlands).

Mode of Action

Basically the same as for 1.07237 BPLS Agar. The brilliant green concentration is, however, much lower, growth is therefore not that strongly inhibited.

Typical Composition (g/litre)

Peptone from meat 10.0; meat extract 5.0; yeast extract 3.0; di-sodium hydrogen phosphate 1.0; sodium dihydrogen phosphate 0.6; lactose 10.0; sucrose 10.0; phenol red 0.09; brilliant green 0.0047; agar-agar 12.0.

Preparation

Suspend 51.5 g/litre, heat gently with frequent agitation and bring to boil to dissolve completely. Afterwards pour plates at about 50°C.

■ **Do not autoclave!**

pH: 6.9 ± 0.2 at 25 °C.

The plates are clear and red.

Experimental Procedure and Evaluation

Optimal yields of salmonellae are obtained if an enriched culture is first prepared in Tetrathionate Broth Base acc. to MULLER-KAUFFMANN (Merck Cat. No. 1.10863.), which should be incubated for 18-24 hours at 43°C. The material is then streaked onto the surface of BPLS agar modified in such a way that single, isolated colonies are formed.

Incubation: 24 hours at 35 °C, aerobically.

Suspected Salmonella colonies should be subjected to further tests.

Appearance of Colonies	Microorganisms
Red, surrounded by a bright red zone	Lactose- and sucrose-negative: Salmonella, Proteus (no swarming), Pseudomonas (small, crenate colonies) and others.
Yellow, surrounded by a yellow zone	Lactose- or sucrose-positive: E. Coli, Enterobacter, possibly Citrobacter, Klebsiella and others.

Literature

DIN Deutsches Institut für Normung e.V.: Untersuchung von Fleisch und Fleischerzeugnissen. Nachweis von Salmonellen. Referenzverfahren. - **DIN 10160**.

DIN Deutsches Institut für Normung e.V.: Mikrobiologische Milchuntersuchung. Nachweis von Salmonellen. Referenzverfahren. - **DIN 10181**.

ISO International Organization for Standardization: Meat and meat products. Detection of Salmonellae. Reference method. - **International Standard ISO 6579** (1993).

EDEL, W., a. KAMPELMACHER, E.H.: Salmonella isolation in nine European laboratories using a standardized technique. - **BULL. Wld. Hith. Org., 41**; 297-306 (1969).

KAUFFMANN, F.: Weitere Erfahrungen mit dem kombinierten Anreicherungsverfahren für Salmonellenbacillen. - **Z. Hyg. Infekt. Krhn., 117**; 26-32 (1935).

READ, R.B., a. REYES, A.L.: Variation in planting efficiency of Salmonellae in eight lots of Brilliant Green Agar. - **App. Microbiol., 16** ; 746-748 (1968).

VASSILIADIS, P., TRICHOFOULOS, D., PAPADAKIS, J. KALAPOTHAKI, V., a. SERIE, CH.: Brilliant green deoxycholate agar as an improved selective medium for the isolation of salmonella. - **Ann. soc. belge med. trop. 59**, 117-120 (1979).

Ordering Information

Product	Merck Cat. No.	Pack size
BPLS Agar, mod. (Brilliant-green Phenol-red Lactose Sucrose Agar, modified)	1.10747.0500	500 g

BPLS Agar, mod.
(Brilliant-green Phenol-red Lactose Sucrose Agar, modified)

Quality control (spiral plating method)

Test strains	Inoculum (cfu/ml)	Recovery rate (%)	Colony colour	Culture medium
Salmonella typhimurium ATCC 14028	10^3-10^5	≥ 40	red / pink	red
Salmonella choleraesius ATCC 13312	10^3-10^5	≥ 40	red / pink	red
Salmonella enteritidis NCTC 5188	10^3-10^5	≥ 40	red / pink	red
Escherichia coli ATCC 25922	10^3-10^5	Not limited	yellow	yellow
Proteus vulgaris ATCC 13315	10^3-10^5	Not limited	yellow	yellow
Staphylococcus aureus ATCC 25923	< 10^5	≤ 0.01		
Enterococcus faecalis ATCC 33186	< 10^5	≤ 0.01		
Bacillus subtilis ATCC 6633	< 10^5	≤ 0.01		

Salmonella choleraesuis ATCC 13312

Salmonella typhimurium ATCC 14028

BPLS Agar (USP)
(Brilliant-green Phenol-red Lactose Sucrose Agar)

Selective agar for the isolation of Salmonella with the exception of S. typhosa and Shigella from pathological materials, faeces, urine, foodstuffs, pharmaceutical materials, etc.

IVD *in vitro diagnosticum –*
For professional use only

See also General Instruction of Use
Warnings and precautions see ChemDAT®
(www.chemdat.info)

This medium complies with the recommendations of the United States Pharmacopeia XXVI (2003) and the European Pharmacopeia II.

Principle
Microbiological method

Mode of Action
This culture medium contains lactose, whose degradation to acid is indicated by the pH indicator phenol red, which changes its colour to yellow. The indicator exhibits a deep red colour in the alkaline range. The growth of the accompanying Gram-positive microbial flora, Salmonella typhi and Shigella is largely inhibited by brilliant green. The growth of Salmonella is, however, improved by the richer nutrient base. Increased growth of accompanying microorganisms is considerably prevented by raising the concentration of brilliant green. Salmonellae are not able to ferment either lactose or sucrose. Thus in contrast to BPL agar, the sucrose contained in this medium allows identification of accompanying, weakly lactose-positive or lactose-negative, but sucrose-positive microorganisms.

Typical Composition (g/litre)
Peptone from meat, peptic 5.0; peptone from casein 5.0; yeast extract 3.0; sodium chloride 5.0; lactose 10.0; sucrose 10.0; phenol red 0.08; brilliant green 0.0125; agar-agar 13.0.

Preparation and Storage
Usable up to the expiry date when stored dry and tightly closed at +15 to +25°C. Protect from light.

After first opening of the bottle the content can be used up to the expiry date when stored dry and tightly closed at +15 to +25°C.

Suspend 51 g/litre, autoclave (15 min at 121°C), pour plates.

pH: 6.9 ± 0.2 at 25 °C.

The plates are clear and red-brown.

Specimen
e.g. Stool, urine .

Clinical specimen collection, handling and processing, see general instructions of use.

Experimental Procedure and Evaluation
Inoculate the plates with the sample material itself or material taken from an enriched culture. Tests should also be performed with less inhibitory culture media.

Incubation: 24 hours at 35 °C aerobically.

Appearance of Colonies	Microorganisms
Pink surrounded by a red zone	Lactose- and sucrose-negative: Salmonella and others
Yellow-green surrounded by a yellow-green zone	Lactose- or sucrose-positive: E. coli, Citrobacter, Proteus vulgaris, Klebsiella and others. Occasionally complete inhibition of growth.

Literature

European Pharmacopeia II, Chapter VIII. 10.

United States Pharmacopeia XXVI, Chapter "Microbial Limit Tests", 1995.

Ordering Information

Product	Merck Cat. No.	Pack size
BPLS Agar (USP) (Brillant-green Phenol-red Lactose Sucrose Agar)	1.07232.0500	500 g
Merckoplate® BPLS Agar (USP)	1.00855.0020	1 x 20 plates

Quality control

Test strains	Recovery rate (%)	Colony colour	Medium colour
Salmonella typhimurium ATCC 14028	≥ 40	red	red
Salmonella choleraesius ATCC 13312	≥ 40	red	red
Salmonella enteritidis NCTC 5188	≥ 40	red	red
Escherichia coli ATCC 25922	not limited	yellow	yellow
Staphylococcus aureus ATCC 25923	≤ 0.01		
Enterococcus faecalis ATCC 33186	≤ 0.01		
Bacillus subtilis ATCC 6633	≤ 0.01		

Brain Heart Agar

For the cultivation of various fastidious pathogenic microorganisms.

These culture media comply with the recommendations given in the Standard Methods for the Examination of Water and Wastewater (1992). The broth satisfies the requirements of the DIN Norm 10163 for the examination of meat and LMBG (German Food and Consumer Goods Law) for the examination of foods.

Mode of Action

These culture media are based on the principle of the ROSENOW broth containing small pieces of brain tissue (ROSENOW 1919) and can be used to cultivate many fastidious bacteria such as streptococci, pneumococci, meningococci, etc. Addition of ascites permits the cultivation of gonococci.

Brain heart broth is especially suited for the cultivation of staphylococci for the plasma coagulase test and for setting up blood cultures. The growth of anaerobic or microaerophilic bacteria is considerably improved by adding small quantities of agar-agar (approx. 0.05-0.2 %) to the broth.

QUEIROZ et I. (1987) developed a selective agar for cultivating Campylobacter pylori on the basis of brain heart agar. It is called Belo Horizonte Medium /BHM).

Brain heart agar is suited for the cultivation not only of bacteria but also of pathogenic fungi. Growth of the accompanying bacterial flora can be almost completely suppressed by adding 20I.U. penicillin and 40 µg streptomycin per ml of culture medium. If this medium is to be used for the selective isolation of fastidious fungi (especially of Histoplasma capsulatum and Blastomyces), from mixinfected samples, 0.05 µg cycloheximide/ml and 0.5 µg chloramphenicol/ml should be added.

This medium is less suited for identifying hemolytic forms when blood has been added due to its glucose content.

Typical Composition (g/litre)

Nutrient substrate (brain extract, heart extract and peptones) 27.5; D(+)glucose 2.0; sodium chloride 5.0; di-sodium hydrogen phosphate 2.5; agar-agar 15.0.

Preparation

Suspend 52 g Brain Heart Agar/litre, autoclave (15 min at 121°C).

pH: 7.4 ± 0.2 at 25 °C.

The broth is clear and brown, the agar is clear sometimes slightly opalescent and brown.

Experimental Procedure and Evaluation

Depend on the purpose for which the media are used.

Literature

American Public Health Association, American Water Works Association and Water Pollution Control Federation: Standard Methods for the Examination of Water and Wastewater, 20th ed., Washington, 1998.

Bundesgesundheitsamt: Amtliche Sammlung von Untersuchungsverfahren nach § 35 LMBG. Beuth Verlag Berlin, Köln.

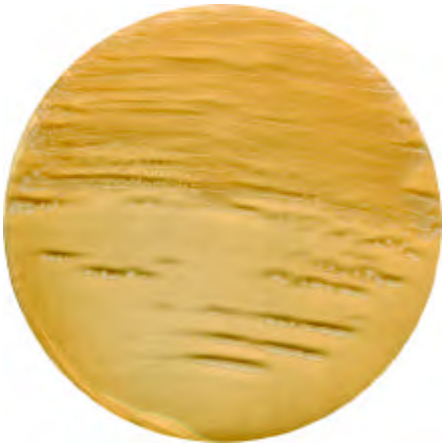
DIN Deutsches Institut für Normung e.V.: Mikrobiologische Untersuchung von Fleisch und Fleischerzeugnissen. Bestimmung Koagulase-positiver Staphylokokken. Reeferenzverfahren - **DIN 10163**.

QUEIROZ, D.M.M., MENDES, E.N., a. ROCHA, G.A.: Indicator medium for isolation of Campylobacter pylori. - **J. Clin. Microbiol., 25**; 2378-2379 (1987).

ROSENOW, E.C.: Studies on elective localization. Focal infection with special reference to Oral sepsis. - **Journ. Dental Res., 1**; 205-249 (1919).

Ordering Information

Product	Merck Cat. No.	Pack size
Brain Heart Agar	1.13825.0500	500 g



Staphylococcus aureus ATCC 25923

Quality control

Test strains	Growth
Streptococcus pyogenes ATCC 12344	good / very good
Streptococcus pneumoniae ATCC 6301	good / very good
Staphylococcus aureus ATCC 25923	good / very good
Erysipelothrix rhusiopathiae ATCC 19414	good / very good
Lactobacillus acidophilus ATCC 4356	fair / good
Clostridium perfringens ATCC 10543	good / very good (anaerobically)
Clostridium sporogenes ATCC 11437	good / very good (anaerobically)

Brain Heart Broth

For the cultivation of various fastidious pathogenic microorganisms.

These culture media comply with the recommendations given in the Standard Methods for the Examination of Water and Wastewater (1992). The broth satisfies the requirements of the DIN Norm 10163 for the examination of meat and LMBG (German Food and Consumer Goods Law) for the examination of foods.

Mode of Action

These culture media are based on the principle of the ROSENOW broth containing small pieces of brain tissue (ROSENOW 1919) and can be used to cultivate many fastidious bacteria such as streptococci, pneumococci, meningococci, etc. Addition of ascites permits the cultivation of gonococci.

Brain heart broth is especially suited for the cultivation of staphylococci for the plasma coagulase test and for setting up blood cultures. The growth of anaerobic or microaerophilic bacteria is considerably improved by adding small quantities of agar-agar (approx. 0.05-0.2 %) to the broth.

QUEIROZ et I. (1987) developed a selective agar for cultivating Campylobacter pylori on the basis of brain heart agar. It is called Belo Horizonte Medium /BHM).

Typical Composition (g/litre)

Nutrient substrate (brain extract, heart extract and peptones) 27.5; D(+)glucose 2.0; sodium chloride 5.0; di-sodium hydrogen phosphate 2.5.

Preparation

Suspend 37 g Brain Heart Broth/litre, autoclave (15 min at 121 °C).

pH: 7.4 ± 0.2 at 25°C.

The broth is clear and brown.

Experimental Procedure and Evaluation

Depend on the purpose for which the media are used.

Literature

American Public Health Association, American Water Works Association and Water Pollution Control Federation: Standard Methods for the Examination of Water and Wastewater, 20th ed., Washington, 1998.

Bundesgesundheitsamt: Amtliche Sammlung von Untersuchungsverfahren nach § 35 LMBG. Beuth Verlag Berlin, Köln.

DIN Deutsches Institut für Normung e.V.: Mikrobiologische Untersuchung von Fleisch und Fleischerzeugnissen. Bestimmung Koagulase-positiver Staphylokokken. Reeferenzverfahren - **DIN 10163**.

QUEIROZ, D.M.M., MENDES, E.N., a. ROCHA, G.A.: Indicator medium for isolation of Campylobacter pylori. - J**. Clin. Microbiol., 25**; 2378-2379 (1987).

ROSENOW, E.C.: Studies on elective localization. Focal infection with special reference to Oral sepsis. - **Journ. Dental Res., 1**; 205-249 (1919).

Ordering Information

Product	Merck Cat. No.	Pack size
Brain Heart Broth	1.10493.0500	500 g

Quality control

Test strains	Incubation	Conditions	Growth
Streptococcus pyogenes ATCC 19615	24 h/35 °C	aerobic / anaerobic	good / very good
Streptococcus pneumoniae ATCC 6305	24 h/35 °C	aerobic / anaerobic	good / very good
Pseudomonas aeruginosa ATCC 27853	24 h/35 °C	aerobic	good / very good
Candida albicans ATCC 60193	48 h/35 °C	aerobic	good / very good
Bacteroides fragilis ATCC 25285	2-5 d/35 °C	anaerobic	good / very good
Haemophilus influenzae ATCC 10211	2-5 d/35 °C	microaerophilic	good / very good
Staphylococcus aureus ATCC 25923	24 h / 35 °C	aerobic	good / very good

Brillant-green 2 %-Bile Broth

BRILA-Broth

For the selective enrichment and enumeration of Escherichia coli and other faecal coliform organisms in water, milk, foodstuffs and other materials by determining the coli titre or by the MPN method.

This culture medium complies with the recommendations of the International Dairy Federation (Internationaler Milchwirtschaftsverband) (FIL-IDF) (1985), the Standard Methods for Examination of Water and Wastewater (1998), the International Organization for Standardization (ISO) (1979) and the DIN Norm 10172.

Mode of Action

Bile and brilliant green almost completely inhibit the growth of the undesired microbial flora including lactose-degrading clostridia (e.g. Cl. perfringens) (MACKENZIE et al. 1948). The fermentation of lactose with gas formation indicates the presence of E. coli and other faecal coliform organisms and is established by using DURHAM tubes. Other non-faecal coliform bacterial also grow in this medium but mostly do not produce any gas.

Typical Composition (g/litre)

Peptone 10.0; lactose 10.0; ox bile, dried 20.0; brilliant green 0.0133.

Preparation

Suspend 40 g/litre, fill into test tubes fitted with DURHAM tubes, autoclave (15 min at 121°C).

pH: 7.2 ± 0.2 at 25 °C.

The prepared broth is clear and green.

Experimental Procedure and Evaluation

Inoculate the tubes.

Incubation: 24-48 hours at 35 °C or at the specified temperature (aerobically).

The E. coli titre refers to the smallest volume of sample material in which gas formation can be detected. Differentiation of the culture should also be performed to confirm the results obtained.

Literature

American Public Health Association, American Water Works Association and Water Pollution Control Federation: Standard Methods for the Examination of Water and Wastewater, 18th ed. Washington, 1992.

DIN Deutsches Institut für Normung e.V.: Mikrobiologische Milchuntersuchung. Bestimmung der coliformen Keime. Referenzverfahren. - **DIN 10172**.

Internationaler Milchwirtschaftsverband: Zählung coliformer Bakterien in Milch und Milchprodukten. **Internationaler Standard FIL-IDF, 73** (1985).

International Organization for Standardization: Meat and meat products - Detection and enumeration of presumptive coliform bacteria and presumptive Escherichia coli (Reference method). - **International Standard ISO/DIS 3811** (1979).

MACKENZIE, E.F.W., TAYLOR, W.E., a. GILBERT, W.E.: Recent experiments in the rapid identification of Bacterium coli type I. - J**. Gen. Microbiol., 2**; 197-204 (1948).

Ordering Information

Product	Merck Cat. No.	Pack size
Brillant-green 2 %-Bile Broth	1.05454.0500	500 g

Quality control

Test strains	Incubation Temperature	Growth	Gas
Escherichia coli ATCC 25922	35 °C	+	+
Escherichia coli ATCC 25922	44 °C	+	+
Escherichia coli ATCC 11775	35 °C	+	+
Escherichia coli ATCC 11775	44 °C	+	+
Citrobacter freundii ATCC 8090	35 °C	+	+
Citrobacter freundii ATCC 8090	44 °C	poor	none / poor
Staphylococcus aureus ATCC 6538-P		inhibited	-
Micrococcus luteus ATCC 10240		inhibited	-
Bacillus cereus ATCC 11778		inhibited	-
Lactobacillus plantarum ATCC 8014		inhibited	-

BROLAC Agar (Bromothymol-blue Lactose Agar)

Elective culture medium, free from inhibitors, for separating lactose-positive from lactose-negative colonies, used especially for Enterobacteriaceae.

Mode of Action

BROLAC agar contains lactose which, when degraded to acid, causes the pH indicator bromothymol blue to change its colour to yellow. Alkalinization produces a blue colouration.

Typical Composition (g/litre)

Peptone from meat 3.5; peptone from casein 3.5; sodium chloride 5.0; lactose 15.5; bromothymol blue 0.04; agar-agar 13.0.

Preparation

Suspend 40.5 g/litre, autoclave (15 min at 121 °C), pour plates.

pH: 7.0 ± 0.2 at 25 °C.

The plates are clear and green to green-blue.

Experimental Procedure and Evaluation

Inoculate by thinly speading the sample material on the surface of the plates.

Incubation: 24 hours at optimum temperature, usually 35°C aerobically.

Appearance of Colonies	Microorganisms
Green to blue, sometimes surrounded by a blue zone	Lactose-negative: Salmonella, Shigella, Serratia, Proteus, Providencia and others.
Golden yellow, surrounded by a yellow zone	Lactose-positive: Escherichia, Coliform bacteria and others.

Ordering Information

Product	Merck Cat. No.	Pack size
BROLAC Agar (Bromothymol-blue Lactose Agar)	1.01639.0500	500 g

Quality control

Test strains	Growth	Colour change to yellow
Escherichia coli ATCC 25922	good / very good	+
Klebsiella pneumoniae ATCC 13883	good / very good	+
Salmonella typhimurium ATCC 14028	good / very good	-
Proteus vulgaris ATCC 13315	good / very good	-
Staphylococcus aureus ATCC 25923	fair / very good	+ / -
Enterococcus faecalis ATCC 33186	fair / very good	+

BROLACIN Agar (Bromothymol-blue Lactose Cystine Agar)

(C.L.E.D. Agar)

For the enumeration, isolation and preliminary identification of microorganisms in urine.

IVD *in vitro diagnosticum –*
For professional use only

See also General Instruction of Use
Warnings and precautions see ChemDAT®
(www.chemdat.info)

Diagnosis of asymptomatic urinary tract infections depends on the detection of a significant bacteriuria, which is defined at the presence of at least 100,000 bacteria in 1 ml of morning urine.

Principle

Microbiological method

Mode of Action

This culture medium promotes the growth of all microorganisms found in urine. It is also an excellent universal culture medium owing to its wide spectrum of nutrients, lack of inhibitors and the fact that it allows a certain degree or differentiation between the colonies. It contains lactose as a reactive compound which, when degrated to acid, causes bromothymol blue to change its colour to yellow. Alkalinization produces a deep blue colouration. The lack of electrolytes suppresses the swarming of Proteus (SANDYS 1960).

Typical Composition (g/litre)

Peptones 7.0; yeast extract 2.0; meat extract 2.0; L-cystine 0.128; lactose 10.0; bromothymol blue 0.03; agar-agar 12.0.

Preparation and Storage

Usable up to the expiry date when stored dry and tightly closed at +15 to +25°C. Protect from light.

After first opening of the bottle the content can be used up to the expiry date when stored dry and tightly closed at +15 to +25°C.

Suspend 33 g/litre, autoclave (15 min at 121°C), pour plates.

pH: 7.3 ± 0.2 at 25 °C.

The plates are clear and bluish green.

Specimen

e.g. Urine.

Clinical specimen collection, handling and processing, see general instructions of use.Experimental Procedure and Evaluation

Inoculate by spreading a defined quantity (up to 1 ml) of the urine sample (dilute if necessary) or material to be tested on the surface of the plate. Incubation: 24 hours at 35 °C aerobically.

Appearance of Colonies	Microorganisms
Large, golden yellow, surrounding medium is yellow	Escherichia coli, lactose-positive Citrobacter and others
Large, golden yellow, usually mucoid, surrounding medium is yellow	Enterobacter, Klebsiella and others
Large, colouless, surrounding medium is blue	Proteus, Serratia and others
Large, brownish centre, surrounding medium is blue	Pseudomonas
Pale yellow, small, opaque	Streptococci
Deep yellow, very small, opaque	Staphylococci

Literature

SANDYS, G.H.: A new method of preventing swarming of Proteus sp. with a description of a new medium suitable for use in routine laboratory practice. - **J. Med. Lab. Technol., 17**; 224-233 (1960)

Ordering Information

Product	Merck Cat. No.	Pack size
BROLACIN Agar (Bromothymol-blue Lactose Cystine Agar)	1.10638.0500	500 g
Merckoplate® Brolacin Agar (C.L.E.D. Agar)	1.10411.0001	1 x 20 plates

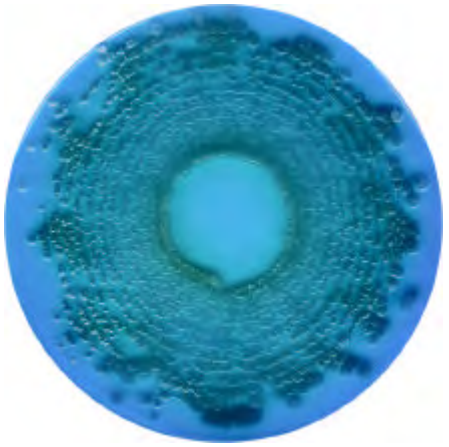
BROLACIN Agar (Bromothymol-blue Lactose Cystine Agar)

(C.L.E.D. Agar)

Quality control (spiral plating method)

Test strains	Inoculumn (cfu/ml)	Recovery (rate (%)	Colour change	Swarming
Escherichia coli ATCC 11775	10^3-10^5	≥ 70	yellow	
Salmonella typhimurium ATCC 13311	10^3-10^5	≥ 70	blue	
Shigella flexneri ATCC 29903	10^3-10^5	≥ 70	blue	
Proteus mirabilis ATCC 29906	10^3-10^5	≥ 70	blue	none/moderate
Proteus vulgaris ATCC 8427	10^3-10^5	≥ 70	blue	none / poor
Pseudomonas aeruginosa ATCC 27853	10^3-10^5	≥ 70	blue	
Staphylococcus aureus ATCC 6538	10^3-10^5	≥ 70	yellow	

Escherichia coli ATCC 11775



Pseudomonas aeruginosa ATCC 27853

Bromocresol-purple Azide Broth

For confirming the presence of enterococci, particularly in the bacteriological analysis of water according to HAJNA and PERRY (1943 an HAJNA (1951).

This broth should be employed after performing a preliminary test with Azide Dextrose Broth.

Mode of Action

Sodium azide inhibits the entire accompanying bacterial flora including those species which may have grown in the preliminary test. Enterococci ferment the glucose present in the medium to give acid which is detected by the pH indicator bromocresol purple - the acid causes the indicator to change its colour to yellow. According to HAJNA (1951), enterococcal glucose fermentation is improved by the addition of glycerol.

Typical Composition (g/litre)

Peptone from casein 10.0; yeast extract 10.0; D(+)glucose 5.0; sodium chloride 5.0; di-potassium hydrogen phosphate 2.7; potassium dihydrogen phosphate 2.7; sodium azide 0.5, bromocresol purple 0.032.

Preparation

Suspend 36 g/litre, adding 5 ml/litre glycerol if desired, dispense into test tubes, autoclave under mild conditions (15 min at 115°C).

pH: 7.0 ± 0.2 at 25 °C.

The prepared broth is clear and violet.

Experimental Procedure and Evaluation

Inoculate the culture medium massively on account of the high degree of inhibition.

Incubation: up to 48 hours at 35 °C aerobically.

Colony growth with turbidity and a change in colour to yellow: Enterococci.

Literature

HAJNA, A.A.: A buffered azide glucose-glycerol broth for presumptive and confirmative tests for fecal Streptococci. - **Publ. Health Lab., 9**; 80-81 (1951).

HAJNA, A.A., a. PERRY, C.A.: Comparative Study of Presumptive and Confirmative Media for Bacteria of the Coliform Group and for Fecal Streptococci. - **Am. J. Publ. Health, 33**; 550-556 (1943).

Ordering Information

Prodcut	Merck Cat. No.	Pack size
Bromocresol-purple Azide Broth	1.03032.0500	500 g
Azide Dextrose Broth	1.01590.0500	500 g
Glycerol (about 87 %)	1.04094.0500	500 ml

Quality control

Test strains	Growth	Colour change to yellow
Streptococcus agalactiae ATCC 13813	none / poor	-
Enterococcus faecalis ATCC 11700	good / very good	+
Enterococcus faecalis ATCC 19433	good / very good	+
Enterococcus hirae ATCC 8043	good / very good	+
Streptococcus bovis DSMZ 20065	good / very good	+ (poor)
Staphylococcus aureus ATCC 25923	none	
Escherichia coli ATCC 25922	none	
Pseudomonas aeruginosa ATCC 27853	none	-

Brucella Agar

Modified medium according to WUNDT (1957) for the isolation and cultivation of Brucella (especially for the pathogenic strains Bruc. melitensis, Bruc. abortus and Bruc. suis) from clinical specimens and foodstuffs of animal origin.

This culture medium can be utilized as it is or as a base for the preparation of special culture media. It complies with the recommendations of WHO (1953) and HAUSLER and KOONITZ in Diagnostic Procedures (1970).

Mode of Action

KUZDAS and MORSE (1953), RENOUX (1954) and WEED (1957) demonstrated that, in the case of heavily contaminated sample material, the growth of accompanying microbial flora can be suppressed by addition of bacitracin, polymyxin, cycloheximide and possibly ethyl violet. Circulin, which also has been recommended originally, is no longer used (ALTON and JONES 1967).

The various Brucella species can be differentiated by exploiting the fact that they show different sensitivities towards the dyes thionine and fuchsin. Differential culture media can be prepared by adding these two compounds to Brucella agar.

Typical Composition (g/litre)

Peptone from meat 10.0; peptone from casein 10.0; yeast extract 2.0; D(+)glucose 1.0; sodium chloride 5.0; agar-agar 13.0.

Preparation

Suspend 41 g/litre, autoclave (15 min at 121°C), pour plates.

pH: 7.0 ± 0.2 at 25 °C.

The plates are clear and yellowish-brown.

Preparation of Brucella differential agar: Sterilize Brucella agar, cool, adjust pH to 6.7 ± 0.1. To 1 litre add 1 ml (1:100,000), 2 ml (= 1:50,000) or 4 (=1:25,000) of an aqueous 1 % thionine or basic fuchsin solution, mix. The solutions should first be heated for 20minutes in a boiling water bath.

Preparation of Brucella selective agar: Sterilize the Brucella agar, cool to 45-50 °C, add filter-sterilized solutions of the following compounds:

Bacitracin	25.000 IU/litre
Polymyxin B sulfate	6.000 IU/litre
Cycloheximide	100 mg/litre
and if required ethyl violet	1.25 mg/litre

Experimental Procedure and Evaluation

Spread the sample material or material from an enriched culture e.g. in Tryptose Broth thinly over the surface of the Brucella agar. If the specimen is heavily contiminated with other bacteria, inoculate selective Brucella agar, too.

Incubation: For the primary culture, incubate in a 10 % carbon dioxide atmosphere for 4-5 days at 35 °C until growth can be seen. If there is no growth, renew the carbon dioxide atmosphere and incubate for up to 21 days.

Prepare subcultures on Brucella agar from individual colonies and incubate as directed above.

Brucella colonies have a diameter of 2-7 mm, are spheroid in shape, pale amber in colour, moist, slightly opalescent and translucent. These characteristics may vary due to changes in pH or moisture content. Examination of gram-stained smears under the microscope shows the presence of short, rod-shaped bacteria.

Further tests should be performed to differentiate between the Brucella species (WUNDT 1958, CRUICKSHANK 1948, FAO/WHO 1964, JONES and WUNDT 1971).

Literature

ALTON, G.G., a. JONES, L.M.: Laboratory techniques in Brucellosis (WHO, Geneva, 1967)

CRUICKSHANK, J.C.: A Simple Method for Testing Dye Sensitivity of Brucella Species. - **J. Path. Bact., 60**; 328-329 (1948).

CARRERE, L. RENOUX, G., et QUATREFAGES, H.: Apropos de l'action de certaines peptones (Tryptose sur les Brucella). - **Ann. Inst. Pasteur, 80**. 321-322 (1951)

FAO/WHO: 1. Techn. Rep. Expert Panel on Brucellosis (WHO Techn. Rep. Ser. 37, Genf (1951)

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HAUSLER, W.J., a. KOONITZ, F.P.: Brucellosis (in: Diagnostic Procedures for Bacterial Mycotic and Parasitic Infections; 5th ed.; APHA, New York 1970).

HUDDLESON, I.F.: Brucellosis in man and animals (Commonwealth Fund, New York, 1943).

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KUZDAS, C.D., a. MORSE, E.V.: A Selective Medium for the Isolation of Brucellae from Contaminated Materials. - **J. Bact., 66**; 502-504 (1953).

RENOUX, G.: Sur un milieu sélectif pour l'isolement de Brucella melitensis. - **Ann. Inst. Pasteur, 87**; 325-333 (1954).

SCHUCHARDT, K.T., RODE, L.J., OGLESBY, G., a. LANKFORD, C.E.: The development of peptone toxicity for Brucellae with aging and the correlation of this toxicity with the probable oxidation of cystine. - **J. Bact., 60**; 655-660 (1950).

SPINK, W.W.: The Nature of Brucellosis. (Univ. Minnesota Press., Minneapolis, USA, 1956).

WEED, L.A.: The use of a selective medium for isolation of Brucella from contaminated surgical specimens. - **Amer. J. Clin. Path., 27**; 482-485 (1957).

WUNDT, W.: Untersuchungen zur Entwicklung leistungsfähiger Brucellennährböden. - **Zbl. Bakt. I. Orig., 169**; 393-402 (1957)

WUNDT, W.: Untersuchungen über die Eignung von Peptonen zur Prüfung der Schwefelwasserstoffbildung von Brucellen. - **Zeitschr. f. Hyg., 144**; 425-435 (1958). BAIRD-PARKER, A.C.: An improved diagnostic and selective medium for isolating coagulase positive Staphylococci. - **J. Appl. Bact., 25**; 12-19 (1962).

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DIN Deutsches Institut für Normung e.V.: Nachweis Koagulase-positiver Staphylokokken. Referenzverfahren für Milchpulver. - **DIN 10163**.

European Pharmacopeia II, Chapter VII, 10.

Internationaler Milchwirtschaftsverband; Nachweis Koagulase-positiver Staphylokokken in Milchpulver (Referenzmethode). - **Internationaler Standard 60 A** (1978).

Brucella Agar

ISO International Organization for Standardization: Dried milk-Enumeration of Staphylococcus aureus. Colony count technique. - Draft Proposal ISO/PP **8869** (1984).

ISO International Organization for Standardization: Meat and meat products - Detection and enumeration of Staphylococcus aureus (Reference methods). - **Draft International Standard ISO/DIS 5551** (1977).

NISKANEN, A., a. AALTO, M.: Comparison of selective media for coagulase-positive enterotoxigenic Staphylococcus aureus. - **Appl Envir. Microbiol., 35**; 1233-1236 (1978)

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STADHOUDERS, J., HASSINGS, F., a. VAN AALSTEN-VAN MAREN, N.O.: A pour-plate method for the detection and enumeration of coagulase-positive Staphylococcus aureus in the BAIRD-PARKER Medium without egg-yolk. - **Netz. Milk Diary J., 30**; 222-229 (1976)

United States Pharmacopeia XXIII, Chapter "Microbial limit Tests", 1995.

Ordering Information

Product	Merck Cat. No.	Pack size
Brucella Agar	1.10490.0500	500 g
Anaeroclip®	1.14226.0001	1 x 25
Anaerocult® c	1.16275.0001	1 x 10
Anaerocult® c mini	1.13682.0001	1 x 25
Thionine (acetate) Certistain®	1.15929.0025	25 g
Tryptose Broth	1.10676.0500	500 g
Bacitracin	CN Biosciences	
Polymyxin-B-sulfate	CN Biosciences	

Manufacturer	Product
Matheson, Colman a. Bell, Norwood (Cincinnati) Ohio, USA	Ethyl violet

Quality control

Test strains	Growth
Brucella abortus	good
Brucella melitensis	good
Brucella suis	good
Escherichia coli ATCC 25922	good
Listeria monocytogenes ATCC 19118	good

Bryant Burkey Broth with Resazurine and Lactate

Medium for the selective enrichment of lactate fermenting Clostridia spp. (Cl. tryobutyricum) which are responsible for "late blowing" in brine salted semi-hard cheese

The medium is used to enumerate the spores of lactic acid fermenting Clostridia spores in silage, milk and dairy products. During milking process low numbers of butyric acid fermenting bacteria (BAB) originating from silage are introduced into the raw milk. When the contaminated milk is used for cheese production, cheese brines become contaminated with heat resistant Clostridia spores. During the ripening of salt brined, semi- and hard cheeses (for example, Gouda, Edammer, Emmentaler, Gruyere, and Parmesan) "late blowing gasogenic Clostridia ferment lactate into butyric acid, acetic acid and gas (CO2 and H2). The gas swells the cheese and is responsible for a defect termed "late blowing" or butyric swelling. The blown up cheese has moreover a bad taste. The main species causing this butyric swelling defect is Co. tyrobutyricum. Other Clostridia belonging to the butyric acid fermenting bacteria (BAB) are Cl. butyricum or Cl. sporogenes. The causative Clostridia spp. are anaerobic Gram-positive microorganisms forming heat resistant endospores, which survive pasteurisation but not UHT or sterilisation of milk.

Mode of Action

Vegetative cells are killed by a heat treatment (75 °C for 10 min.). Resazurin is a redox indicator and monitors the oxygen level. The nutrient composition of the basal medium, particularly the high quality of the peptones creates the conditions for a rapid growth of lactate fermenting Clostridia spp. Sodium acetate promotes the spore germination, which is activated by the heat treatment of the sample. Lactateis the substrate for the Clostridia spp. producing gas. A strong gas production is visualised by the raising of the paraffin plug.

Typical Composition (g/litre)

Peptone from casein 15.0; yeast extract 5.0; meat extract 7.5; sodium acetate 5.0; cysteine HCl 0.5; resazurin 0.0025; calcium lactate 5.0.

Preparation

Dissolve 38 g in 1000 ml of demin. water

Autoclave (15 min. at 121°C).

Cool to 45-50 °C and dispense in bottles or tubes.

pH: 5.9 ± 0.1 at 25 °C.

The prepared non-boiled broth is pink and the boiled broth is colourless. A pink colour indicates the presence of oxygen.

Expermimental Procedure and Evaluation

The MPN method is employed in the examination on lactate fermenting Clostridia spp. Tubes of medium are boiled where appropriate (100°C for 10 min.) to regenerate anaerobiosis and cooled down to 25-30 °C. Colourless tubes are inoculated with sample or sample dilutions and overlaid with 2cm of sterile (121°C for 20 min.) melted (58-60°C) paraffin. The tubes are heat treated (75°C for 10 min.) to kill vegetative microorganisms and cooled down to 37°C to solidify the paraffin.

The inoculated medium is incubated at 37 °C for up to 7 days. The tubes are evaluated every 48 h. Tubes with growth and gas formation indicated by a raised paraffin plug are considered positive. The MPN index is used to calculate the number of Clostridia.

Further biochemical identification verfies the presence of Cl. tyrobutyricum.

Literature

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Bryant, M.P. & Burkey, L.A.: 1953 Cultural methods and some characteristics of some more numerous gropus of bacteria with bovine rumen. J Dairy Science **36**, 205-217.

Cerf, O. & Bergere, J.L. 1968 La numeration des spores de Clostridium et son application au lait et aux produits laiters. Numeration des differents groups de Clostridium. Le Lait **48**, 501-509.

Franknet, J & de Carheil, M. 1983 Les tests de controle des germes butyriques. La technique laiterie **977**, 15-28.

Ordering Information

Product	Merck Cat. No.	Pack size
Bryant Burkey Broth with Resazurine and Lactate	1.01617.0500	500 g
Paraffin	1.07158.1000	1 kg

good growth gasformation (+)

no growth

Bryant Burkey Broth with Resazurine and Lactate

Quality control

Test strains	Growth	Gas Formation
Clostridium tyrobutyricum W 7	good / very good	+
Clostridium tyrobutyricum DSMZ 663	good / very good	+
Clostridium perfringens ATCC 10543	good / very good	+
Escherichia coli ATCC 25922	good / very good	- (poor)
Staphylococcus aureus ATCC 25923	good / very good	-
Pseudomonas aeruginosa ATCC 27853	none	-

Buffered Listeria Enrichment Broth Base acc. to FDA/BAM 1995

For the selective enrichment of Listeria spp.

This medium complies with the modifications made by FDA/ BAM (1995).

Mode of Action

The enrichment broth is a modification of the formulation of Tryptic Soy Broth (CASO) with the addition of 6 g/l yeast extract and by increasing its buffering strength. Dextrose is the carbohydrate source. Sodium chloride maintains the osmotic balance of the medium. Phosphate acts as a buffer. Sodium pyruvate mediates the recovery of sublethally damaged Listeria spp.

The addition of acriflavine, cycloheximide and nalidixic acid inhibits the growth of the accompanying flora.

Typical Composition (g/litre)

Tryptic Soy Broth 30 g; yeast extract 6.0; di-sodium hydrogen phosphate 9.6; potassium dihydrogen phosphate 1.35; sodium pyruvate 1.1.

Preparation

Suspend 24 g in 500 ml of demin. water, dissolve and dispense 225 ml aliquots. Autoclave (15 min at 121 °C).

pH: 7.3 ± 0.2 at 25 °C.

The prepared broth is clear.

Experimental Procedure and Evaluation

Inoculate Listeria Enrichment broth (usually add 25 g of a representative sample to 225 ml broth) and homogenize.

For the recovery of sublethally injured Listeria spp. incubate at 30°C for 4 h.

Thereafter, the appropriate aliquot of supplement (0.5 ml from a vial of Listeria enrichment supplement reconstituted by adding of 1 ml sterile demin. water) is added. The sample is thoroughly mixed and incubation is continued for another 44 h at 30°C.

After 24 and 48 h of incubation streak a loopful of incubated broth onto both Oxford and Palcam agar (alternatively LPM agar). Incubate Oxford and Palcam agar at 35 °C, LPM agar at 30°C for 24-48 h.

Literature

LOVETT, J., FRANCES, D.W., a. HUNT, J.M.: Listeria in raw milk, detection, incidence and pathogenecity. - **Journal of Food Protection, 50**; 188-192 (1987).

International Diary Federation: Milk and milk products-detection of Listeria monocytogenes. - IDF Provesional International Standard No. 143 International Diary Federation, Brussels (1990).

HITCHINS, A.D.: Listeria monocytogenes - In FDA-Bacteriological Analytical Manual 8th EDITION, AOAC International Arlington V.A. (1995).

SWAMINATHAN, B., ROCOURT, J., a. BILLE, J.: Listeria - In MURRAY, P.R., BARRON, E.J., PFALLER, M.A., TANOVER, F.C. a. YOLKEN, R.H. (Eds.) - Manual of Clinical microbiology, 6th ed. American Society of Microbiology, Washington D.C., 342-343 (1995).

FLOWERS, R.S., ANDREWS, W., DONNELLY, C.W., a. KOENING, E.: Pathogens in milk and milk products. - In MARSHAL, R.T. (ed.) Standard methods for the examination of dairy products, 16th ed. American Public Health Association, Washington D.C. (1993).

Ordering Information

Product	Merck Cat. No.	Pack size
Buffered Listeria Enrichment Broth (Base) acc. to FDA/BAM 1995	1.09628.0500	500 g
Listeria Selective Enrichment Supplement acc. to FDA-BAM 1995/ IDF-FIL	1.11781.0001	1 x 16 vials
OXFORD Listeria Selective Agar (Base)	1.07004.0500	500 g
OXFORD Listeria Selective Supplement	1.07006.0001	1 x 13 vials
PALCAM Listeria Selective Agar (Base)	1.11755.0500	500 g
PALCAM Listeria Selective Supplement acc. to VAN NETTEN et al.	1.12122.0001	1 x 16 vials

Quality control

Test strains	Growth
Listeria monocytogenes ATCC 19114	good
Listeria monocytogenes ATCC 19116	good
Listeria innocua ATCC 33090	good
Staphylococcus aureus ATCC 25923	good
Enterococccus faecalis ATC 19433	good
Escherichia coli ATCC 25922	none

Buffered Peptone Water (BPW)

For the preliminary, non-selective enrichment of bacteria, particularly pathogenic Enterobacteriaceae, from foodstuffs and other materials.

This culture media complies with the recommendations of the International Standard Organisation ISO (ISO 6579-2002). Horizontal method for the detection of salmonella spp.

Mode of Action

The broth is rich in nutrients and produces high resuscitation rates for subletally injured bacteria and intense growth. The phosphate buffer system prevents bacterial damage due to changes in the pH of the medium.

Typical Composition (g/litre)

Peptone from casein 10.0; sodium chloride 5.0; disodium hydrogen phosphate dodecahydrate 9.0; potassium dihydrogen phosphate 1.5.

Preparation

Suspend 25.5 g/l, if desired, dispense into suitable containers, autoclave (15 min at 121 °C).

pH: 7.0 ± 0.2 at 25 °C.

The prepared broth is clear and yellowish.

Experimental Procedure and Evaluation

Inoculate the culture medium with the sample material.

Incubation: 16 - 20 hours at 37°C aerobically.

Transfer material from the resulting culture to a selective enrichment culture medium recommended by the appropriate standard.

Literature

DIN Deutsches Institut für Normung e.V.: Mikrobiologische Milchuntersuchung. Nachweis von Salmonellen. Referenzverfahren. – **DIN 10181**.

DIN Deutsches Institut für Normung e.V.: Untersuchung von Fleisch und Fleischerzeugnissen. Nachweis von Salmonellen. Referenzverfahren. -DIN10160.

International Standard Organisation: Detection of salmonellae (Reference method). International Standard ISO 6579 (2002).

International Standard Organisation: Milk and Milk Products -Detection of Salmonella spp. ISO 6785 / IDF 93 (2001)

Ordering Information

Product	Merck Cat. No.	Pack size
Buffered Peptone Water (BPW)	1.07228.0500	500 g
Buffered Peptone Water (BPW)	1.07228.5000	5 kg

Quality control

Test strains	Growth
Salmonella typhimurium ATCC 14028	good / very good
Escherichia coli ATCC 25922	good / very good
Enterococcus faecalis ATCC 33186	good / very good
Pseudomonas aeruginosa ATCC 27853	good / very good
Salmonella enteritidis ATCC 13076	good / very good

Calcium Caseinate Agar acc. to FRAZIER and RUPP, modified

A modification of the selective agar proposed by FRAZIER and RUPP (1928) for the detection and enumeration of proteolytic microorganisms (proteolytes) in foodstuffs and other materials.

Mode of Action

This medium contains casein which is degraded by the proteolytes to form clearer zones surrounding the colonies in the otherwise turbid medium.

Typical Composition (g/litre)

Peptone from meat 4.0; meat extract 2.0; peptone from casein 2.0; calcium caseinate 3.5; calcium chloride dihydrate 0.2; tri-potassium citrate monohydrate 0.35; di-sodium hydrogen phosphate anhydrous 0.105; potassium dihydrogen phosphate 0.035; sodium chloride 5.0; agar-agar 13.0.

Preparation

Suspend 30.2 g/litre completely (if necessary use a mixer), place in a cold water bath and while frequently shaking heat slowly until the suspension boils, boil for about 10 minutes, autoclave (15 min at 121 °C). Mix thoroughly while pouring to suspend the precipitate. 5-10 g skim milk powder/litre can be added before heating to increase turbidity.

pH: 7.0 ± 0.2 at 25 °C.

The plates are turbid and yellowish-brown.

Experimental Procedure and Evaluation

Inoculate by the pour-plate method or by spreading the sample on the surface of the medium.

Incubation: 2-3 days at 35 °C aerobically.

Count the proteolyte colonies (surrounded by clear zones). The plates can be flooded with 5 to 10 % acetic acid to facilitate recognition of the zones.

Literature

FRAZIER, W.C., a. RUPP, P.: Studies on the proteolytic bacteria of milk. I. A medium for the direct isolation of caseolytic milk bacteria. - **J. Bact. 16**; 57-63 (1928).

Ordering Information

Product	Merck Cat. No.	Pack size
Calcium Caseinate Agar acc. to FRAZIER and RUPP, modified	1.05409.0500	500 g
Acetic acid min. 96 %	1.00062.1000	1 l
Skim milk powder	1.15363.0500	500 g

Quality control

Test strains	Growth	Clear zone
Bacillus cereus ATCC 11778	good / very good	+
Pseudomonas aeruginosa ATCC 27853	good / very good	+
Proteus vulgaris ATCC 13315	good / very good	-
Escherichia coli ATCC 25922	good / very good	-
Enterobacter cloacae ATCC 13047	good / very good	-

Enterobacter cloacae ATCC 13047

Proteus vulgaris ATCC 13315

Campylobacter Blood-Free Selective Agar Base (modified CCDA)

Medium for the isolation of Campylobacter from foods.

The use of Campylobacter Blood-Free Selective Agar is specified by the UK Ministry of Agriculture, Fisheries and Food (MAFF) in a validated method for isolation of Campylobacter from foods.

Mode of Action

Campylobacter Blood-Free Selective Agar supports the growth of most enteric Campylobacters. Addition of CCDA Selective Supplement inhibits growth of Enterobacteriaceae, Yeasts and Fungis, it makes it more selective for C.jejuni, C.coli and C.lari and an increased recovery rate has been achieved when incubated at 37 °C rather than at 42 °C.

Typical Composition (g/litre)

Peptone 20,0; Casein hydrolysate 3,0; activated charcoal 4,0; Sodium chloride 5,0; Sodium desoxycholate 1,0; Sodium pyruvate 0,25; Ferrous sulphate 0,25; Agar-Agar 12,0.

Preparation

Dissolve 22,75 g in 500 ml of demin. water and heat to boiling until completely dissolved.

Autoclave (15 min. at 121° C).

Cool to 45–50 °C. Aseptically add the content of 1 vial of CCDA Selective Supplement. Mix well and pour into sterile Petridishes.

pH: 7.4 ± 0.2 at 25 °C.

The prepared medium is black.

The prepared plates can be stored for up to 2 weeks at 2-8 °C.

Experimental Procedure and Evaluation

Inoculate by spreading the sample material on the surface of the plates. Plates must be dried directly prior to inoculation in order to prevent presence of condensing water on the surface and swarming of the bacteria.

Incubation: 24-48 hours in an O_2-deficient, CO_2-enriched atmoshere which can be produced in an anaerobic jar with the aid of Anaerocult® C or in the special incubation bag with the aid of Anaerocult® C mini. Prevent drying out of the surface of the plates during incubation!

Literature

BOLTON, F.J., HUTCHINSON, D.N., a. COATES, D.: **J. Clin. Microbiol.19**, 169-171, (1984)

HUTCHINSON, D.N. a. BOLTON, F.J.: **J. Clin. Path. 34**, 956-957, (1984).

MAFF, : Validated Methods for the Analysis of Foodstuffs: Method for the detection of thermotolerant Campylobacter in Foods (v30); **J. Assoc. Publ. Analysts 29**, 253-262; (1993).

BOLTON, F.J., HUTCHINSON, D.N., a. PARKER, G.: **Eur. J. Clin. Microbiol. Infect. Dis.7**, 155-160, (1988).

Ordering Information

Product	Merck Cat. No.	Pack size
Campylobacter Blood-Free Selective Agar Base (modified CCDA)	1.00070.0500	500 g
Anaerobic jar	1.16387.0001	1 ea
Anaeroclip®	1.14226.0001	1 x 25
Anaerocult® C	1.16275.0001	1 x 10
Anaerocult® C mini	1.13682.0001	1 x 25
CCDA Selective Supplement	1.00071.0001	16 vials

Quality control

Test strains	Recovery rate after 48 hours
Campylobacter jejuni ATCC 33291	≥ 70 %
Campylobacter jejuni ATCC 29428	≥ 70 %
Campylobacter coli ATCC 33559	≥ 70 %
E. coli ATCC 25922	≥ 0.01 %
Candida albicans ATCC 10231	≤ 20 %

CCDA Selective Supplement

Additive for the preparation of Campylobacter Blood Free Selective Agar for the enrichment of Campylobacter from foods (modified CCDA acc. to Preston).

Mode of Action

CCDA Selective Supplement is a mixture of two antibiotics in lyophilized form.

Amphotericin largely reduces the growth of Yeasts and Moulds.

Cefoperazone especially inhibits Enterobacteriaceae.

Composition (per vial)

Amphotericin B 5 mg; Cefoperazone 16mg

Preparation

The lyophilisate is to dissolve in the original vial by adding of 2 ml of sterile distilled water. Mix gently to dissolve completely.

Add contents of a vial (2 ml) aseptically to 500 ml of sterile Campylobacter Blood Free Selective Agar (Base) cooled to 45-50 °C. Mix well.

Ordering Information

Product	Merck Cat. No.	Pack size
CCDA Selective Supplement	1.00071.0001	1 x 16 vials
Campylobacter Blood Free Selective Agar (Base)	1.00070.0500	500 g

Campylobacter Selective Agar Base

Medium proposed by SKIRROW (1977) for the isolation of Campylobacter from clinical material in human and veterinary medicine as well as from contaminated foodstuffs, water etc.

IVD *in vitro diagnosticum –*
For professional use only

See also General Instruction of Use
Warnings and precautions see ChemDAT®
(www.chemdat.info)

Campylobacter fetus is the causative agent of enzootic abortions and enteritis in domestic livestock (MÜLLER 1980). Campylobacter jejuni and Campylobacter coli in particular are largely responsible for the Campylobacter enteritis which affects humans (SKIRROW 1977, BUTZLER and SKIRROW 1979, BOKKENHEUSER et al. 1979, BLASER et al 1980). In man, Campylobacter is most commonly transmitted by foodstuffs derived from infected animals, water or direct contact with infected animals (ROBINSON et al. 1982, STERN and KOTULA 1982, CHRISTOPHER et al. 1983).

Principle

Microbiological method

Mode of Action

A nutrient-rich culture medium and an O_2-deficient, CO_2-enriched atmosphere ensure that Campylobacter grows well. The antibiotics which are added as a Campylobacter selective supplement largely inhibit the accompanying microbial flora.

Typical Composition (g/litre)

Peptone-protein mixture 21.0; electrolyte 5.0; starch, soluble 1.0; agar-agar 13.0

Also to be added:

blood 50-70 ml; Campylobacter Selective Supplement 5 vials.

Composition (per vial)

Vancomycin 2.0 mg; polymyxin 50.0 µg; trimethoprim 1.0 mg.

Preparation and Storage

Usable up to the expiry date when stored dry and tightly closed at +15 to +25°C. Protect from light.

After first opening of the bottle the content can be used up to the expiry date when stored dry and tightly closed at +15 to +25°C.

Preparation

Suspend 40 g/litre, autoclave (15 min at 121 °C), cool to 45-50 °C, add 5-7% defibrinated blood (sheep, horse) and mix in 1vial of Campylobacter Selective Supplement per 200ml culture medium, pour plates.

pH: 7.3 ± 0.2 at 25 °C.

Before adding blood, the prepared medium is clear and yellowish-brown; afterwards light red and non-hemolytic.

Specimen

e.g. Stool,
Clinical specimen collection, handling and processing, see general instructions of use.

Experimental Procedure and Evaluation

Inoculate by spreading the sample material on the surface of the plates.

Incubation: 24-48 hours in an O_2-deficient, CO_2-enriched atmosphere which can be produced in an anaerobic jar with the aid of Anaerocult® C or in the special incubation bag with the aid of Anaerocult® C mini. The Campylobacter species can be classified, to some extend, according to the dependence of their growth at different temperatures (see Table).

Campylobacter species	Incubation temperature		
	25 °C	37 °C	42 °C
C. fetus ssp. fetus	+	+	-
C. jejuni/coli	-	+	+
C. fetus spp. venerealis	+	+	-

Literature

BLASER, M.J., LAFORCE, F.M., WILSON, N.A., a. WANG, W.-LL.: Reservoirs for human campylobacteriosis. - **J. Infect. Diseases, 141**; 665-669 (1980).
BOKKENHEUSER, V.D., RICHARDSON, N.J., BRYNER, J.H., ROUX, D.J., SCHUTTE, A.B., KOORNHOF, H.J., FREIMAN, I., a. HARTMAN, E.: Detection of enteric campylobacteriosis in children. - **J. Clin. Microbiol., 9**; 227-232 (1979).
BUTZLER, J.P., a. SKIRROW, M.S.: Campylobacter enteritis. - **Clin. Gastroenterol., 8**; 737-765 (1979).
CHRISTOPHER, F.M., SMITH, G.C., a. VANDERZANT, C.: Examination of poultry giblets, raw mild and meat for Campylobacter fetus susp. jejuni. - **Archiv Lebensmittelhyg., 33**; 151 (1982).
HERBERT, G.A., HOLLIS, D.G., WEAVER, R.E., LAMBERT, M.A., BLASER, M.J., a. MOSS, C.W.: 30 Years of Campylobacter: Biochemical characteristics a biotyping proposal for Campylobacter jejuni. - **J. Clin. Microbiol., 15**; 1065-1073 (1983).
MÜLLER, H.E.: Campylobacter fetus-infektionen - eine Übersicht. - **Hyg. + Med., 5**; 26-30 (1980).
ROBINSON, D.A., a. JONES, D.M.: Milkborne Campylobacter infection. - **Brit. Med. J., 282**; 1374-1377 (1981).
SKIRROW, M.B.: Campylobacter enteritis: a "new" disease. - **Brit. Med., 2** ; 9-11 (1977).
STERN, M.J., a. KOTULA, A.W.: Survival of Campylobacter jejuni inoculated into Ground Beef. - **Appl. Envirnm. Microbiol., 44**; 1150-1153 (1982).
VERON, M., a. CHATELAIN, R.: Taxonomic Study of the Genus Campylobacter Sebald and Véron and Designation of the Neotype Stain for the Type Species Campylobacter fetus (Smith and Taylor) Sebald and Véron. - **Int.J.Bacteriol., 23**; 122-134 (1973).

Campylobacter Selective Agar Base

Ordering Information

Product	Merck Cat. No.	Pack size
Campylobacter Selective Agar Base	1.02248.0500	500 g
Merckoplate® Campylo-bacter selective agar	1.13579.0001	1 x 20 plates
Anaerobic jar	1.16387.0001	1 ea
Anaeroclip®	1.14226.0001	1 x 25
Anaerocult® C	1.16275.0001	1 x 10
Anaerocult® C mini	1.13682.0001	1 x 25
Campylobacter Selective Supplement	1.02249.0001	1 x 16 vials
Plate basket	1.07040.0001	1 ea
Defibrinated sheep or horse blood		

Quality control

Test strains	Growth	Clear zone
Campylobacter jejuni ATCC 33560	(42 °C)	good / very good
Campylobacter fetus ATCC 27374	(35 °C)	good / very good
Campylobacter coli ATCC 43478	(42 °C)	good / very good
Escherichia coli ATCC 25922	(42 °C)	none / poor
Enterobacter cloacae ATCC 13047	(42 °C)	none / poor
Proteus mirabilis ATCC 29906	(42 °C)	none / poor

Campylobacter Selective Supplement

Additive for the preparation of Campylobacter Selective Agar (Merck Cat. No. 1.02248.0500) acc. to SKIRROW (1977).

IVD *in vitro diagnosticum –*
For professional use only

See also General Instruction of Use
Warnings and precautions see ChemDAT®
(www.chemdat.info)

Principle

Microbiological method

Mode of Action

Campylobacter Selective Supplement is a mixture of three different lyophilized antibiotics. It supresses the growth of fecal accompanying bacteria during the culture of Campylobacter strains.

See also General Instruction of Use.

Composition (per vial)

Vancomycin 2.0 mg; polymyxin 50.0 µg; trimethoprim 1.0 mg.

Experimental Procedure

The lyophilisate is dissolved in the original vials by adding sterile, destilled water (ca. 2 ml).

In the preparation of Campylobacter Selective Agar, the dissolved contents of one vial is evenly mixed into 200 ml of sterile, still liquid medium cooled to about 45-50°C.

Storage

Usable up to the expiry date when stored dry and tightly closed at +2 to +8°C.

After first opening of the bottle the content should be used completely.

Literature

SKIRROW, M.B.: Campylobacter enteritis: a "new" disease.
- **Brit. Med. J., 6078**; 9-11 (1977).

Ordering Information

Product	Merck Cat. No.	Pack size
Campylobacter Selective Supplement	1.02249.0001	1 x 16 vials

Candida Elective Agar acc. To NICKERSON

For the isolation and preliminary differentiation of Candida and other yeasts according to NICKERSON (1953).

IVD *in vitro diagnosticum –*
For professional use only

CE

See also General Instruction of Use
Warnings and precautions see ChemDAT®
(www.chemdat.info)

Principle

Microbiological method

Mode of Action

This culture medium contains, in addition to a nutrient base consisting of yeast extract, glycine and glucose, "bismuth sulfite indicator" which largely suppresses the growth of accompanying microorganisms. Candida and most other yeasts develop normally, they reduce bismuth sulite and become brown to black in colour. BARR and COLLINS (1966) recommended addition of 2 mg neomycin sulfate/litre to improve the inhibition of the accompanying bacterial flora.

Typical Composition (g/litre)

Yeast extract 1.0; peptone from soymeal 2.0; glycine 10.0; D(+)glucose 10.0; bismuth sulfite indicator 2.0; agar-agar 15.0.

Preparation and Storage

Usable up to the expiry date when stored dry and tightly closed at +15 to +25° C. Protect from light.

After first opening of the bottle the content can be used up to the expiry date when stored dry and tightly closed at +15 to +25°C.

Suspend 40 g/litre, shake well to ensure uniform distribution of the resulting precipitate, pour plates.

■ **Do not autoclave!**

pH: 6.5 ± 0.2 at 25 °C.

The prepared plates are opalescent and yellowish-white in colour.

Specimen

e.g. Vaginal Swabs.

Clinical specimen collection, handling and processing, see general instructions of use.

Experimental Procedure and Evaluation

Take a specimen from the mycelial growth with a platinum loop or make a pharyngeal or vaginal smear using a cotton wool swab, spread sample material in the surface of the medium.

Incubation: 4 days at 28°C aerobically and if necessary at 35 °C.

Brown to black, smooth colonies with a pasty appearance are usually yeasts.

Similarly coloured bacterial colonies or yeast-like fungi do not often grow on this medium and can be differentiated by microscopic examination. Dermatophytes and mould appear seldom on this culture medium and can easily be recognized by the aerial mycelium.

Further tests should be performed to differentiate the yeasts and particularly to identify Candida albicans. Biochemical methods for identifying Candida species have been described by MARTIN and SCHNEIDAU (1970).

Literature

BARR, F.S., a. COLLINS, G.F.: A rapid method for the isolation and identification of Candida. - **J. Southern Med. Assoc., 59**; 694-695 (1966).

NICKERSON, W.J.: Reduction of inorganic substances by yeasts. I. Extracellular reduction of sulfite by species of Candida. - **J. Infect. Dis., 93**; 43-56 (1953).

NICKERSON, W.J.: Biology of Pathogenic Fungi. - Chronica Botanica Comp. Waltham (1947).

MARTIN, M.V., a. SCHNEIDAU, J.D.: A simple and reliable assimilation test for the identification of Candida species. - **Am. J. Clin. Path., 53**; 875-879 (1970).

Ordering Information

Product	Merck Cat. No.	Pack size
Candida Elective Agar acc. to NICKERSON	1.10456.0500	500 g
Candida Elective Agar acc. to NICKERSON	1.10456.5000	5 kg
Merckoplate® Candida Elective Agar acc. to NICKERSON	1.10412.0001	1 x 20 plates

Candida Elective Agar acc. To NICKERSON

Quality control

Test strains	Growth	Recovery rate	Colony colour
Candida albicans ATCC 10231	good / very good	≥ 70 %	brown / black
Candida albicans 1021	good / very good		brown / black
Candida glabrata DSMZ 70614	fair / very good		brown
Saccharomyces cerevisiae ATCC 7752	none / poor		
Proteus mirabilis ATCC 29906	none / poor		
Enterobacter cloacae ATCC 13047	none / poor		
Pseudomonas aeruginosa ATCC 27853	none / poor		
Escherichia coli ATCC 25922	none		

Candida albicans ATCC 10231

Candida glabrata DSMZ 70614

CATC Agar (Citrate Azide Tween® Carbonate) Base

Selective agar proposed by BURKWALL and HARTMANN (1964) and modified by REUTER (1968) for the identification of enterococci in meat, meat products, dairy products and other foodstuffs.

In a series of comparative studies performed by BELZER (1983), the best results were obtained with CATC agar.

Mode of Action

The high concentrations of citrate and azide almost completely inhibit the growth of the accompanying microbial flora. Enterococci reduce the colourless 2,3,5-triphenyltetrazolium chloride to a red formazan, their colonies thus become red in colour.

Typical Composition (g/litre)

Peptone from casein 15.0; yeast extract 5.0; potassium dihydrogen phosphate 5.0; sodium citrate 15.0; polyoxyethylene sorbitanmonooleate (Tween® 80) 1,0; agar-agar 15.0.

Also to be added:

Sodium carbonate 2.0; 2,3,5-triphenyltetrazolium chloride 0.1; sodium azide 0.4

Preparation

Suspend 56 g/litre, autoclave (15 min at 121 °C). At a temperature of 50 °C mix in 20 ml of a 10 % sodium carbonate solution/litre, 10 ml of a 1 % 2,3,5-triphenyltetrazolium chloride solution/litre and 4 ml of a 10 % sodium azide solution/litre, each filter-sterilized. Pour plates.

pH: 7.0 ± 0.2 at 25 °C.

The plates are clear and yellow.

Experimental Procedure and Evaluation

Inoculate by spreading the sample material thinly on the surface of the culture medium.

Incubation: 24 hours at 35 °C, aerobically.

Appearence of Colonies	Microorganisms
Red	Ent. faecalis, Ent. faecium, Str. zymogenes, Str. liquefaciens
Colourless	Accompanying microorganisms

Literature

BELZER, R.: Vergleichende Untersuchungen von Enterokokkenselektivnährböden. – Inaug. Dissert., Univ. München, 1983.

BURKWALL, M.K., a. HARTMAN, P.A.: Comparison of direct plating media for the isolation and enumeration of enterococci in certain frozen foods. – Appl. Microbiol., 12; 18-23 (1964).

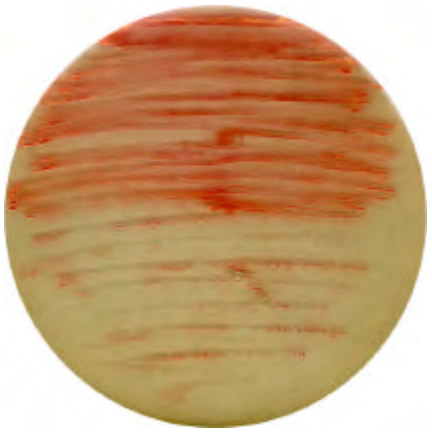
REUTER, G.: Erfahrungen mit Nährböden für die selektive mikrobiologische Analyse von Fleischerzeugnissen. – Arch. f. Lebensmittelhyg., 19; 53-57 and 84-89 (1968).

SARASWAT, D.S., CLARK, W.S. Jr., a. REINBOLD, G.W.: Selection of medium for the isolation and enumeration of enterococci in dairy products. – J. Milk Food Techn., 26; 114-118 (1963).

Ordering Information

Product	Merck Cat. No.	Pack size
CATC Agar (Citrate Azide Tween® Carbonate) Base	1.10279.0500	500 g
2,3,5-Triphenyltetrazolium chloride	1.08380.0010	10 g
Sodium azide purified	1.06688.0100	100 g
Sodium carbonate	1.06392.0500	500 g

Enterococcus faecalis ATCC 11700



Enterococcus faecium ATCC 6057

CATC Agar (Citrate Azide Tween® Carbonate) Base

Quality control

Test strains	Growth	Red colonies
Streptococcus pyogenes ATCC 12344	none	-
Streptococcus agalactiae ATCC 13813	none	-
Enterococcus faecalis ATCC 11700	good	+
Enterococcus faecalis ATCC 33186	good	+
Enterococcus faecium ATCC 6057	good	±
Streptococcus bovis DSM 20065	none / poor	-
Escherichia coli ATCC 25922	none	
Staphylococcus aureus ATCC 25923	none	

CAYE Broth modified acc. to EVANS

Medium for the culture of pathogenic E.coli to enhance verotoxin production during growth.

Principle

Microbiological method.

Mode of Action

Casaminoacids and yeast extract promote the growth of verotoxin producing E.coli. Trace elements and the high pH-value additionally support the formation of verotoxins.

Typical Composition (g/litre)

Casaminoacids 20.0; yeast extract 6.0; D(+)glucose 2.5; sodium chloride 2.5; di-potassium hydrogen phosphate 8.71; magnesium sulphate 0.05; manganese chloride 0.005.

Preparation

Suspend 7,95 g in 200 ml demin. water and heat in a boiling water bath or in a steam jet, swirling regularly, until the medium has completely dissolved.

Autoclave (15 min. at 121° C).

Cool the medium down to room temperature. Add aseptically one vial of the prepared CAYE broth Supplement to the CAYE broth; mix. Fill 1 ml under agitation into sterile tubes.

pH: 8.5 ± 0.2 at 25 °C.

The prepared broth is light brown and clear. Occuring precipitates do not have any effect to the properties of the medium.

Experimental Procedure and Evaluation

Pick up 5-6 typical colonies from the isolation medium and inoculate into the prepared tube.

Incubation: 6 hours at +37 °C without stirring.

Literature

Franke, V., Hahn, G., A. Tolle, A.: Vorkommen und Nachweis von Enterotoxin-bildenden E. Coli-stämmen In Milch und Milchprodukten. - **Zbl.Bakt. Hyg. A 257**; 51-59 (1984).

KÖHLER, B., KARCH, H., a. SCHMIDT, H.: Antibacterials that are used as growth promoters in animal husbandry can affect the release of Shiga toxin 2-converting bacteriophages and Shiga toxin 2 from Escherichia coli strains. - **Microbiology 146** ; 1085-1090 (2000).

Ordering Information

Product	Merck Cat. No.	Pack size
CAYE Broth modified acc. to EVANS	1.00060.0100	100 g
CAYE Broth Supplement	1.00051.0001	1 x 16 vials

Quality control

Test strains	Verotoxin production	
Escherichia coli O157:H7 43889	VT 1 negative	VT 2 positive
Escherichia coli O157:H7 43890	VT 1 positive	VT 2 negative
Escherichia coli O157:H7 43888	VT 1 negative	VT 2 negative
Escherichia coli O157:H7 43895	VT 2 positive	VT 2 positive

CAYE Broth Supplement

Additive for the preparation of CAYE Broth or modified Brain Heart Agar for the detection of verotoxins from pathogenic E.coli.

Mode of Action

CAYE Broth Supplement enhances the production and release of verotoxins produced by pathogenic E.coli during growth.

Composition (per vial)

Verotoxin inducer 10 µg

Preparation

The content is dissolved in the original vial by adding 1ml of sterile deionized or distilled water.

The content of 1 vial CAYE Broth Supplement is added to 200ml of CAYE Broth or to 100 ml of Brain Heart Agar after it has cooled down to about + 45 °C.

Experimental Procedure and Evaluation

Broth method

Pick up 1-5 typical colonies from the isolation medium and inoculate a tube with 1ml of the prepared CAYE Broth with CAYE Broth Supplement.

Incubation: 6 hours at +37 °C without stirring.

Agar plate method

Streak one colony onto the surface of a plate containing brain heart agar with CAYE Broth Supplement.

Incubation: 18-24 hours at +37°C aerob.

Ordering Information

Product	Merck Cat. No.	Pack size
CAYE Broth Supplement	1.00051.0001	1 x 16 vials
Brain Heart Agar	1.13825.0500	500 g
CAYE Broth mod. acc. to EVANS	1.00060.0100	100 g

CHAPMAN Agar
(Staphylococcus Selective Agar No. 110 acc. to CHAPMAN)

For the isolation and differentiation of staphylococci in foodstuffs and other materials according to CHAPMAN (1946, 1948, 1952).

Mode of Action

Only those microorganisms which display a high salt tolerance can grow on this culture medium; these include staphylococcal colonies, which can be differentiated on the basis of mannitol degradation, gelatinolysis and pigment production.

SMUCKLER and APPLEMAN (1964) recommend the addition of sodium azide (65 mg/litre) to improve the inhibition of Bacillus species.

Typical Composition (g/litre)

Peptone from casein 10.0; yeast extract 2.5; di-potassium hydrogen phosphate 5.0; gelatin 30.0; lactose 2.0; D(-)mannitol 10,0; sodium chloride 75.0; agar-agar 12.0.

Preparation

Suspend 146.5 g/litre, autoclave (15 min at 121 °C). Pour plates.

pH: 7.0 ± 0.2 at 25 °C.

The plates are clear and yellowish-brown.

Experimental Procedure and Evaluation

Inoculate the plates by spreading the sample on the surface of the medium.

Incubation: 48 hours at 35 °C aerobically.

Pigment-forming colonies are golden yellow, non-pigmented colonies are white.

Formation of acid from mannitol is indicated by a colour change to yellow, when drops of a 0.04 % bromothymol-blue solution are applied to colony sites; the individual colonies should first be removed with a platinum loop.

According to STONE (1935), gelatinolysis is an indicator of toxicity and it is shown by the appearance of clear zones around the colonies about 10 minutes after applying drops of a saturated ammonium sulfate solution or a 20 % sulfosalicylic acid solution.

Further tests should be performed to confirm the results.

Literature

CHAPMAN, G.H.: A single culture medium for selective isolation of plasma coagulating staphylococci and for improved testing of chromogenesis, plasma coagulation, mannitol fermentation and the Stone reaction. – **J. Bact., 51**; 409-410 (1946).

CHAPMAN, G.H.: An improved Stone medium for the isolation and testing for food-poisoning staphylococci. – **Food Res., 13**; 100-105 (1948).

CHAPMAN, G.H.: A simple method for making multiple tests of a microorganism. – **J. Bact. 63**; 147 (1952).

SMUCKLER, S.A., a. APPLEMAN, M.D.: Improved staphylococcus medium no. 110. – **Appl. Microbiol. 12** ; 355-359 (1964).

STONE, R.V.: A cultural method for classifying staphylococci as of the "food poisoning" type. – **Proc. Soc. Exptl. Biol. Med., 33**; 185-187 (1935).

Ordering Information

Product	Merck Cat. No.	Pack size
CHAPMAN Agar (Staphylococcus Selective Agar No. 110 acc. to CHAPMAN)	1.05469.0500	500 g
5-sulfosalicylic acid dihydrate	1.00691.0100	100 g
Ammonium sulfate	1.01217.0100	100 g
Bromothymol blue indicator	1.03026.0005	5 g
Sodium azide purified	1.06688.0100	100 g

Staphylococcus aureus ATCC 25923

Staphylococcus epidermidis ATCC 12228

CHAPMAN Agar
(Staphylococcus Selective Agar No. 110 acc. to CHAPMAN)

Quality control

Test strain	Growth
Staphylococcus aureus ATCC 25923	good/ very good
Staphylococcus aureus ATCC 6538	good / very good
Staphylococcus simulans ATCC 11631	good / very good
Staphylococcus epidermidis ATCC 12228	fair / very good
Escherichia coli ATCC 25922	none
Proteus vulgaris ATCC 13315	none
Pseudomonas aeruginosa ATCC 27853	none
Streptococcus pyogenes ATCC 12344	none

China-blue Lactose Agar

Elective culture medium for differentiating between lactose-positive and lactose-negative microorganisms and for determination of the microbial count in milk (BRANDL and SOBECK-SKAL 1963).

Mode of Action

This culture medium is free from inhibitors and contains lactose as a reactant. Degradation of lactose to acid is indicated by a colour change of the pH indicator, china blue, from colourless to blue.

Typical Composition (g/litre)

Meat extract 3:0; peptone from casein 5.0; sodium chloride 5.0; lactose 10.0; china blue 0.375; agar-agar 12.0.

Preparation

Suspend 35.5 g/litre, autoclave (15 min at 121 °C).

pH: 7.2 ± 0.2 at 25 °C.

The plates are clear and pale blue.

Experimental Procedure and Evaluation

Inoculate the culture medium by the streaking or pour-plate methods. The method employed depends on the purpose for which the medium is used.

Incubation: 24-48 hours at 35 °C aerobically.

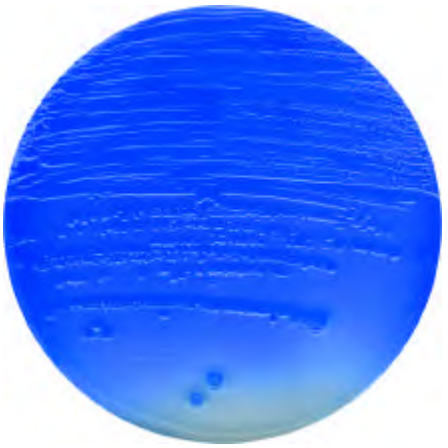
Appearance of Colornies	Microorganisms
Blue	Lactose-positive: e.g. E. coli, coliform bacteria, staphylococci, streptococci and others
Colourless	Lactose-negative: e.g. Salmonella, Serratia, Proteus and others

Literature

BRANDL, E., u. SOBECK-SKAL, E.; Zur Methodik der Keimzahlbestimmung in Milch mit Chinablau-Lactoseagar. – **Milchwiss. Ber., 13** (1963).

Ordering Information

Product	Merck Cat. No.	Pack size
China-blue Lactose Agar	1.02348.0500	500 g



Escherichia coli ATCC 25922

Quality control

Test strains	Growth	Colour change to blue
Escherichia coli ATCC 25922	good / very good	+
Proteus mirabilis ATCC 29906	good / very good	-
Pseudomonas aeruginosa ATCC 27853	good / very good	-
Enterococcus faecalis ATCC 11700	good / very good	+ (poor)
Streptococcus agalactiae ATCC 13813	moderate	+
Staphylococcus epidermidis ATCC 12228	moderate	+
Bacillus cereus ATCC 11778	good / very good	-

Chromocult® dehydrated culture media

Culture media for the rapid identification of bacteria using chromogenic substrates.

Mode of Action

A method for the rapid identification of characteristic bacterial enzymes is provided by use of chromogenic substrates. In Chromocult® culture media, these chromogenic substrates are already integrated into the growth medium. The culture medium base is composed that on one hand the growth of the target bacteria is specifically encouraged, while at the same time an optimum activity of the characteristic enzymes takes place. The identification of the enzyme activity is greatly simplified by the addition of chromogenic substrates to the culture medium. Then, a direct identification, using the characteristic colony colouring on the culture medium itself is possible without the application of further additives. Furthermore, this colouring remains stable for several days, unaffected by the pH-value, temperature or light. Because the colouring does not diffuse into the culture medium, a differentiation of positive single colonies is possible, even when high microbial counts are present. Furthermore, the choice of appropriate chromogenic substrates permits the visualisation of a whole series of different enzyme activities with different colours in one culture medium.

Quality control of the culture medium

The quality control of Chromocult® Dehydrated Culture Media includes, apart from the usual quality parameters for dehydrated culture media, the colony colouring as an important additional criterion.

A

Chromocult® Coliform Agar

Selective agar for the simultaneous detection of total coliforms and E. coli in drinking water and processed food samples.

The approval by US-EPA is pending.

Mode of Action

In the first instance, the interaction of selected peptones, pyruvate, sorbitol and phosphate buffer guarantees rapid colony growth, even for sublethally injured coliforms. The growth of Gram-positive bacteria as well as some Gram-negative bacteria is largely inhibited by the content of Tergitol® 7 which has no negative effect on the growth of the coliform bacteria.

For the second stage, Merck has developed a new combination of two chromogenic substrates which allow for the simultaneous detection of total coliforms and E. coli.

E. coli identification

The characteristic enzyme for coliforms, ß-D-galactosidase cleaves the Salmon-GAL substrate and causes a salmon to red colour of the coliform colonies.

E. coli identification

The substrate X-glucuronide is used for the identification of ß-D-glucuronidase, which is characteristic for E. coli.

E. coli cleaves both Salmon-GAL and X-glucuronide, so that positive colonies take on a dark-blue to violet colour. These are easily distinguished from other coliform colonies which have a salmon to red colour. As part of an additional confirmation of E.coli, the inclusion of tryptophane improves the indole reaction, thereby increasing detection reliability when it is used in combination with the Salmon-GAL and X-glucuronide reaction.

Typical Composition (g/litre)

Peptones 3.0; sodium chloride 5.0; sodium dihydrogen phosphate 2.2; di-sodium hydrogen phosphate 2.7; sodium pyruvate 1.0; tryptophane 1.0; agar-agar 10.0; Sorbitol 1.0; Tergitol® 7 0.15; chromogenic mixture 0.4.

Preparation

Suspend 26.5 g in 1 litre of demin. water by heating in a boiling water bath or in free flowing steam. Stir the content to assist dissolution (approx. 35 mn). Some turbidity may occur, but this does not effect the performance!

■ **Do not autoclave! Do not overheat!**

pH: 6.8 ± 0.2 at 25 °C.

Note: After autoclaving add E. coli / Coliform Supplement to the medium cooled to 45-50 °C if the sample material contains high gram-positive bacteria, Pseudomonas or Aeromonas spp.

The plates are opalescent to turbid and yellowish. Store in a refrigerator and protect from light. To prevent plates from drying, seal in plastic pouches or bags.

Shelf-life under these conditions: 6months.

Experimental Procedure and Evaluation

Inoculate the medium by the pour plate method or by spreading the sample material on the surface of the plates. In addition the membrane-filter-technique can also be used.

Incubation: 24 hours at 35 °C aerobically.

E. coli: dark-blue to violet colonies (Salmon-GAL and X-glucuronide reaction).

Total coliforms: salmon to red colonies (Salmon-GAL reaction) and dark-blue to violet colonies (E. coli).

Other Gram-negatives: colourless colonies, except for some organisms which possess ß-D-glucuronidase activity. These colonies appear light-blue to turquoise.

In order to confirm E. coli, coat the dark-blue to violet colonies with a drop of KOVACS' indole reagent. If the reagent turns to a cherry-red colour after some seconds, a positive indole formation confirms the presence of E. coli.

Membrane-filter method:

The simultaneous detection of total coliforms and E. coli using Chromocult® Coliform Agar (CCA) relies on the production of specific colony colours. OSSMER et. al (1999) reported on the effect of the type and brand of membrane filters on the growth and colour formation of coliforms and E. coli on CCA. The best performance was obtained when using filters of cellulose-mixed-ester material, s. a. Gelman GN6 or Schleicher and Schüll ME25. For the validation of membrane filters it is advised to use one of these filters as reference.

Literature

FRAMPTON, E.W., RESTAINO, L. a. BLASZKO, L.: Evaluation of ß-glucuronidase substrate 5-bromo-4-chloro-3-indol-ß-D-glucuronide (X-GLUC) in a 24 hour direct plating method for Escherichia coli. – **J. Food Protection, 51**; 402-404 (1988).

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LE MINOR, L. a. HAMIDA, F. BEN: Advantages de la recherche de la ß-galactosidase sur celle de la fermentation du lactose en milieu complexe dans le diagnostic bactériologique, en particulier des Enterobacteriaceae. – **Ann. Inst. Pasteur (Paris), 102**; 267-277 (1962).

MANAFI, M. a. KNEIFEL, W.: A combined chromogenic-fluorogenic medium for the simultaneous detection of total coliforms and E. coli in water. – **Zentralabl. Hyg. 189**; 225-234 (1989).

OSSMER, R., SCHMIDT, W., MENDE, U.: Chromocult® Coliform Agar – Influence of Membrane Filter Quality on Performance. – XVII Congresso de la Sociedad, Granada (1999).

New Zealand Dairy Industry: Microbiological Methods Manual, Section 48: Product Test Methods – Enteric Indicator Organisms. – NZTM 2; 48.5.1-48.5.10 (1998).

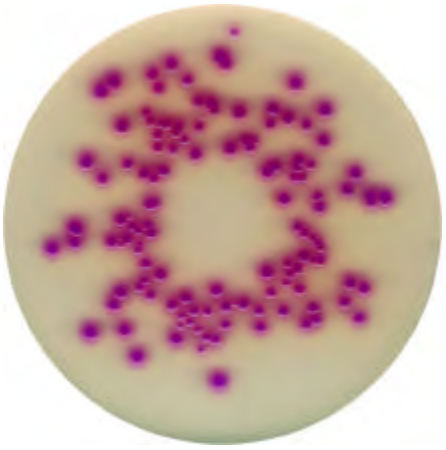
Chromocult® Coliform Agar

Ordering Information

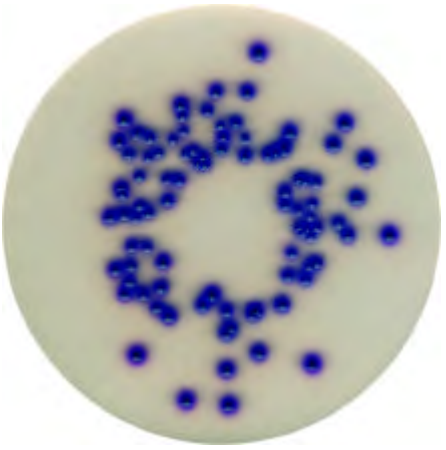
Product	Merck Cat. No.	Pack size
Chromocult® Coliform Agar	1.10426.0500	500 g
Bactident® Indole (dropper bottle)	1.11350.0001	1 x 30 ml
E. Coli/Coliform Selective-Supplement	1.10156.0001	1 x 16 vials
KOVÁCS Indole Reagent	1.09293.0100	100 ml
Cellulose-mixed-ester-GNG membrane filters	Gelman 66278	
Cellulose-mixed-ester-ME 25/21	Schleicher & Schüll 406870	

Quality control

Test strains	Recovery rate %	Growth	Colony colour	Salmon-GAL	X-Glucuronide	Indole
Escherichia coli ATCC 11775	≥ 70	good/very good	dark-blue to violet	+	+	+
Citrobacter freundii ATCC 8090	≥ 70	good/very good	salmon to red	+	-	-
Escherichia coli DSMZ 502	≥ 70	good/very good	blue to violet	+	-	+
Salmonella enteritidis ATCC 13076	not limited	fair/very good	colourless	-	-	-
Enterococcus faecalis ATCC 19433	≤ 0.01	none				



Citrobacter freundii ATCC 8090



Escherichia coli ATCC 11775

Chromocult® Coliform Agar ES (Enhanced Selectivity)

Selective agar for the simultaneous detection and colony count of total coliforms and E.coli in non-processed foods as well as surface water samples.

Mode of Action

The combination of suitable peptones and the buffering using MOPS allow rapid growth of coliforms and an optimal transformation of the chromogenic substrates. The amount of bile salts and propionate largely inhibit growth of Gram-positive and Gram-negative accompanying flora.

The simultaneous detection of total coliforms and E.coli is achieved using the combination of two chromogrenic substrates. The substrate Salmon™-β-D-GAL is split by β-D-galactosidase, characteristic for coliforms, resulting in a salmon to red colouration of coliform colonies. The detection of the β-D-glucuronidase, characteristic for E.coli, is cleaved via the substrate X-β-D-glucuronide, causing a blue colouration of positive colonies.

As E.coli splits Salmon™-β-D-GAL as well as X-β-D-glucuronide, the colonies turn to a dark violet colour and can be easily differentiated from the other coliforms being salmon-red.

Typical Composition (g/litre)

Peptone 5.0; potassium chloride 7.5; MOPS 10.0; bile salts 1.15; propionate 0.5; Agar-Agar 10.0; 6-Chloro-3-indoxyl-beta-D-galactopyranoside 0.15; isopropyl-beta-D-thiogalactopyranoside 0.1; 5-bromo-4-chloro-3-indoxyl-beta-D-glucuronic acid 0.1.

Preparation

Suspend 34.5 g in 1000 ml of demin. water and heat to boiling with frequent agitation until completely dissolved (approximately 45 minutes).

■ **Do not autoclave, do not overheat.**

The medium is cooled to 45-50 °C (appearance of a precipitate if exceeding 2 hours) and poured into plates.

pH: 7.0 ± 0.2 bei 25 °C

The plates are clear and colorless. When stored at +4 °C ± 2°C, the shelf life of plates is 2 weeks.

Sample Preparation

To eliminate an interference between the coloration of colifirms/E.coli and the sample (e.g. low pH) it is recommended to use a 1:10 dilution of the sample into a buffered solution (e.g. Peptone Water buffered or Sodium chloride peptone broth buffered).

Application

Inoculate the medium using the pour-plate-method, surface spreading or membrane-filter-technique. The type of membrane filter affects the performance of the medium (growth and colouration of colonies). Best results were obtrained using membrane filters of cellulose-mixed-ester material, e.g. Gelman GN-8 (OSSMER, 1999).

Incubation: 24 hours at 35-37 °C.

Results

E.coli: dark blue to violet colonies (Salmon™-β-D-GAL and X-β-D-glucuronide reaction).

Some E.coli (3-4 %) are β-glucuronidase-negative and grow as salmon-red colonies, e.g. E.coli O157 strains.

Total coliforms: Salmon to red colonies (Salmon™-β-D-GAL reaction) and dark blue to violet colonies (E.coli).

Accompanying flora: colourless/turquoise colonies.

Literature

FRAMPTON, E. W., RESTAINO, L. and BLSZKO, L. 1988, Evaluation of the β-glucuronidase substrate 5-bromo-4-chloro-3-indolyl-β-D-glucuronide (X-GLUC) in a 24 hour direct plating method for Escherichia coli. – **J. Food Protection 51**; 402-404

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MANAFI, M. and KNEIFEL, W. 1989, A combined chromogenic-fluorogenic medium for the simultaneous detection of total coliforms and E.coli in water. – **Zentralbl. Hyg. 189**; 225-234

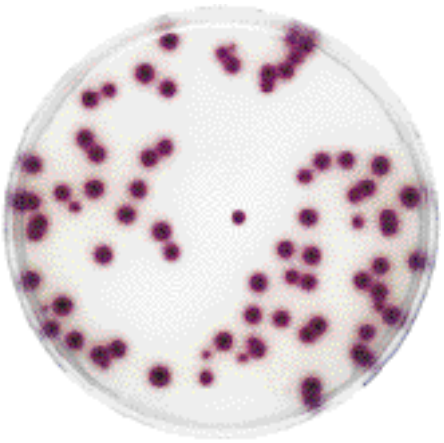
Ordering Information

Product	Merck Cat. No.	Pack size
Chromocult® Coliform Agar ES (Enhanced Selectivity)	1.00850.0500	500 g
Peptone Water (buffered)	1.07228.0500	500 g
Sodium chloride peptone broth (buffered)	1.10582.0500	500 g

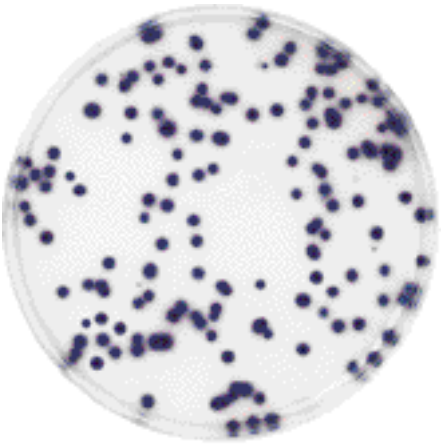
Chromocult® Coliform Agar ES (Enhanced Selectivity)

Quality control

Test strains	Inoculum (c.f.u./plate)	Recovery rate %	Colony colour
E.coli ATCC 11775	30 – 300	≥ 70	dark blue to violet
Citrobacter freundii ATCC 8090	30 – 300	≥ 70	salmon-red
Enterobacter cloacae ATCC 13047	30 – 300	≥ 70	salmon-red
Aeromonas hydrophila ATCC 7966	1000 - 2000	≤ 1	
Serratia liquefaciens ATCC 27592	1000 - 2000	≤ 0.01	
Staphylococcus aureus ATCC 25923	1000 - 2000	≤ 0.01	
Lactococcus lactis ATCC 19435	1000 - 2000	≤ 0.01	
Bacillus subtilis ATCC 6633	1000 - 2000	≤ 0.01	



Citrobacter freundii ATCC 8090



E.coli ATCC 11775

Chromocult® Enterococci Agar

Selective culture medium for the isolation, differentiation and enumeration of Enterococci in water, foodstuffs and other materials.

Mode of Action

The presence of Enterococci, especially E. faecalis, E. faecium, E.durans and E.hirae, serves as an indicator for faecal contamination.

Growth of Enterococci is stimulated by selected peptones, phosphates and addition of Tween® 80. Enterococci cleave the unique chromogenic substrates in the medium. This produces red colonies allowing an easy detection of Enterococci.

Sodium azide and ox bile inhibit most accompanying microbial flora. Non-Enterococci produce colourless, blue/violet or turquoise colonies. These colonies are easily distinguished from the red coloured colonies Enterococci produce.

Typical Composition (g/litre)

Peptones 10.0; sodium chloride 5.0; sodium azide 0.2; di-potassium hydrogenphosphate 3.4; potassium di-hydrogen-phosphate 1.6; ox bile 0.5; Tween® 80 1.0; chromogenic-mixture 0.25; agar-agar 11.0

Preparation

Suspend 33.0 g in 1 litre of demin. water by heating in a boiling water bath or in a flowing steam. Stir the contents to assist dissolution (approx. 45 minutes), let the medium cool to 45-50 °C and pour into plates.

■ **Do not autoclave! Do not overheat!**

pH. 7.0 ± 0.2 at 25 °C

The plates are clear and slightly yellow. If stored at +4 ± 2 °C and protected from light the plates are stable for 2 weeks.

Experimental Procedure

Inoculate the medium by the pour-plate-method or by spreading the sample material on the surface of the plates. In addition the membrane-filter-technique can also be used.

The type of membrane filter affects the performance of the medium (growth and colouration of colonies). Best results were obtained using membrane filters of cellulose-mixed-ester material, e.g. Gelman GN-6 (OSSMER, 1999).

Incubation: 24 ± 4hours at 35-37 °C.

If this will neither result in a colour change nor in visible growth continue the incubation up to 44 ± 4 hours.

Evaluation

Enterococci:

Red colonies with a diameter of 0.5 to 2 mm

Non-Enterococci:

colourless	(e.g. Aerococcus viridans ATCC 29503)
blue/violet	(e.g. Aerococcus viridans ATCC 10400)
turquoise	(e.g. Streptococcus equi ATCC 33398)

Literature

DOTT, H. W., HAVEMEISTER, G., MÜLLER, H. E. and SACRÈ, C. 1982, Faecal streptococci as indicator organisms of drinking water. – **Zbl. Bakt. Hyg., I. Abt. Orig. A 252**; 154-165

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LITSKY, W., MALLMANN, W. L. and Fifield, C. W. 1953, A new medium for the detection of enterococci in water. – **Amer. J. Pbl. Hlth. 43**; 873-879

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SNYDER, M. L. and LICHSTEIN, H. C. 1940, Sodium azide as an inhibiting substance for gram-negative bacteria. – **J. Infect. Dis. 67, 113-115**

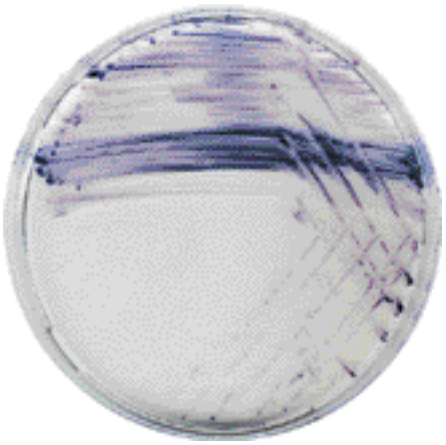
Ordering Information

Product	Merck Cat. No.	Pack size
Chromocult® Enterococci Agar	1.00950.0500	500 g

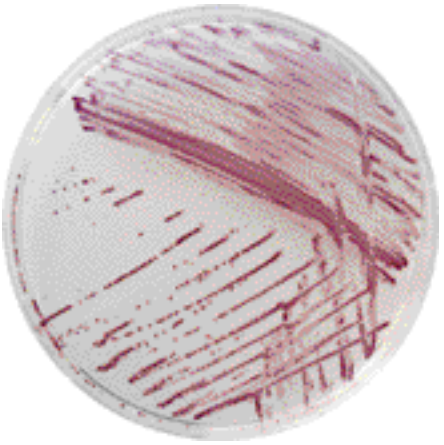
Chromocult® Enterococci Agar

Quality control

Test strains	Inoculum (c.f.u./plate)	Growth	Colony colour
Enterococcus faecalis ATCC 19433	30 – 300	good	red
Enterococcus faecium ATCC 882	30 – 300	good	red
Enterococcus durans ATCC 6056	30 – 300	good	red
Enterococcus hirae ATCC 8043	30 – 300	good	red
Aerococcus viridans ATCC 10400	1000 - 2000	fair / none	blue / violet
Bacillus cereus ATCC 11778	1000 - 2000	-	-
Escherichia coli ATCC 11775	1000 - 2000	-	-
Pseudomonas aeruginosa ATCC 27853	1000 - 2000	-	-



Aerococcus viridans ATCC 25903



Aerococcus viridans ATCC 10400

Chromocult® Enterococci Broth

Use as a test for enterococci and also for their selective enrichment in the bacteriological water examination.

Mode of Action

The presence of enterococci (as well as the less frequent D-streptococci), which account for most of the faecal streptococci, serves as an indicator for faecal contamination. This is, in some respect, more specific than the presence of coliforms which may originate from non-faecal sources, whereas enterococci can come only from faeces of human or animal origin.

The concentration of sodium-azide present in this medium largely inhibits the growth of the accompanying, and especially the Gram-negative microbial flora while sparing the enterococci.

The substrate X-GLU (5-bromo-4-chloro-3-indolyl-ß-D-glucopyranoside) is cleaved, stimulated by selected peptones, by the enzyme ß–D-glucosidase which is characteristic for enterococci. This results in an intensive blue-green colour of the broth. Azide, at the same time, prevents a false positive result by most other ß-D-glucosidase positive bacteria. Therefore, the colour-change of the broth largely confirms the presence of enterococci and D-streptococci in water.

Typical Composition (g/litre)

Peptones 8.6; sodium chloride 6.4; sodium azide 0.6; 5-bromo-4-chloro-3-indolyl-ß-D-glucopyranoside (X-GLU) 0.04; Tween® 80 2.2.

Preparation

Suspend 18 g (single-strength) or 36 g (double-strength) in 1 litre of demin. water, dispense into suitable vessels, autoclave (15 min at 121 °C).

pH: 7.5 ± 0.2 at 25 °C.

The prepared broth is clear and yellowish.

Experimental Procedure

Small sample volumes (up to 1 ml) can be added to the single-strength broth. Larger volumes (10 ml or more) should be diluted with the aliquot volume of double-strength broth to give the normal concentration.

Incubation: 24 ± 4 hours at 35 °C or 44 °C aerobically. If there is no colour-change nor visible growth, continue the incubation up to 44 ± 4 hours.

Evaluation

A strong blue-green colour of the broth indicates the presence of enterococci and D-streptococci. The observed turbidity from growth may be very weak.

Literature

ALTHAUS, H., DOT, W., HAVEMEISTER, G., MÜLLER, H.E., a. SACRÉ, C.: Faecal streptococci as indicator organisms of drinking water. – **Zbl. Bakt. Hyg., I. Abt. Orig. A 252**; 154-165 (1982).

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SNYDER, M.L. a. LICHTSTEIN, H.C.: Sodium azide as an inhibiting substance for gram-negative bacteria. – **J. Infect. Dis. 67**; 113 (1940).

Ordering Information

Product	Merck Cat. No.	Pack size
Chromocult® Enterococci Broth	1.10294.0500	500 g

No growth

Enterococcus faecalis

Quality control

Test strains	Growth	Colour change to blue-green
Enterococcus faecalis ATCC 11700	fair / good	+
Enterococcus faecalis ATCC 19433	fair / good	+
Enterococcus faecium ATCC 6057	fair / good	+
Streptococcus bovis DSMZ 20480	not limited	+
Staphylococcus aureus ATCC 25923	fair / good	-
Aeromonas hydrophila DSMZ 30187	none / poor	-
Escherichia coli ATCC 25922	none / poor	-
Pseudomonas aeruginosa ATCC 27853	none / poor	-

Chromocult® TBX (Tryptone Bile X-glucuronide) Agar

Selective agar for the detection and enumeration of Escherichia coli in foodstuffs, animal feed and water.

The medium complies with the recommendations of ISO 16649-2, 2000.

Mode of Action

The presence of the enzyme β-D-glucuronidase differentiates most E. coli ssp. from other coliforms. E. coli absorbs the chromogenic substrate 5-bromo-4-chloro-3-indolyl-β-D-glucuronide (X-β-D-glucuronide). The enzyme β-glucuronidase splits the bond between the chromophore 5-bromo-4-chloro-3-indolyle- and the β-D-glucuronide. E. coli colonies are coloured blue-green.

Growth of accompanying Gram-positive flora is largely inhibited by the use of bile salts and the high incubation temperature of 44°C.

Typical Composition (g/litre)

Peptone 20.0; bile salts No. 3 1.5; X-β-D-glucuronide 0.075; agar-agar 15.0.

Preparation

Suspend 36.6 g in 1 litre of demin. water by heating in a boiling water bath or in flowing steam until the medium is completely dissolved. Autoclave at 121 °C for 15 min. Cool to 45-50 °C in a water bath, mix gently and pour 15 ml in sterile Petridishes.

pH: 7.2 ± 0.2 at 25 °C.

The prepared medium is clear and yellowish. If stored at +2 to +8°C and protected from light plates or medium in bottles are stable for 4 weeks.

Experimental Procedure

The pour plate or membrane filtration technique can be used to inoculate the medium.

Pour plate technique:

Pipette 1 ml of a homogenate or appropriate 10-fold dilution into a sterile Petridish, add 15 ml of the medium (cooled to 45-50°C) and mix gently.

Processed samples:

For the recovery of sublethally injured E. coli, plates are incubated at 37 °C or 30 °C for 4 h. After this resuscitation step incubation is continued at 44 °C for another 18-20 h.

Fresh or raw samples:

Plates are incubated at 44 °C for 18-24 h aerobically.

Membrane filtration technique:

Filter an aliquot of a liquid sample through a Cellulose-mix-ester Membrane e.g. Gelman GN 6.

In processed samples sublethally injured E. coli cells can occur:

For the recovery of sublethally injured E. coli the membrane filter is transferred to Glutaminate Agar (DEV Glutaminate Broth to which 15 g agar per litre is added) and incubate at 37 °C or 30°C for 4 h. After this resuscitation step transfer the membrane-filter to Chromocult® TBX Agar and incubate at 44 °C for another 18-20 h.

Fresh or raw samples:

Transfer the membrane-filter to Chromocult® TBX agar and incubate at 44 °C for 18-24 h.

Results:

E. coli colonies are blue-green (X-β-D-glucuronide reaction).

Attention:

β-Glucuronidase-negative E. coli strains (3-4 %) form colourless colonies, e.g. E. coli 0157, or they cannot grow at elevated temperature of 44 °C, e.g. E. coli 0157:H7.

Literature

International Standard ISO 16649-2: Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of presumptive Escherichia coli; Part 2: Colony-count technique at 44 °C using 5-bromo-4-chloro-3-indolyl-ß-D-glucoronic acid (1999).

Ordering Information

Product	Merck Cat. No.	Pack size
Chromocult® TBX (Tryptone Bile X-glucuronide) Agar	1.16122.0500	500 g
Agar-agar purified	1.01614.1000	1 kg
DEV Glutaminate Broth	1.10687.0500	500 g
Cellulose-mixed-ester-GNG membrane filters	Gelman 66278	

Chromocult® TBX (Tryptone Bile X-glucuronide) Agar

Quality control using the spiral plate method

Test strains	Inoculum (cfu/ml)	Colony colour	Recovery rate
Escherichia coli DSMZ 502	10^3-10^5	blue-green	≥ 70 %
Citrobacter freundii ATCC 8090	≥ 10^5	-	≤ 0,01 %
Enterococcus faecalis ATCC 19433	≥ 10^5	-	≤ 0,01 %

Escherichia coli ATCC 25922

Escherichia coli DSMZ 502

Clostridium perfringens Supplement

Additive for the preparation of TSC-Agar (Base), MERCK Cat. No. 1.11972.0500.

Mode of Action

D-Cycloserine inhibits the accompanying bacterial flora and causes the colonies which develop to remain smaller. It also reduces a diffuse and thus disturbing blackening around the Clostridium perfringens colonies. 4-Methylumbelliferylphosphate (MUP) is a fluorogenic substrate for the alcaline and acid phosphatase. The acid phopshatease is a high specific indicator for Clostridium perfringens.

The acid phosphatase splits the fluorogenic substrate MUP forming 4-methylumbelliferone which can be identified as it fluorescence in long wave UV light. Thus a strong suggestion for the presence of Clostridium perfringens can be obtained.

Typical Composition

200 mg D-Cycloserine; 50 mg 4-methylumbelliferyl-phosphate disodium salt.

Preparation

Add 3 ml of sterile demin. water to 1 vial and dissolve the mixture. To prepare 500 ml of TSC Agar, add the dissolved mixture to the sterile culture medium base cooled to a temperature of 50°C. Mix the supplement homogeneously into the culture medium by carefully swirling.

pH of the ready-to-use medium: 7.4 ± 0.2 at 25 °C.

The ready plates (incl. supplelment) are clear and light brown.

Experimental Procedure and Evaluation

Inoculate by the pour plate technique only.

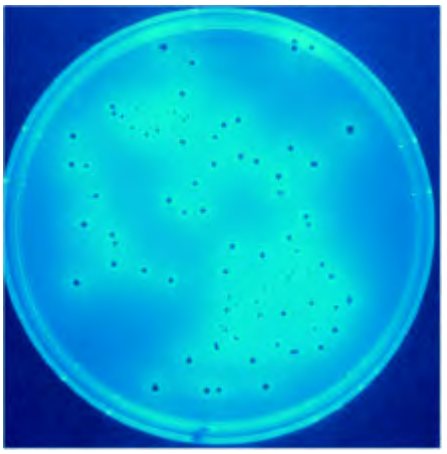
Incubate: 18-24 hours at 44 °C under anaerobic conditions (e.g. Anaerocult® A, Anaerocult® A mini, or Anaerocult® P).

Fluorescence can be detected with an UV lamp; light blue fluorescencing black colonies indicate Clostridium perfringens.

For Quality control please refer to TSC-Agar (Merck Cat. No.1.11972.).

Ordering Information

Product	Merck Cat. No.	Pack size
Clostridium perfringens Supplement	1.00888.0001	1 x 16 vials
TSC Agar, Base	1.11972.0500	500 g
Anaerocult® A	1.13829.0001	1 x 10
Anaerocult® A mini	1.01611.0001	1 x 25
Anaerocult® P	1.13807.0001	1 x 25
UV Lamp (366 nm)	1.13203.0001	1 ea



TSC-Agar, Base with Clostridium perfringens Supplement
light blue fluorescencing black colonies indicate Clostridium perfringens

Columbia Agar (Base)

This superior, complete medium proposed by ELLNER et al. (1966) can be used for the cultivation of even fastidious microorganisms and also as a base for the preparation of various special culture media.

IVD *in vitro diagnosticum –*
For professional use only

See also General Instruction of Use
Warnings and precautions see ChemDAT®
(www.chemdat.info)

This culture medium can be utililzed to prepare blood or boiled blood agar ("chocolate agar"), special inhibitors must be added for selective cultivation. Columbia agar base can be used to prepare lactose milk egg-yolk agar for the isolation of fastidious clostridia (ELLNER et al. 1966). AL-JUMAILI and BINT (1981) recommended the addition of blood, cycloserine and cefoxitin to Columbia agar (base) for the isolation of Clostridium difficile. It can also be employed in the so-called Corynebacterium diphtheriae toxicity (virulence) test according to HERMANN et al. (1958) when using the agar plate diffusion method described by ELEK (1949). GREENWOOD et al. (1977) used it to prepare Vaginalis agar for the cultivation of Gardnerella vaginalis. BANNERMANN and BILLE used it to make Acriflavin-Ceftacidim Agar (AC Agar) for the selective cultivation of Listeria from foodstuffs.

Principle

Microbiological method

Typical Composition (g/litre)

Special nutrient substrate 23.0; starch 1.0; sodium chloride 5.0; agar-agar 13.0.

Preparation and Storage

Usable up to the expiry date when stored dry and tightly closed at +15 to +25° C. Protect from light.

After first opening of the bottle the content can be used up to the expiry date when stored dry and tightly closed at +15 to +25°C.

Suspend 42 g/litre, autoclave (15 min at 121°C).

Cool to 45-50 °C before mixing in heat-sensitive additives.

pH: 7.3 ± 0.2 at 25 °C.

The plates are clear and yellowish-brown. After blood is added, they are bright red and non-hemolytic

Preparation of blood agar: Mix 5 ml blood homogeneously with 95 ml sterile culture medium base. Pour plates.

Preparation of gentamicin blood agar: Mix 100 ml defibrinated sheep blood and 0.11 ml gentamicin solution homogeneously with 900 ml sterile culture medium base. Pour plates.

Preparation of boiled agar: Add 10 ml blood to 90 ml sterile culture medium base. Heat the mixture in a water bath for about 10 minutes to 80 °C swirling all the time until the medium becomes chocolate brown in colour, pour plates.

Preparation of lactose milk egg-yolk agar: Dissolve 42 g dehydrated culture medium, 12 g lactose, 1 g agar-agar in 1 litre demineralized water. Mix in 33 ml/litre of a 0.1 % aqueous solution of neutral red, adjust the pH to 7.0 and autoclave (15min at 121 °C). Cool to 45-50 °C, add approximately 35 ml egg-yolk emulsion/litre and 10 g dried milk/litre and mix homogeneously. Pour plates.

Specimen

e.g. Blood.

Clinical specimen collection, handling and processing, see general instructions of use.

Experimental Procedure and Evaluation

Depend on the purpose for which the medium is used.

Literature

AL-JUMAILI, I.J., a. BINT, A.J.: Simple method of isolation and presumptive identification of Clostridium difficile. - **Zbl. Bakt., I. Abt. Orig. A, 250**; 152-146 (1981).

BANNERMANN, E.S., a. BILLE, J.: A new selective medium for isolating Listeria spp. from heavily contaminated material. - **Appl. Environ. Microbiol., 43**; 165-167 (1988).

BLACK, W.A., a. VAN BUSKIRK, F.: Gentamicin blood agar used as a general-purpose selective medium. - **Appl. Microbiol., 25**; 905-907 (1973).

ELEK, S.D.: The plate virulence test for diphtheria. - **J. Clin. Pathol., 3**; 250-258 (1949).

ELLNER, P.D., STOESSEL, C.I., DRAKEFORD, E., a. VASI, .:A new culture medium for medical bacteriology. - **Amer. J. Clin. Path., 29**; 181-183 (1958).

GREENWOOD, J.R., PICKETT, M.J., MARTIN, W.J., a. MACK, E.G.: Haemophilus vaginalis (Corynebacterium vaginale): method for isolation and rapid biochemical identification. - **Health Lab. Sci., 14**; 102-106 (1977).

HERMANN, G.J., MOORE, M.S., a. PARSONS, E.J.: A substitute for serum in the diphtheria in vitro toxigenicity test. - **Amer. J. Clin. Path., 29**; 181-183 (1958).

HUNT, D.E., JONES, J.V., a. DOWELL, V.R.: Selective medium for the isolation of Bacteroides gingivalis. - **J. Clin. Microbiol., 23**; 441-445 (1986).

KARMALI, M.A., SIMOR, A.E., ROSCOE, M., FLEMING, P.C., SMITH, S.S., a. LANE, J.: Evaluation of a blood-free, charcoal-based selective medium for the isolation of Campylobacter organisms from feces. - **J. Clin. Microbiol., 23**; 456-459 (1986).

KUNZE, M.: COLUMBIA-Agar-Grundsubstrat als Nährmedium für Mykoplasmen. - **Zbl. Bakt. I. Abt. Orig., 216**; 271-272 (1971).

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THOMPSON, J.S.: Colistin-oxolinic acid blood agar: a selective medium for the isolation of Gardnerella vaginalis. - **J. Clin. Microbiol., 21**;843 (1985).

ZAADHOF, K.J., u. TERPLAN, G.: Zur Diagnose von Galtstreptokokken im TKT-Medium und CAMP-Test unter Verwendung des Columbia-Agar-Substrats. - **Arch. Lebensmittelhyg., 22**; 114-115 (1971).

Columbia Agar (Base)

Ordering Information

Product	Merck Cat. No.	Pack size
Columbia Agar (Base)	1.10455.0500	500 g
Columbia Agar (Base)	1.10455.5000	5 kg
Agar-agar purified	1.01614.1000	1 kg
Egg-yolk emulsion sterile	1.03784.0001	10 x 100 ml
Gentamicin solution	1.11977.0001	10 ml
Lactose monohydrate	1.07657.1000	1 kg
Neutralred indicator	1.01369.0025	25 g
Skim milk powder	1.15363.0500	500 g
Defibrinated blood		

Quality control (spiral plating method)

Test strains	Inoculum (cfu/ml)	Growth		Hemolysis	Bacitrain test
		without blood (%)	with blood (%		
Escherichia coli ATCC 25922	10^3-10^5	≥ 70	≥ 70		
Staphylococcus aureus ATCC 25923	10^3-10^5	≥ 70	≥ 70	β	-
Streptococcus pyogenes ATCC 12344	10^3-10^5	≥ 70	≥ 70	β	+
Streptococcus pyogenes ATCC 21059	10^3-10^5	≥ 70	≥ 70	β	+
Streptococcus pneumoniae ATCC 6301	10^3-10^5	≥ 70	≥ 70	α	-
Enterococcus faecalis ATCC 19433	10^3-10^5	≥ 70	≥ 70	-	-
Bacillus cereus ATCC 11778	10^3-10^5	≥ 70	≥ 70	β	

F

Count Agar Sugar-free acc. to FIL-IDF

For determining the count of so-called infective microorganisms in butter and other dairy products.

This culture medium complies with the recommendations of the Fédération Internationale de Laiterie - International Dairy Federation (Internationaler Milchwirtschaftsverband), (1985, 1991).

Mode of Action

"Infective microorganisms" are defined as those organisms which are not directly involved in the microbiological production of a dairy product or which do not belong to its specific flora. This culture medium does not contain any fermentable carbohydrates and has relatively little nutrient value so that these microorganisms can be cultivated selectively.

Typical Composition (g/litre)

Peptone from gelatin 7.5; peptone from casein 7.5; sodium chloride 5.0; agar-agar 15.0.

Preparation

Suspend 35 g/litre, autoclave (15 min at 121 °C).

pH: 7.5 ± 0.2 at 25 °C.

The plates are clear and yellowish.

Experimental Procedure and Evaluation

The instructions given in the international Standard of the FIL-IDF should be followed so that the results can be compared at an international level.

Incubation: 48 hours at 35 °C, followed by 48 hours at 20 °C.

Do not count pin-point colonies.

Literature

International Dairy Federation: Methods of sampling milk and milk products. **- International Standard, FIL/IDF 50 B** (1985).

Internationaler Milchwirtschaftsverband: Zählung von Infektionskeimen in Butter. **- Internationaler Standard, 153** (1991).

Internationaler Milchwirtschaftsverband: Zählung von Infektionskeimen in Sauermilcherzeugnissen. **- Internationaler Standard FIL/IDF, 153** (1991).

Ordering Information

Product	Merck Cat. No.	Pack size
Count Agar Sugar-free acc. to FIL-IDF	1.10878.0500	500 g

Quality control (spiral plating method)

Test strains	Inoculum (cfu/ml)	Recovery rate (%)
Escherichia coli ATCC 25922	10^3-10^5	≥ 70
Staphylococcus aureus ATCC 25923	10^3-10^5	≥ 70
Enterococcus faecalis ATCC 11700	10^3-10^5	≥ 70
Pseudomonas aeruginosa ATCC 27853	10^3-10^5	≥ 70
Bacillus cereus ATCC 11778	10^3-10^5	≥ 70
Candida albicans ATCC 10231	10^3-10^5	≥ 70

CT-Supplement

Additive for the preparation of CT-SMAC Agar (Cat. No. 1.09207.0500).

Composition (per vial)

Cefixim	0.025 mg
Potassium Tellurite	1.25 mg

Experimental Procedure

The lyophilisate is dissolved in the original vial by adding about 1 ml of sterile distilled water.

In the preparation of CT-SMAC Agar, the dissolved content of 1 vial is evenly mixed into 500 ml of sterile, still liquid medium (Cat. No. 1.09207.) cooled to 45-50 °C.

Literature

ZADIK, P.M., P.A. CHAPMAN, and C.A. SIDDONS, Use of tellurite for the selection of verocytotoxigenic Escherichia coli 0157. **– J. Med. Microbiol., 39**; 1550-158 (1993).

Ordering Information

Product	Merck Cat. No.	Pack size
CT-Supplement	1.09202.0001	1 x 16 vials
Sorbitol-Mac Conkey Agar	1.09207.0500	500 g

Quality control (SMAC Agar with CT-Supplement)

Test strains	Recovery rate	Colony colour	Sorbitol
E. coli 0157:H7 ATCC 35150	≥ 60	colourless	-
E. coli ATCC 11775	≤ 0.01 %	-	-
Serratia marcescens ATCC 14756	≤ 0.01 %	-	-
Bacillus cereus ATCC 11778	≤ 0.01 %	-	-

CULTURA Mini-Incubator

Multi-functional:
for the incubation of
- 18 dip-slides or
- 12 Petri-dishes or
- blood culture bottles or
- identification systems (any kind)

Ease of use:
- fixed-temperature 35°C
- variable control (range: 25-40°C)
- visual control guaranteed (clear Plexiglass-door)
- easy to clean

Reliable:
- high build quality
- space saving
- safe handling (VDE/TÜV);
  CE and GS-certified acc. to international regulations)
- maintenance free

Complete:
- all in one pack (Mini-Incubator, Multirack, thermometer)

Key-stone:
- central principle of a Microbiology-laboratory

Technical data

External dimensions:	W x H x D = 310 x 155 x 168
Internal dimensions:	W x H x D = 220 x 120 x 150
Weight (net):	1.1 kg
Consumption:	26 W/220 V 26 W/110 V
Temperature range:	25-45 °C (fix: 35 °C)
Temperature accuracy:	± 1 °C

Ordering Information

Product	Merck Cat. No.	Pack size
CULTURA Mini-Incubator (26 W/220 V)	1.13311.0001	1 Incubator 1 Multirack 1 Thermo-meter
CULTURA Mini-Incubator (26 W/110 V)	1.15533.0001	1 Incubator 1 Multirack 1 Thermo-meter
MULTIRACK (spare-rack) for CULTURA Mini-Incubator	1.13312.0001	

CULTURA Mini-Incubator

ww

CZAPEK-DOX Agar

Elective agar proposed by CZAPEK (1902-1903) and DOX (1910) for the cultivation of fungi and soil bacteria.

Mode of Action

This culture medium contains sucrose as its sole carbon source and nitrate as its sole nitrogen source. Fungi grow well in this medium, but the only bacteria which are able to develop are the non-fastidous soil-bacteria. According to RAPER and FENELL (1965), addition of 1 % corn-steep liquor promotes the growth and sporulation of most Aspergillus species. WARCUP (1950) recommends addition of 5 g yeast extract/litre and a pH value of 4.0 for the isolation of soil fungi. The accompanying bacterial flora can also be inhibited by adding 30 mg streptomycin/litre and 2 mg aureomycin/litre (WARCUP 1963).

Typical Composition (g/litre)

Sucrose 30.0; sodium nitrate 3.0; magnesium sulfate 0.5; potassium chloride 0.5; iron(III)sulfate 0.01; di-potassium hydrogen phosphate 1.0; agar-agar 13.0.

Preparation

Suspend 48 g/litre, autoclave (15 min at 121 °C), pour plates.

pH: 7.3 ± 0.2 at 25 °C.

The plates are turbid and whitish.

Experimental Procedure and Evaluation

Inoculate by spreading the sample material thinly on the surface of the culture medium.

Incubation: usually 1 week at 28 °C aerobically. The optimal incubation temperature for Penicillium, Aspergillus and Candida are 20-25 °C, 30 °C and 28 ° C, respectively.

Literature

American Public Health Association, American Water Works Association and Water Pollution Control Federation: Standard Methods for the Examination of Water and Wastewater, 18th ed., Wahington, 1992.

CZAPEK, F.: Untersuchungen über die Stickstoffgewinnung und Eiweißbildung der Pflanzen. **- Beitr. Chem. Physiol. u. Pahtol., 1**; 540-560, 3; 47-66 (1902-1903).

DOX, A.W.: The intracellular enzymes of Penicillium and Aspergillus with special references to those of P. camenberti. **- U.S. Dept. Agr. Bur. Anim. Ind. Bull., 120**; 170 pp (1910).

RAPER, K.B., a. FENELL, D.J.: The genus Aspergillus (The Williams a. Wilkins Comp., Baltimore, 1965).

WARCUP, J.H.: The soil-plate method for isolation of fungi from soil. **-Nature, 166**; 117-118 (1950).

WARCUP, J.H.: Occurence of dormant ascospores in soil. **- Nature, 197**; 1317-1318 (1963).

Ordering Information

Product	Merck Cat. No.	Pack size
CZAPEK-DOX Agar	1.05460.0500	500 g

Quality control

Test strains	Growth
Bacillus cereus ATCC 11778	poor / fair
Bacillus subtilis ATCC 6633	fair / good
Candida albicans ATCC 10231	fair / good
Escherichia coli ATCC 25922	poor / fair
Penicillium commune ATCC 10428	fair / good
Aspergillus niger ATCC 16404	good / very good
Saccharomyces cerevisiae ATCC 7752	none / poor
Candida glabrata DSMZ 70614	poor / fair
Staphylococcus aureus ATCC 25923	none

DCLS Agar (Deoxycholate Citrate Lactose Sucrose Agar)

Selective agar for the isolation and differentiation of pathogenic Enterobacteriaceae from various materials.

IVD *in vitro diagnosticum –*
For professional use only

See also General Instruction of Use
Warnings and precautions see ChemDAT®
(www.chemdat.info)

According to the comprehensive studies of BURKHARDT et al. (1968) and BURKHARDT (1977), this culture medium has proved to be of value, when screening for Salmonella, Shigella. THAI and CHEN (1955) also reported that pathogenic Yersinia (Pasteurellae), e.g. Yers. pestis and Yers. pseudotuberculosis grow on this medium.

Principle

Microbiological method

Mode of Action

The composition and mode of action of this culture medium largely corresponds with LEIFSON agar. The addition of sucrose allows the differentiation of lactose- and sucrose-negative Salmonella, Shigella and Arizona colonies from lactose-negative, sucrose-positive bacteria such as Proteus vulgaris, Serratia etc. When compared with LEIFSON agar, this medium has the advantage that, owing to its sucrose content, there is no danger of obtaining false-positive results when trying to detect pathogenic Enterobaceriaceae.

Typical Composition (g/litre)

Peptone from meat 5.0; meat extract 5.0; lactose 10.0; sucrose 10.0; tri-sodium citrate-2-hydrate 6.0; sodium thiosulfate 4.0; sodium deoxycholate 3.0; ammonium iron(III)citrate 1.0; neutral red 0.02; agar-agar 13.0.

Preparation and Storage

Usable up to the expiry date when stored dry and tightly closed at +15 to +25° C. Protect from light.

After first opening of the bottle the content can be used up to the expiry date when stored dry and tightly closed at +15 to +25° C.

Suspend 57 g/litre rapidly and completely, cool rapidly, pour plates so as to obtain thick layers.

- **Do not autoclave.**

pH: 7.5 ± 0.2 at 25 °C.

The plates are clear and reddish-brown.

- **Storage: up to 1 week at +2 to +8 °C.**

Specimen

e.g. Stool.

Clinical specimen collection, handling and processing, see general instructions of use.

Experimental Procedure and Evaluation

Dry the surface of the poured plates and inoculate.

Incubation: 24 hours at 35 °C aerobically.

Literature

BURKHARDT, F.: Zur Leistungsfähigkeit des durch Saccharose-Zuatz modifizierten Leifson-Nährbodens **- Zbl. Bakt. Hyg., I. Orig., 239**; 488-492 (1977).

BURKHARDT, F., SUNTHORNSARATUL. A., EKACHAMPAKA, P., a. KREEPANICH, K.: Epidemiological studies on cholera vibrios and other enteropathogenes (Salmonella, Shigella) in two slum areas of Bangkok. **-Bull. Dep. Med. Science, Bangkok, 10**, 1-25 (1968).

THAI, E., a. CHEN, T.H.: Two simple tests for the differentiation of plague and Pseudotuberculosis bacilli. **- J. Bact., 69**; 103-104 (1955).

Ordering Information

Product	Merck Cat. No.	Pack size
DCLS Agar (Deoxycholate Citrate Lactose Sucrose Agar)	1.10270.0500	500 g

DCLS Agar (Deoxycholate Citrate Lactose Sucrose Agar)

Quality control

Test strains	Growth	Colony colour	Precipitate
Enterobacter cloacae ATCC 13047	fair / good	red	+
Escherichia coli ATCC 25922	none / poor	red	
Klebsiella pneumoniae ATCC 13883	fair / good	red	+
Salmonella typhimurium ATCC 14028	good / very good	colourless	
Salmonella choleraesuis ATCC 13312	good / very good	colourless	
Salmonella enteritidis ATCC 13076	good / very good	colourless	
Shigella flexneri ATCC 12022	fair / good	colourless	
Proteus vulgaris ATCC 13315	fair / good	colourless / red	±
Proteus mirabilis ATCC 14273	fair / good	colourless	
Pseudomonas aeruginosa ATCC 27853	fair / good	colourless	
Staphylococcus aureus ATCC 25923	none		
Bacillus cereus ATCC 11778	none		

Salmonella enteritidis ATCC 13076

Enterobachter cloacae ATCC 13407

HA

Deoxycholate Lactose Agar

Selective agar for the enumeration and isolation of Coliform bacteria from milk, water, ice-cream and other materials.

This culture medium complies with the recommendations of the APHA (1992) for the examinations of foods.

Mode of Action

The concentrations of deoxycholate and citrate are so low that the coliform bacteria can grow normally whereas the accompanying Gram-positive bacteria are largely inhibited. Degradation of lactose to acid is detected by the pH indicator neutral red which changes its colour to red, and by a zone of precipitation caused by bile acid.

Typical Composition (g/litre)

Peptones 10.0; lactose 10.0; sodium chloride 5.0; sodium citrate 2.0; sodium deoxycholate 0.5; neutral red 0.033; agar-agar 12.5.

Preparation

Suspend 40 g/litre in demin. water and heat in a boiling water bath or a current of steam. **Do not autoclave,** pour plates.

pH: 7.1 ± 0.2 at 25 °C.

The plates are clear and red-brown.

- **The culture medium should be prepared and used the same day.**

Experimental Procedure and Evaluation

Inoculate the culture medium by the spread-plate or pour-plate method.

Incubation: 24 hours at 35 °C aerobically.

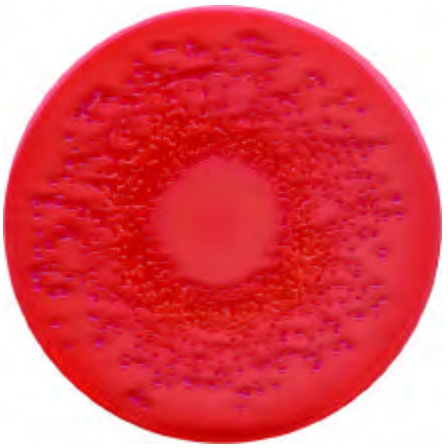
Appearance of Colonies	Microorganisms
Red, surrounded by zone of precipitate	Lactose-positive: Escherichia coli
Pale with a pink centre and surrounded by a zone of precipitate	Weakly lactose-positive: Enterobacter, Klebsiella and others
Colourless	Lactose-negative: Salmonella, Shigella, Proteus, Pseudomonas and others

Literature

American Public Health Association: Compendium of Methods for the Microbiological Examinations of Foods. 3rd ed., 1992.

Ordering Information

Product	Merck Cat. No.	Pack size
Deoxycholate Lactose Agar	1.02894.0500	500 g



Enterobacter cloacae
ATCC 13047

Salmonella enteritidis
ATCC 13076

Deoxycholate Lactose Agar

Quality control

Test strains	Inoculum (cfu/ml)	Recovery rate (%)	Colony colour	Precipitate
Escherichia coli ATCC 25922	10^3-10^5	≥ 40	red	+
Klebsiella pneumoniae ATCC 13883	10^3-10^5	≥ 40	pink /red	±
Enterobacter cloacae ATCC 13047	10^3-10^5	≥ 40	pink / red	±
Salmonella typhimurium ATCC 14028	10^3-10^5	≥ 40	colourless	-
Salmonella enteritidis ATCC 13076	10^3-10^5	≥ 40	colourless	
Shigella flexneri ATCC 12022	10^3-10^5	≥ 40	colourless	
Proteus mirabilis ATCC 14273	10^3-10^5	≥ 40	colourless	
Enterococcus faecalis ATCC 11700	>10^5	≥ 0.01		
Staphylococcus aureus ATCC 25923	>10^5	≥ 0.01		

Dermatophytes Selective Agar (DTM) acc. to TAPLIN

Culture medium proposed by TAPLIN et al. (1969, 1970) for the isolation and rapid differentiation of dermatophytes from specimens including those infected with other microorganisms.

IVD *in vitro diagnosticum –*
For professional use only

See also General Instruction of Use
Warnings and precautions see ChemDAT®
(www.chemdat.info)

The comparative studies of MERTZ et al. (1970) demonstrated that the selectivity of Dermatophytes selective agar (DTM) is superior to that of other media used for cultivating fungi. According to ALLEN et al. (1970), this medium offers the advantage that the dermatophytes grow rapidly and produce an unmistakable colour change.

Principle

Microbiological method.

Mode of Action

This culture medium contains the pH indicator phenol red and the selective inhibitors cycloheximide, gentamicin and chlorotetracycline, which partly suppress the growth of bacteria, yeasts and moulds. When grown on DTM, most dermatophytes produce basic metablites, which bring about an alkalinzation of the acidic culture medium, causing the phenol red to change its colour from yellow to red. This colour change may, however, occasionally be caused by other microorganisms, too. Many moulds produce acidic metabolites, which do not change the colour of the culture medium. According to the authors, it is thus possible to differentiate rapidly between dermatophytes and other fungi with a high degree of accuracy (approx. 97 %).

Typical Composition (g/litre)

Peptone from soymeal 10.0; D(+)glucose 10.0; cycloheximide 0.5; gentamicin sulfate 0.1; chlorotetracycline 0.1; phenol red 0.2; agar-agar 17.0.

Preparation and StorageCat. No. 1.10896. Dermatophytes Selective Agar (DTM) acc. to TAPLIN (500 g).

Usable up to the expiry date when stored dry and tightly closed at +15 to +25° C. Protect from light.

After first opening of the bottle the content can be used up to the expiry date when stored dry and tightly closed at +15 to +25° C.

Suspend 38 g/litre, autoclave under mild conditions (10 min at 121 °C), pour plates or prepare slant tubes.

pH: 5.5 ±0.2 at 25 °C.

The plates are clear and yellow-orange.

Specimen

e.g. Nails, hair, skin.

Clinical specimen collection, handling and processing, see general instructions of use.

Experimental Procedure and Evaluation

Inoculate the surface of the culture medium with specimens obtained by appropriate methods.

Incubation: 7 days, possibly up to 3 weeks at approx. 28 °C aerobically.

Literature

ALLEN, A.M., DREWRY, R.A., a. WEAVER, R.E.: Evaluation of a new color indicator media for diagnosis of dermatophytosis. **-Arch. Derm., 102**; 68-70 (1970).

MERTZ, W.G., BERGER, C.L., a. SILVA-HUTNER, M.: Media with pH-indicator for the isolation of dermatophytes. **- Arch. Derm., 99**; 203-209 (1969).

TAPLIN, D., ALLEN, A.M., a. MERTZ, P.M.: Experience with a new indicator medium (DTM) for the isolation of dermatophyte fungi, in "Proceedings of the International Symposium of Mycoses", scientific publication 205. Washington, D.C. Pan American Health Organization, 55-58 (1970).

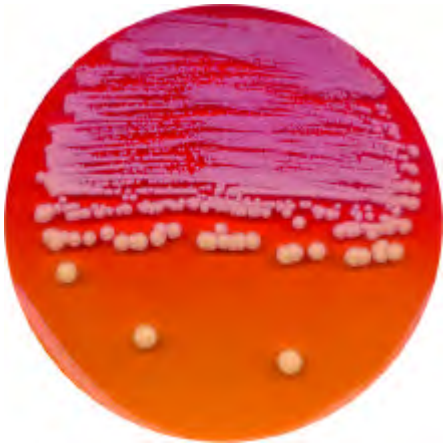
Ordering Information

Product	Merck Cat. No.	Pack size
Dermatophytes Selective Agar (DTM) acc. to TAPLIN	1.10896.0500	500 g
Merckoplate® Dermatophytes selective Agar (DTM) acc. to TAPLIN	1.10422.0001	1 x 20 plates

Dermatophytes Selective Agar (DTM) acc. to TAPLIN

Quality control (incubation: 7 days at 28 °C, aerobic)

Test strains	Growth	Colour change to red
Trichophyton mentagrophytes ATCC 18748	poor / good	+
Trichophyton rubrum ATCC 28188	poor / good	+
Microsporum gallinae ATCC 12108	poor / good	+
Microsporum canis ATCC 36299	poor / good	+
Geotrichum candidum DSMZ 1240	fair / good	±
Candida albicans ATCC 10231	good / very good	+
Aspergillus niger ATCC 16404	none / poor	
Penicillium commune ATCC 10428	none / poor	
Saccharomyces cerevisiae ATCC 9763	none	
Bacillus cereus ATCC 11778	none	
Escherichia coli ATCC 25922	none	
Staphylococcus aureus ATCC 25923	none	



Candida albicans
ATCC 10231

Geotrichum candidum
DSMZ 1240

DEV Gelatin Agar

For determining the total microbial count and detecting gelatin-liquefying microorganisms in water according to the German methods for the examination of water and the German drinking water regulations (1990).

Typical Composition (g/litre)

Peptone from meat 10.0; meat extract 10.0; sodium chloride 5.0; gelatin 10.0; agar-agar 15.0.

Preparation

Suspend 50 g/litre, autoclave (15 min at 121 °C), pour plates.

pH: 7.3 ± 0.2 at 25 °C.

■ **Do not overheat!**

The plates are clear and yellowish-brown.

Experimental Procedure and Evaluation

According to the German methods, the medium is inoculated by the pour plate method and incubated for 44 ± 4 hours at

20 ± 2 °C. To evaluate the plates, flood them with a saturated solution of ammonium sulfate; clear zones then appear around the gelatin-liquefying colonies.

Literature

Deutsche Einheitsverfahren zur Wasser-, Abwasser- und Schlammuntersuchung. - VCH Verlagsgesellschaft, D-6940 Weinheim.

Verordnung über Trinkwasser und über Wasser für Lebensmittelbetriebe vom 12. Dezember 1990. **- Bundesgesetzbl.: Teil I**; 2613-2669 (1990).

Ordering Information

Product	Merck Cat. No.	Pack size
DEV Gelatin Agar	1.10685.0500	500 g
Ammonium sulfate	1.01217.0100	100 g

Quality control (spiral plating method)

Test strains	Inoculum (cfu/ml)	Recovery rate
Escherichia coli ATCC 25922	10^3-10^5	≥ 70 %
Proteus vulgaris ATCC 13315	10^3-10^5	≥ 70 %
Staphylococcus aureus ATCC 25923	10^3-10^5	≥ 70 %
Enterococcus faecalis ATCC 11700	10^3-10^5	≥ 70 %
Bacillus cereus ATCC 11778	10^3-10^5	≥ 70 %
Pseudomonas aeruginosa ATCC 27853	10^3-10^5	≥ 70 %
Aeromonas hydrophila ATCC 7966	10^3-10^5	≥ 70 %

Escherichia coli ATCC 25922

Aeromonas hydrophila ATCC 7966

DEV Glutamate Broth

For selective enrichment and determining the titre of coliform bacteria in the bacteriological analysis of water. It complies with the German Standard. Methods for water and sludge examination as well as the German drinking water regulation (1990) and the regulations for the examination of food (LMBG).

By adding agar-agar it is possible to make Glutamate Nutrient Agar for the examination of foodstuffs acc. to ISO 16649, see Chromocult® TBX agar.

Mode of Action

The proliferation of enterococci is almost completely inhibited by the lack of certain nutrient substances essential for their growth. Lactose-positive organisms cause a colour change to yellow.

Typical Composition (g/litre)

Casein hydrolysate 1.0; lactose 10.0; sodium L(+)glutamate 6.36; sodium formate 0.25; L(+)arginine monohydrochloride 0.02; L(+)aspartic acid 0.024; L(-)cystine 0.02; di-potassium hydrogen phosphate 0.9; ammonium chloride 2.5; magnesium sulfate 0.1; calcium chloride 0.01; iron(III) citrate 0.01; thiaminium dichloride 0.001; nicotinic acid 0.001; pantothenic acid 0.001; bromocresol purple 0.01.

Preparation

Suspend 21 g or 42 g/litre, dispense into culture tubes fitted with DURHAM tubes, autoclave (15 min at 121 °C).

pH: 6.7 ± 0.2 at 25 °C.

The medium is clear and purple.

Preparation of Glutamate Nutrient Agar: Dissolve 21 g/litre together with 15 g/litre agar-agar and autoclave (15 min at 121°C)

Experimental Procedure and Evaluation

According to the German regulations, the inoculated broth is incubated for 20 ± 4 hours at 42 ± 0.5 °C. Otherwise it is applied according to the required purpose.

If lactose-positive microorganisms grow, the broth colour changes to yellow.

Literature

Bundesgesundheitsamt: Amtliche Sammlung von Untersuchungsverfahren nach § 35 LMBG. - Beuth Verlag Berlin, Köln.

Deutsche Einheitsverfahren zur Wasser-, Abwasser- und Schlammuntersuchung. - VCH Verlagsgesellschaft, D-6940 Weinheim

Ordering Information

Product	Merck Cat. No.	Pack size
DEV Glutamate Broth	1.10687.0500	500 g
Agar-Agar, granulated	1.01614.1000	1 kg

Quality control; incubation: 24 h at 35 °C

Test strains	Growth	Colour change to yellow	Gas formation
Escherichia coli ATCC 25922	good / very good	+	+
Escherichia coli ATCC 11775	good / very good	+	+
Klebsiella pneumoniae ATCC 13883	good / very good	+	+
Salmonella typhimurium ATCC 14028	good / very good	-	-
Enterococcus faecalis ATCC 11700	none / poor	-	-
Pseudomonas aeruginosa ATCC 27853	good / very good	-	-

DEV Nutrient Agar

For determining the total microbial count in water according to the German Standard Methods (Deutsche Einheitsverfahren), the German Drinking Water Regulations (Trinkwasser-Verordnung) (1990) and the German regulation for food examination (LMBG).

Typical Composition (g/litre)

Peptone from meat 10.0; meat extract 10.0; sodium chloride 5.0; agar-agar 18.0.

Preparation

Suspend 43 g/litre, autoclave (15 min at 121 °C).

pH: 7.3 ± 0.2 at 25 °C.

The plates are clear and yellowish-brown.

Any possible turbity of the medium has no impact on the microbiological performance!

Experimental Procedure and Evaluation

According to the German regulations, the medium is inoculated by the pour plate method and incubated at 20 ± 2 or 35 ± 1 °C for 44 ± 4 hours, aerobically.

Literature

Bundesgesundheitsamt: Amtliche Sammlung von Untersuchungsverfahren nach § 35 LMBG. Beuth Verlag Berlin, Köln.

Verordnung über Trinkwasser und über Wasser für Lebensmittelbetriebe vom 12. Dezember 1990. **- Bundesgesetzbl., Teil I**; 2613-2629 (1990).

Ordering Information

Product	Merck Cat. No.	Pack size
DEV Nutrient Agar	1.11471.0500	500 g
DEV Nutrient Agar	1.11471.5000	5 kg

Quality control (spiral plating method)

Test strains	Inoculum (cfu/ml)	Recovery rate (%)
Escherichia coli ATCC 25922	10^3-10^5	≥ 70
Klebsiella pneumoniae ATCC 13882	10^3-10^5	≥ 70
Serratia marcescens ATCC 14756	10^3-10^5	≥ 70
Proteus vulgaris ATCC 13315	10^3-10^5	≥ 70
Aeromonas hydrophila ATCC 7966	10^3-10^5	≥ 70
Enterococcus faecalis ATCC 11700	10^3-10^5	≥ 70
Bacillus subtilis ATCC 6633	10^3-10^5	≥ 70
Pseudomonas aeruginosa ATCC 27853	10^3-10^5	≥ 70

Enterococcus faecalis ATCC 19433

Escherichia coli ATCC 25922

DEV Tryptophan Broth

This culture medium is intended for growing intermediate cultures in the differentiation of coliform bacteria when carrying out the bacteriological analysis of water as recommended in the German Standard Methods and the German drinking water regulation (1990).

The broth complies with the German regulations for the examination of foods.

Typical Composition (g/litre)

Peptone from meat 10.0; DL-tryptophan 1.0; sodium chloride 5.0.

Preparation

Suspend 16 g/litre, dispense into suitable containers, autoclave (15 min at 121 °C).

pH: 7.2 ± 0.2 at 25 °C.

The prepared broth is clear and yellowish-brown.

Experimental Procedure and Evaluation

The broth is inoculated with the pure culture to be tested and incubated according to DEV 4 to 6 hours at 35 °C aerobically.

Literature

Bundesgesundheitsamt: Amtliche Sammlung von Untersuchungsverfahren nach § 35 LMBG. - Beuth Verlag Berlin, Köln.

Deutsche Einheitsverfahren zur Wasser-, Abwasser- und Schlammuntersuchung. - VCH Verlagsgesellschaft, D-69469 Weinheim.

Verordnung über Trinkwasser und über Wasser für Lebensmittelbetriebe vom 12. Dezember 1990. **- Bundesgesetzbl., Teil I**; 2613-2629 (1990).

Ordering Information

Product	Merck Cat. No.	Pack size
DEV Tryptophan Broth	1.10694.0500	500 g
Bactiden® Indole (dropper bottle)	1.11350.0001	1 x 30 ml
KOVÁCS Indole Reagent	1.09293.0100	100 ml

Quality control

Test strains	Growth	Indole formation
Escherichia coli ATCC 25922	good / very good	+
Escherichia coli ATCC 11775	good / very good	+
Klebsiella pneumoniae ATCC 13883	good / very good	-
Proteus mirabilis ATCC 14153	good / very good	-
Salmonella typhimurium ATCC 14028	good / very good	-

HA

Dextrose Casein-peptone Agar

Medium proposed by WILLIAMS (1936) for the identification and enumeration of Bacillus species, especially of "flat sour" bacteria (TANNER 1944), in foodstuffs.

This medium complies with the recommendations of the NCA (National Canners Association 1954, 1956), and the APHA (1992) for examining foods.

Mode of Action

Bacterial colonies, which metabolize dextrose to form acid, cause the indicator bromocresol purple in their immediate surroundings to change its colour to yellow.

Typical Composition (g/litre)

Peptone from casein 10.0; D(+)glucose 5.0; bromocresol purple 0.04; agar-agar 12.0..

Preparation

Suspend 27 g/litre, autoclave (15 min at 121 °C).

pH: 6.8 ± 0.2 at 25 °C.

The medium is clear and purple.

Experimental Procedure and Evaluation

The culture medium is usually inoculated by the pour-plate method.

Detection of spores: Add the sample material to the culture medium, heat (30 minutes in free-flowing steam) and pour plates.

Detection of mesophilic bacteria: Incubate up to 72 hours at 35°C.

Detection of thermophilic bacteria: Incubate up to 48 hours at 55-60 °C.

Typical flat-sour colonies have a smooth edge, a diameter of 2-3mm with an opaque central spot and are usually surrounded by a yellow zone. Neighbouring colonies which cause alkalinization of the culture medium can mask the yellow colouration.

Literature

American Public Health Association Inc.: Compendium of Methods for the Microbiological Examination of Foods. - **3rd ed.**, 1992.

National Canners Association: A Laboratory Manual of the Canning Industry. **- 1st ed., Washington** 1954.

National Canners Association: Ibid. **- 2nd ed., Washington** 1956, 2-9.

TANNER, F.W.: "The Microbiology of Foods." Champaign III., Gerard Press, **2nd ed.** 1944, 693-722; 762-763; 1127-1128.

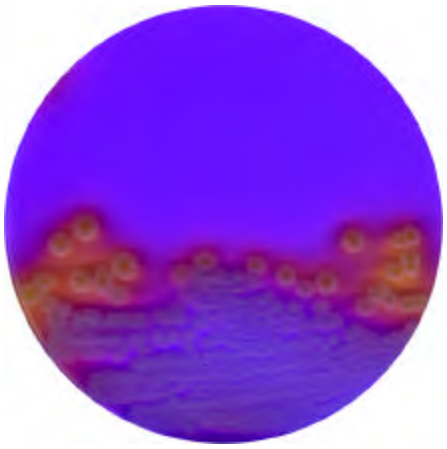
WILLIAMS, O.B.: Tryptone medium for the detection of flat sour spores. **-Food Research, I (3)**, 217-221 (1936).

Ordering Information

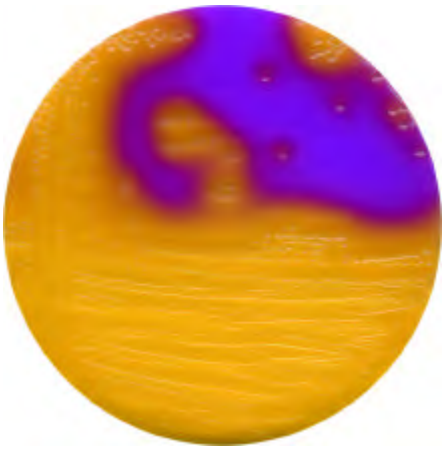
Product	Merck Cat. No.	Pack size
Dextrose Casein-peptone Agar	1.10860.0500	500 g

Quality control

Test strains	Growth	Colour change to yellow
Staphylococcus aureus ATCC 25923	fair / very good	+
Enterococcus faecalis ATCC 11700	good / very good	+
Bacillus cereus ATCC 11778	good / very good	+
Bacillus subtilis ATCC 6633	good / very good	+ (after 24 h usually weak)
Escherichia coli ATCC 25922	good / very good	+
Alcaligenes faecalis ATCC 19209	fair / very good	-
Bacillus stearothermophilus ATCC 7953	good / very good (60 °C)	+
Bacillus coagulans DSMZ 1	good / very good	+



Bacillus cereus ATCC 11778



Escherichia coli ATCC 25922

DHL Agar acc. To SAKAZAKI

Deoxycholate hydrogen sulfide lactose agar is used for the detection and isolation of pathogenic Enterobacteriaceae from all types of materials.

IVD *in vitro diagnosticum –*
For professional use only

CE

See also General Instruction of Use
Warnings and precautions see ChemDAT®
(www.chemdat.info)

This medium represents a modified deoxycholate agar as proposed by SAKAZAKI et al. (1960, 1971).

Principle

Microbiological method.

Mode of Action

H_2S production is indicated by a blackening of the colonies due to formation of iron sulfide. Although Proteus is H_2-positive, its colonies are not black. Colonies of Proteus, Morganella, Rettgerella and Providencia are, however, surrounded by dark brown zones, which occur, because these species act on the phenylalanine of the peptone to produce phenylpyruvate, which forms an iron complex with iron(III) ions. The sucrose content of the medium permits differentiation of weakly lactose-positive or lactose-negative, sucrose-positive species from sucrose- and lactose-negative Enterobacteriaceae. The deoxycholate largely suppresses the growth of Gram-positive bacteria and prevents the swarming of Proteus species. This medium provides a rich nutrient base and contains a relatively low concentration of the inhibitor deoxycholate. These properties permit growth of even fastidious strains of Salmonella and Shigella. The colonies formed are considerably larger than those found on other selective culture media. Proteus, Morganella, Rettgerella and Providencia can be differentiated from Salmonella.

Typical Composition (g/litre)

Peptone from casein 10.0; peptone from meat 10.0; meat extract 3.0; lactose 10.0; sucrose 10.0; L-cysteinium chloride 0.2; sodium citrate 1.0; sodium deoxycholate 1.5; sodium thiosulfate 2.0; ammonium iron(III) citrate 1.0; neutral red 0.03; agar-agar 15.0.

Preparation and Storage

Usable up to the expiry date when stored dry and tightly closed at +15 to +25° C. Protect from light.

After first opening of the bottle the content can be used up to the expiry date when stored dry and tightly closed at +15 to +25° C.

Suspend 63.5 g/litre, pour plates to give thick layers (about 20ml per plate).

■ **Do not autoclave.**

pH: 7.2 ± 0.2 at 25 °C.

The plates are clear and red.

Specimen

e.g. Stool.

Clinical specimen collection, handling and processing, see general instructions of use.

Experimental Procedure and Evaluation

Spread the sample or material from an enrichment culture thinly on the surface of the plates.

Incubation: 24-48 hours at 35 °C aerobically.

Appearance of Colonies	Microorganisms
Red surrounded by a zone of precipitate, medium sized, flat	Escherichia coli
Pink with a red centre, often mucoid	Enterobacter, Klebsiella and others
Colourless, sometimes with a black centre	Citrobacter
Colourless, surrounded by a dark brown zone	Proteus mirabilis, Morganella, Rettgerella, Providencia
Red, surrounded by a dark brown zone	Proteus vulgaris
Colourless with a black centre	Salmonella (incl. Arizona)
Colourless, large, flat	Shigella

Literature

SAKAZAKI, R., NAMIOKA, S., OSADA, A., a. YAMADA, C.A.: A problem on the pathogenic role of Citrobacter of enteric bacteria. **- Japan. J. Ex. Med., 30**; 13-22 (1960).

SAKAZAKI, R., TAMURA, K., PRESCOTT, L.M., BENZIC, Z., SANYAL, S.C., a. SINHA, R.: Bacteriological examination of diarrheal stools in Calcutta. **-Indian J. Med. Res., 59**; 1025-1034 (1971).

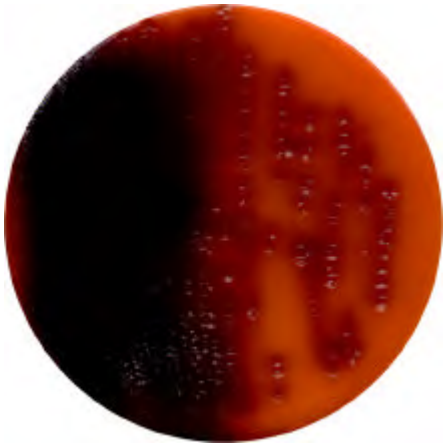
Ordering Information

Product	Merck Cat. No.	Pack size
DHL Agar acc. to SAKAZAKI	1.11435.0500	500 g

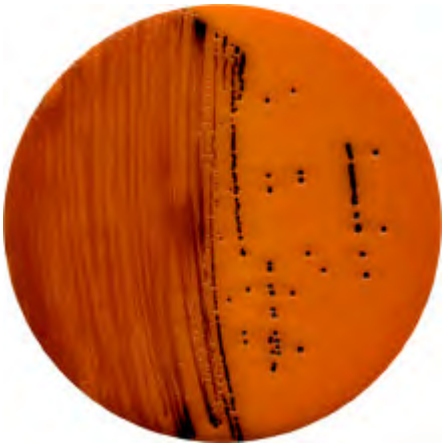
DHL Agar acc. To SAKAZAKI

Quality control; incubation: 24 h at 35 °C

Test strains	Growth	Colony colours	Black centre	Culture Medium
Escherichia coli ATCC 25922	good / very good	red	-	precipitate
Klebsiella pneumoniae ATCC 10031	good / very good	pink	-	
Salmonella typhimurium ATCC 14028	good / very good	colourless	+	-
Salmonella enteritidis ATCC 13076	good / very good	colourless	+	-
Proteus vulgaris ATCC 13315	fair / good	pink	-	brownish zone
Proteus mirabilis ATCC 14153	good / very good	colourless	±	brownish zone
Shigella flexneri ATCC 12022	fair / very good	colourless	-	-
Enterococcus faecalis ATCC 11700	none / poor			
Staphylococcus aureus ATCC 25923	none			
Bacillus cereus ATCC 11778	none			



Proteus mirabilis ATCC 14153



Salmonella enteritidis ATCC 13076

DIASALM Base acc. To VAN NETTEN AND VAN DER ZEE

Diagnostic Salmonella semi-solid Rappaport Vassiliadis (Diasalm) Medium

Diasalm is a diagnostic semi-solid selective motility agar to be used for the isolation of Salmonella spp. in food and environmental samples.

Mode of Action

DIASALM combines the characteristics of semi-solid indole motility agar (SIM Agar) and Rappaport-Vasilliadis (RVS Broth). The selective system exploits the resistance of Salmonella spp. as compared to other Enterobacteriaceae to high osmolarity ($MgCl_2$) and low pH (5.5). The combination of novobiocin and malachite green suppresses the growth of Gram-positive bacteria and most, but not all Gram-negative bacteria. The semi-solid approach simultaneously enriches salmonellae and separates motile salmonellae from most competitive organisms resistant to the selective system. As a result of this on plating agars, salmonellae are rarely overgrown by non salmonellae. Sometimes salmonellae occur mixed with Proteus, Hafnia or Enterobacter spp. as interfering motile Enterobacteriaceae. A diagnostic system consisting of saccharose, lactose and bromocresol purple differentiates salmonellae from lactose and many lactose and saccharose dissimilating organisms. Non motile salmonellae growing at the inoculum spot(s) may produce a grey blackish center.

Typical Composition (g/litre)

Peptone from casein 13.5; peptone from meat 13.5; saccharose 7.5; lactose 0.5; ammonium iron(II) sulfate 0.2; sodium thiosulfate 0.8; potassium dihydrogen phosphate 1.47; magnesium chloride-6-H2O 23.3; malachite green 0.037; bromocresol purple 0.08, agar-agar 2.7.

Preparation

Suspend 64 g in 1 litre of demin. water by heating in a boiling water bath or in a flowing steam until the medium is completely dissolved. Do not autoclave / do not overheat! Dissolve the lyophilisate of 1 vial MSRV Selective Supplement (Cat. No. 1.09874.) by adding 1 ml sterile distilled water and add the solution to the medium cooled to 45-47 °C. Mix gently and pour plates.

pH: 5.5 ± 0.2 at 25 °C.

The prepared medium is clear and dark green. Prepared plates can be stored for up to 1 week at +2 to +8 °C.

The plates must be well dried before use (minimum: 1 h at room temperature).

Experimental Procedure

1. Enrich the sample material in Buffered Peptone Water (incubation: 16-20 h at 35 °C).
2. Inoculate with either 3 drops 83 x 0.03 ml) or 1 drop of 0.1ml of the pre-enrichment culture in the center of DIASALM medium plates.
3. Incubate the plates aerobically in an upright position at 42°C for 12-18 h, but not longer than 24 h.

Evaluation

Motile salmonellae show a purple halo of growth originating from the inoculation spot. Against a white background the purple halo can be surrounded by a light brown black zone. A grey-blackish centre may suggest the presence of non-motile salmonellae, Citrobacter freundii or Proteus spp. On the edge of a typical migration zone a loopful is taken for subculture on plating agars e.g. Rambach agar, BPLS agar, mod. XLD agar. For the confirmation of Salmonella spp. further biochemical serological tests are recommended.

Literature

CURTIS, G.D.W., a. BAIRD, R.M. (eds): Pharmacopoeia of culture media for Food Microbiology: Additional Monographs (II). **- Int. J. of Food Microbiology Vol 1**7; 230-233 (1993)

VAN DER ZEE, H.: Conventional methods for the detection and isolation of salmonella enteritidis. **- Int. J. Food Microbiol., 21**; 41-46 (1994).

PUZICKOVA, V., KARPIKOVA, R., a. PAKROVA, E.: Use of semi-solid medium for the isolation of Salmonella enteritidis.**- Vet. Med. Praha Vol 41 (9)**; 283-288 (1996).

VAN DER ZEE, H., a. VAN NETTEN, P.: Diagnostic selective semi-solid media based on Rappaport-Vassiliadis broth for detection of salmonella spp. and Salmonella enteritidis in foods. **- Proc. Symp. "Salmonella and Salmonellosis" Ploufragen.**; 69-77 (1992).

Ordering Information

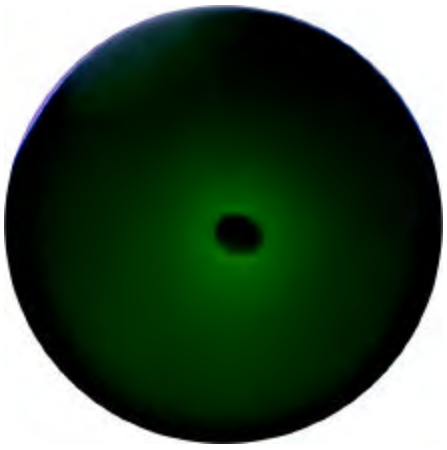
Product	Merck Cat. No.	Pack size
DIASALM Base acc. To VAN NETTEN AND VAN DER ZEE	1.09803.0500	500 g
MSRV Selective-Supplement	1.09874.0001	1 x 16 vials
Peptone Water (buffered)	1.07228.0500	500 g
Peptone Water (buffered)	1.07228.5000	5 kg

DIASALM Base acc. To VAN NETTEN AND VAN DER ZEE

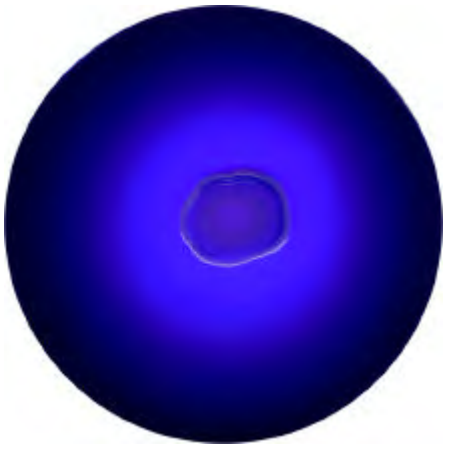
Diagnostic Salmonella semi-solid Rappaport Vassiliadis (Diasalm) Medium

Quality control

Test strains	Growth (swarming)	Motility zone (colour)
Salmonella typhimurium ATCC 14028	+	grey-violet, dark circle
Salmonella abony NCTC 6017	+	grey-violet, dark circle
Salmonella dublin ATCC 15480	+	grey-white, violet, dark circle
Salmonella enteritidis ATCC 13076	+	grey-white, violet, dark circle
Citrobacter freundii ATCC 8090	≤ 20 mm	greenish / yellow
Pseudomonas aeruginosa ATCC 9027	≤ 20 mm	grey-white, violet halo
Enterobacter cloacae ATCC 13047	≤ 20 mm	grey-white
Hafnia alvei ATCC 29926	≤ 20 mm	grey-white
Proteus mirabilis ATCC 29906	≤ 20 mm	grey-white / violet



Citrobacter freundii ATCC 8090



Salmonella enteritidis ATCC 13076

Dichloran Glycerol (DG18) Agar

Selective agar with low water activity (aw) for the enumeration and isolation of xerophilic moulds in dried and semi-dried foods as well as a general purpose medium for counting yeast and moulds in foodstuffs.

Dichloran glycerol (DG 18) agar was formulated by HOCKING and PITT (1980) and is recommended for the enumeration of xerophilic moulds in dried and semi-dried foods, such as dried fruits, meat and fish products, spices, confectionery, cereals, nuts. BECKERS et al. (1982) demonstrated the use of DG 18 as a general purpose medium for counting yeasts and moulds in foodstuffs.

Mode of Action

By reducing the water activity from approx. 0.99 to 0.95 with 18 % (w/w) glycerol and addition of chloramphenicol growth of bacteria is prevented. The inclusion of dichloran serves to inhibit the rapid spreading of mucoraceous fungi and restricts colony sizes of other genera, easing the colony count.

Typical Composition (g/litre)

Peptone 5.0; glucose 10.0; potassium dihydrogen phosphate 1.0; dichloran 0.002; magnesium sulfate 0.5; chloramphenicol 0.1; agar-agar 15.0.

pH:5.6 ± 0.2 at 25 °C.

The prepared plates are clear and yellowish.

Preparation

Suspend 31.6 g in 1 litre of demin water and heat to boiling until completely dissolved. Add 175 ml of glycerol p.a. (Merck Cat. No. 1.04092.) to the medium, mix and autoclave at 121 °C for 15min. Cool to approx. 50 °C, mix well and pour plates.

The appearance of the prepared medium is amber and slightly opalescent. When stored at +2 to +8 °C in the dark, the shelf life of plates is approximately 1 week and in bottles approx. 2months.

Experimental Procedure

Directly inoculate agar plates using surface spreading technique with serial dilutions.

Incubate at 22-25 °C and look for growth after 4, 5 and 6 days.

Interpretation of Results

Count the number of xerophilic colonies per gram of food.

Literature

HOCKING, A.D., and PITT, J.I. (1980) Dichloran-glycerol medium for enumeration of xerophilic fungi from low moisture foods. **Appl. Environm. Microbiol. 39**, 488-492.

BECKERS, H.J., BOER, E., VAN EIKELENBOOM, E., HARTOG, B.J., KUIK, D., MOL, N., NOOITGEDACHT, A.J., NORTHOLD, M.O., and SAMSON, R.A. (1982) Inter. Stand. Org. Document ISO/TC34/SC9/N151.

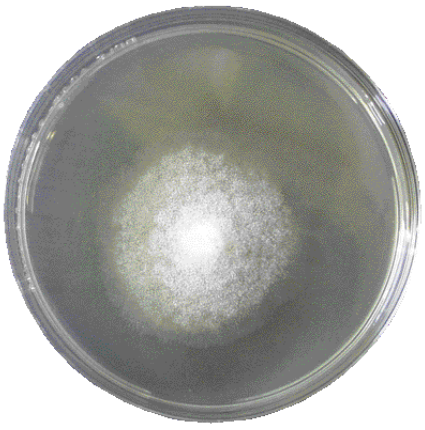
Ordering Information

Product	Merck Cat. No.	Pack size
Dichloran Glycerol (DG18) Agar	1.00465.0500	500 g

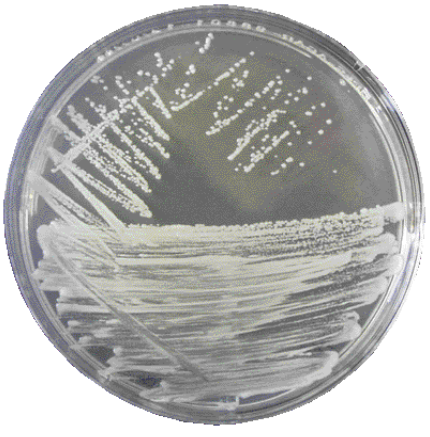
Quality control

Test strains	Growth
Saccharomyces cereviseae ATCC 9763	good / very good
Rhodotorula mucilaginosa DSMZ 70403	good / very good, colony colour: orange
Mucor racemosus ATCC 42647	fair / good
Bacillus subtilus ATCC 6633	none
Escherichia coli ATCC 25922	none

Rhodotorula mucilaginosa



Mucor racemosus



Saccharomyces cerevisiae

Dichloran Rose Bengal Chloramphenicol (DRBC) Agar

Selective agar for the enumeration of food spoiling yeasts and moulds.

Mode of Action

DRBC was developed by KING et al. (1979) and is a modification of Rose-Bengal-Chloramphenicol Agar (RBC from JARVIS (1973). In comparison to RBC, the medium contains Dichloran (0.002 g/l), th pH is lowered to 5.6 and the Rose-Bengal concentration is cut in half (0.025 g/l). This results in an increased inhibition of bacteria and yeasts.

The inclusion of dichloran serves to inhibit the rapid spreading of mucoraceous fungi and restricts colony sizes of other genera, easing the colony count.

Typical Composition (g/litre)

Peptone 5.0; glucose 10.0; potassium dihydrogen phosphate 1.0; dichloran 0.002; magnesium sulfate 0.5; Rose Bengal 0.025; chloramphenicol 0.1; agar-agar 15.0.

pH: 5.6 ± 0.2 at 25 °C.

Preparation

Suspend 31.6 g in 1 litre of demin. water and heat to boiling until completely dissolved. Autoclave the medium at 121 °C for 15min. Cool to approx. 50 °C, mix well and pour plates.

The appearance of the prepared medium is clear and pink. When stored at +2 to +8 °C in the dark, the shelf life of plates is approximately 1 week and in bottles approx. 2 months.

Experimental Procedure

Directly inoculate agar plates using surface spreading technique with serial dilutions.

Incubate at 25 °C and look for growth after 3, 4 and 5 days.

Interpretation of Results

Count the number of colonies per gram of food.

Attention:

Some fungi may be inhibited on this medium. Therefore it is recommended to use Rose Bengal Chloramphenicol Agar (Merck Cat. No. 1.00467.) additionally to examine and identify the complete fungal flora.

Literature

KING, D.A., HOCKING, A.D., and PITT, J.I. (1979) Dichloran-rose bengal medium for enumeration and isolation of moulds from foods. **Appl. Environm. Microbiol. 37**, 959-964.

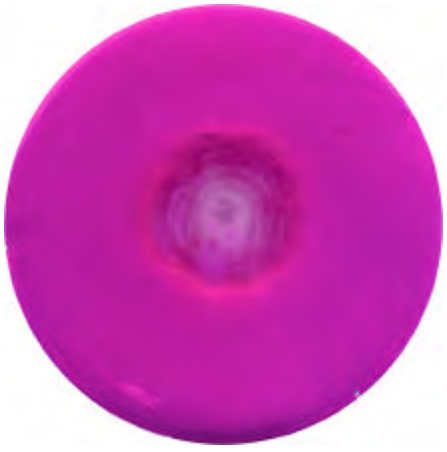
JARVIS, B. 1973 Comparison of an improved rose-bengal-chlortetracycline agar with other media for the selective isolation and enumeration of moulds and yeasts in food. **J. Appl. Bacteriol. 36**, 723-727.

Ordering Information

Product	Merck Cat. No.	Pack size
Dichloran Rose Bengal Chloramphenicol (DRBC) Agar	1.00466.0500	500 g

Quality control

Test strains	Growth
Saccharomyces cereviseae ATCC 9763	good / very good
Rhodotorula mucilaginosa DSMZ 70403	good / very good, colony colour: orange
Mucor racemosus ATCC 42647	fair / good
Bacillus subtilis ATCC 6633	none
Escherichia coli ATCC 25922	none



Mucor racemosus ATCC 42647

Saccharomyces cerevisiae ATCC 9763

Differential Clostridial Agar (DCA) acc. to WEENK

For the enumeration of sulfite-reducing clostridia in dried foods.

Mode of Action

The medium consists of a nutritionally rich base medium, including starch to promote spore germination. Resazurin is added as redox-indicator, turning red at high redox-potential, indicating aerobic conditions. Sulfite and an iron source are added as indicators. Sulfite redicing clostridia produce sulfide from sulfite, which gives a black precipitate with the iron present in the medium. Sulfite reducing clostridia are enumerated as black colonies.

Typical Composition (g/litre)

Peptone from casein 5.0; peptone from meat 5.0; meat extract 8.0; starch 1.0; D(+)glucose 1.0; yeast extract 1.0; cysteinium chloride 0.5; resazurin 0.002; agar-agar 20.0.

Preparation

Suspend 41.5 g in 1 litre of demin. water and autoclave (15 min at 121 °C).

Cool to about 48 °C and aseptically add, just before use, 5 ml/ litre medium freshly prepared ferric(III) ammonium citrate solution (1 g in 5 ml demin. water, heat sterilized: 15 min. at 121°C) and 1.0ml/litre sodium sulfite solution (1.06657.; 2.5g in 10 ml demin. water, filter sterilized).

The complete medium is yellowish to reddish-brown. The medium is to be used immediately. **Do not store.** The base medium can be stored for at least 2 weeks at 4 °C.

pH: 7.6 ± 0.2 at 25 °C.

Experimental Procedure and Evaluation

1 ml sample per plate, pour-plate method. After solidification the plates are overlaid with sterile DCA.

Incubation: At 30 °C for 3 days anaerobically (e.g. with Anaerocult®, Anaerocult® A mini)

Reading of results and interpretation:

Discrete black colonies of 1-5 mm in diameter are considered to be presumptive sulfite-reducing clostridia.

Note: In order to facilitate spore germination, a heat treatment of the spores/sample of 10 minutes at 30 °C before inoculation of the agar is recommended.

Literature

WEENK, G., FITZMAURICE, E., MOSSEL, D.A.A.: Selective enumeration of spores of Clostridium species in dried foods. **- J. Appl. Bact., 70**; 135-143 (1991).

Ordering Information

Product	Merck Cat. No.	Pack size
Differential Clostridial Agar (DCA) acc. to WEENK	1.10259.0500	500 g
Anaerocult® A	1.13829.0001	1 x 10
Anaerocult® A mini	1.01611.0001	1 x 25

Quality control

Test strains	Recovery rate (%)	Growth	Black colonies
Clostridium perfringens ATCC 10543	≥ 70	good / very good	+
Clostridium sporogenes ATCC 19404	≥ 70	good	+
Clostridium bifermentans ATCC 19299	≥ 70	good	+
Clostridium perfringens ATCC 13124	≥ 70	good	+
Bacillus licheniformis ATCC 14580		poor / fair	-

Differential Reinforced Clostridial Broth (DRCM)

Medium proposed by GIBBS and FREAME (1965) for the enumeration of all clostridia by the MPN method in foodstuffs and other materials.

This culture medium was successfully utilized by FREAME and FITZPATRICK (1971) and GIBBS (1973) to isolate and count clostridia. The Institute for Food Technology and Packing of the Technical University of Munich (Institut für Lebensmittel-technologie und Verpackung der TU München) (1976) recommends this medium for the examination of packing materials. It complies with the requirements of the DIN Norm 38411 for the examination of water.

Mode of Action

Differential Reinforced Clostridial Broth represents a development of the Reinforced Clostridial Media proposed by HIRSCH and GRINDSTED (1954) and GIBBS and HIRSCH (1956). The redox indicator resazurin is used to monitor anaerobiosis. Clostridia reduce sulfite to sulfide, the formed iron sulfide causes the culture medium to turn black. As other bacteria can also produce sulfide, vegetative forms must first be removed from the culture by a relevant treatment (e.g. pasteurization), and the anaerobic spore-forming micro- organisms must then be identified. GIBBS and FREAME (1956) inhibited the growth of most non-spore-forming microorganisms by adding polymyxin (70 IU/ml) to the broth.

Typical Composition (g/litre)

Peptone from casein 5.0; peptone from meat 5.0; meat extract 8.0; yeast extract 1.0; starch 1.0; D(+)glucose 1.0; L-cysteinium chloride 0.5; sodium acetate 5.0; sodium di-sulfite 0.5; ammonium iron(II) citrate 0.5; sodium resazurin 0.002.

Preparation

Suspend 27.5 g/litre, dispense into test tubes, autoclave (15 min at 121 °C).

pH: 7.1 ± 0.2 at 25 °C.

The ready-to-use broth in the tube is clear and reddish-brown.

- **The prepared culture medium can be stored for up to 2weeks.**

Experimental Procedure and Evaluation

Inoculate the culture medium, cover with a 3 to 5 mm layer of sterilized paraffin viscous and pasteurize (30 min at 75 °C in a water bath!).

Incubation: at least 7 days at 30 °C.

Microbial growth can usually be seen after 3-4 days. The cultures should be observed for up to 4 weeks as occasionally some time is required for spore germination to start. The cultures should be checked for a black colouration. Further tests should be performed to identify the clostridia.

Literature

Arbeitsgruppe des Instituts für Lebensmitteltechnologie und Verpackung an der Technischen Universität München: Merkblätter für die Prüfung von Packmitteln. Merkblatt 28. "Bestimmung von Clostridiensporen in Papier, Karton, Vollpappe und Wellpappe." **- Verpackungs-Rdsch., 27/20**; Techn. wiss. Beilage, 82-84 (1976).

DIN Deutsches Institut für Normung e.V.: Deutsche Einheitsverfahren zur Wasser-, Abwasser- und Schlammuntersuchung. Mikrobiologische Verfahren (Gruppe K). Nachweis von sulfitreduzierenden sporenbildenden Anaerobiern (K 7). **- DIN 38411**

FREAME, B., a. FITZPATRICK, B.W.F.: The use of Differential Reinforced Clostridial Medium for the isolation and enumeration of Clostridia from food. - In "Isolation of Anaerobes" ed. by SHAPTON, D.A., a. BOARD, R.G., Academic Press, London, New York, 48-55 (1972).

GIBBS, B.M.: The detection of Clostridium welchii in the Differential Clostridial Medium technique. **- J. Appl. Bact., 36**; 23-33 (1973).

GIBBS, B.M., a. FREAME, B.: Methods for the recovery of clostridia from foods. **- J. Appl. Bact., 28**; 95-111 (1956).

GIBBS, B.M., a. HIRSCH, A.: Spore formation by Clostridium species in an artificial medium **- J. Appl. Bact., 19**; 129-141 (1956).

HIRSCH, A., a. GRINSTED, E.: Methods for the growth and enumeration of anaerobic spore-formers from cheese, with observations on the effect on nisin. **- J. Dairy Res., 21**; 101-110 (1954).

Ordering Information

Product	Merck Cat. No.	Pack size
Differential Reinforced Clostridial Broth (DRCM)	1.11699.0500	500 g
Paraffin viscous	1.07160.1000	1 l
Polymyxin-B-sulfate	CN Biosciences	

Quality control

Test strains	Growth	Blacking
Escherichia coli ATCC 25922	good / very good	-
Bacillus cereus ATCC 11778	fair / good	-
Pseudomonas aeruginosa ATCC 27853	poor / fair	-
Clostridium bifermentans ATCC 19299	good / very good	+
Clostridium perfringens ATCC 10543	good / very good	+
Clostridium perfringens ATCC 13124	good / very good	+
Clostridium sporogenes ATCC 11437	good / very good	+
Clostridium sporogenes ATCC 19404	good / very good	+

DNase Test Agar

For detecting microbial DNase (deoxyribonuclease) by the method of JEFFRIES et al. (1957) and for identifying microorganisms, especially DNase-positive staphylococci.

This culture medium complies with the recommendations of the International Organization for Standardisation (ISO) (1977).

Mode of Action

Colonies producing DNase hydrolyse the deoxyribonucleic acid (DNA) content of this medium located in their immediate vicinity. If the medium is then flooded and acidified with 1 N HCl, the DNA precipitates out (turbidity) and clear zones appear around DNase-positive colonies. Some authors recommend instead flooding the medium with toluidine blue solution (STREITFIELD et al. 1962) or the use of DNase test agars containing toluidine blue (SCHREIER 1969) or methyl green (SMITH et al. 1969).

Staphylococci can also be differentiated by exploiting the fact that they metabolize mannitol to form acid, in this case mannitol and a pH indicator must be added to the culture medium.

Typical Composition (g/litre)

Tryptose 20.0; sodium chloride 5.0; deoxyribonucleic acid 2.0; agar-agar 15.0.

Preparation

Suspend 42 g/litre, autoclave (15 min at 121 °C), pour plates.

pH: 7.3 ± 0.2 at 25 °C.

The plates are clear and yellowish-brown.

Addition of mannitol: Prior to autoclaving the culture medium add 10 g mannitol/litre and, as an indicator, 0.025 g bromothymol blue/litre or 0.025 g phenol red/litre and mix thoroughly.

Experimental Procedure and Evaluation

Inoculate by streaking a pure culture of the organism to be tested onto the surface of the test agar. Several strains can be inoculated onto one plate (divide the plate into sectors or make parallel streaks).

Incubation: under optimal conditions (in the case of staphylococci, 24 hours at 35 °C aerobically).

When necessary first check the plates for mannitol fermentation, then carefully flood the surface of the plates with 1 N hydrochloric acid.

Appearance of Colonies	Microorganisms
Mannitol:	
Yellow, surrounded by a yellow zone	Mannitol-positive
Colourless or the same colour as the culture medium	Mannitol-negative
1 N HCl:	
Well defined, clearer zones in an otherwise turbid culture medium	DNase-positive
No clear zones	DNase-negative

Literature

International Organization for Standardization: Meat and meat products -Detection and enumeration of Staphylococcus aureus (Reference methods). **-Draft International Standard ISO/DIS 5551** (1977).

JEFFRIES, C.D., HOLTMANN, D.F., a. GUSE, D.G.: Rapid method for determining the activity of microorganisms on nucleic acid. **- J. Bact., 73**; 590-591 (1957).

SCHREIER, J.B.: Modification of Deoxyribonuclease Test Medium for rapid identification of Serratia marcescens. **- Amer. J. Clin. Pathol., 51**; 711-716 (1969).

SMITH, P.B., HANCOCK, G.A., a. RHODEN, D.L.: Improved Medium for Detecting Deoxyribonuclease-Producing Bacteria. **- Appl. Microbiol., 18**; 991-993 (1969).

STREITFIELD, M.M., HOFFMANN, E.M., a. JANKLOW, H.M.: Evaluation of extra-cellular deoxyribonuclease activity in Pseudomonas. **- J. Bact., 84**; 77-80 (1962).

Ordering Information

Product	Merck Cat. No.	Pack size
DNase Test Agar	1.10449.0500	500 g
Bromothymol blue indicator	1.03026.0005	5 g
D(-)Mannitol	1.05982.0500	500 g
Hydrochloric acid 1 mol/l	1.09057.1000	1 l
Phenol red indicator	1.07241.0005	5 g

DNase Test Agar

Quality control

Test strains	Growth	Clear zones
Staphylococcus aureus ATCC 25923	good / very good	+
Staphylococcus aureus ATCC 6538	good / very good	+
Staphylococcus epidermidis ATCC 12228	good / very good	-
Escherichia coli ATCC 25922	good / very good	-
Serratia marcescens ATCC 14756	good / very good	+
Bacillus cereus ATCC 11778	good / very good	+

Staphylococcus aureus ATCC 25923

Escherichia coli ATCC 25922

EC Broth

For the selective identification of coliform bacteria and Escherichia coli in water, foodstuffs and other materials according to HAJNA and PERRY (1943).

This Escherichia coli broth complies with the recommendations of the Standard Methods for the Examination of Water and Wastewater (1998).

Mode of Action

The lactose content of this medium favours the growth of lactose-positive bacteria, especially of coliform bacteria and E. coli. The bile salts, however, largely inhibit the growth of Gram-positive bacteria or microorganisms which are not adapted to the intestinal environment. Lactose-positive bacteria metabolize lactose with gas formation.

Typical Composition (g/litre)

Peptone from casein 20.0; lactose 5.0; bile salt mixture 1.5; sodium chloride 5.0; di-potassium hydrogen phosphate 4.0; potassium dihydrogen phosphate 1.5.

Preparation

Suspend 37 g or 74 g/litre, fill into test tubes fitted with DURHAM tubes, autoclave (15 min at 121 °C).

pH: 6.9 ± 0.2 at 25 °C.

The prepared broth is clear and yellowish-brown.

Experimental Procedure and Evaluation

Small aliquots (approx. 1 ml) of the sample material are added to the normal-strength broth, large quantities should be mixed with double-strength broth in order to maintain the normal concentration of the broth.

Incubation: 24-48 hours at 44.5 °C aerobically.

Gas formation at 44.5 °C:	Escherichia coli, possibly also other coliform bacteria

Literature

American Public Health Association, American Water Works Association and Water Pollution Control Federation: Standard Methods for Examination of Water and Wasterwater, 20th ed., Washington, 1998.

FISCHBEIN, M., a. SURKIEWICZ, B.F.: Comparison on the recovery of Escherichia coli from frozen foods and nutmeats confirmatory incubation in EC-medium at 44.5 and 45.5 °C. **- Appl. Microbiol., 12**; 127-131 (1964).

HAJNA, A.A., PERRY, C.A.: Comparative study of presumptive and confirmative media for bacteria of the coliform group and for fecal Streptococci. **-Am. J. Publ. Hlth., 33**; 550-556 (1943).

PERRY, C.A., a. HAJNA, A.A.: Further evaluation of EC-medium for the isolation of coliform bacteria and Escherichia coli. **- Am. J. Publ. Hlth., 34**; 735-738 (1944).

TENNANT, A.D., REID, L.E., ROCKWELL, L., a. BYNDE, E.T.: Coliform bacteria in sea water and shellfish. II. The E.C. confirmation test for Escherichia coli **- Can. J. Microbiol., 7**; 733-739 (1961).

Ordering Information

Product	Merck Cat. No.	Pack size
EC Broth	1.10765.0500	500 g

Quality control

Test strains	Growth at 44.5 °C	Gas formation at 44.5 °C
Escherichia coli ATCC 25922	good	+
Escherichia coli ATCC 8739	good	+
Enterobacter cloacae ATCC 13047	none / fair	-
Klebsiella pneumoniae ATCC 13883	none / fair	-
Citrobacter freundii ATCC 8090	none / fair	-
Proteus mirabilis ATCC 14153	none / fair	-
Pseudomonas aeruginosa ATCC 27853	none / poor	-
Clostridium perfringens ATCC 10543	none / poor	-
Enterococcus faecalis ATCC 19433	none / poor	-

E. coli / Coliform Selective Supplement

Additive for the preparation of selective culture media for the detection of E.coli/Coliforms.

Mode of Action

E.coli/Coliform supplement is a mixture of two antibiotics in lyophilized form.

Vancomycin inhibits the growth of gram-positive bacteria, Pseudomonas spp. and Aeromonas spp. are supressed by Cefsulodine.

Composition (per vial)

Vancomycin 2.5 mg; Cefsulodine 2.5 mg

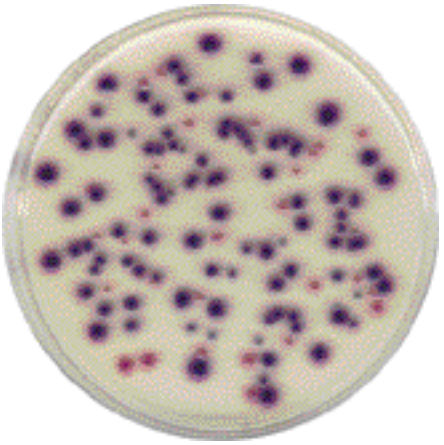
Preparation

The lyophilisate is suspended in the original vial by adding 2 ml sterile distilled water. After short vigorous shaking the solution is clear.

The contents of 1 vial is mixed evenly into 500 ml of sterile medium base cooled to about 45-50 °C.

Ordering Information

Product	Merck Cat. No.	Pack size
E. coli / Coliform Selective Supplement	1.00898.0001	1 x 16 vials



Result* with E.coli/Coliform Selective Supplement

Result' without E.coli/Coliform Selective Supplement

***Chromocult® Coliform Agar inoculated with:**

Escherichia coli (blue)	ATCC 11775
Citrobachter freundii (red)	ATCC 8090
Hafnia alvati	ATCC 29926
Aeromonas hydrophila	ATCC 7966
Pseudomonas aeroginosa	ATCC 27853
Staphylococcus cohnii	ATCC 29974
Bacillus licheniformis	ATCC 14580

Egg-yolk Emulsion (sterile)

Mode of Action

Egg-yolk Emulsion is used as an additive (e.g. Cereus Selective Agar Base acc. to MOSSEL, Merck, Cat. No. 1.05267., and Potassium Thiocyanate Actidione® Sodium Azide Egg-yolk Pyruvate Agar Base, Merck, Cat. No. 1.05395.) and permits the detection of microbial lecithinase activity.

Typical Composition

Sterile egg-yolk 500ml; NaCl 4.25 g; distilled water to give a final volume of 1000ml.

Experimental Procedure

Shake the bottle well to suspend any sediment. Mix 100 ml with 0.9litre of the culture media which has been sterilized and cooled to 45-50°C. Pour plates.

■ **Observe sterile procedure when emptying the bottle!**

Storage

In the refrigerator (+2 °C to +8°C).

Ordering Information

Product	Merck Cat. No.	Pack size
Egg-yolk Emulsion (sterile)	1.03784.0001	10 x 100 ml

Egg-yolk Tellurite Emulsion 20% (sterile)

Mode of Action

Egg-yolk tellurite emulsion is used as an additive for BAIRD-PARKER Agar (Merck, Cat. No. 1.05406.) and permits the detection of lecithinase activity and tellurite reduction.

Typical Composition

Sterile egg-yolk 200ml; NaCl 4.25 g; potassium tellurite 2.1 g; distilled water to give a final volume of 1000ml.

Experimental Procedure

Shake the bottle well to suspend any sediment. Mix 50ml with 950 ml of the reconstituted culture medium which has been sterilized and cooled to 45-50°C. Pour plates.

- **Observe sterile procedure when emptying the bottle!**

Storage: in the refrigerator (+2 °C to +8 °C).

- **Plates prepared with egg-yolk tellurite emulsion, unlike those made with an egg-yolk emulsion and a separate potassium tellurite solution, are stable for about 2 months. The ready plates can be stored int the refrigerator.**

Ordering Information

Product	Merck Cat. No.	Pack size
Egg-yolk Tellurite Emulsion 20% (sterile)	1.03785.0001	10 x 50 ml

EMB Agar (Eosin Methylene-blue Lactose Sucrose Agar)

Selective agar proposed by HOLT-HARRIS and TEAGUE (1916) for the detection and isolation of pathogenic Enterobacteriaceae.

IVD *in vitro diagnosticum –*
For professional use only

See also General Instruction of Use
Warnings and precautions see ChemDAT®
(www.chemdat.info)

Principle

Microbiological method

Mode of Action

The lactose and sucrose contained in this medium allow lactose- and sucrose-negative salmonellae and shigellae to be distinguished from lactose-positive coliform organisms and lactose-negative, sucrose-positive, accompanying flora (e.g. Proteus vulgaris, Citrobacter, Aeromonas hydrophila). The growth of undesired accompanying microorganisms, particularly Gram-positive bacteria, is largely inhibited by the dyes present in the medium.

Typical Composition (g/litre)

Peptones 10.0; di-potassium hydrogen phosphate 2.0; lactose 5.0; sucrose 5.0; eosin Y, yellowish 0.4; methylene blue 0.07; agar-agar 13.5.

Preparation and Storage

Usable up to the expiry date when stored dry and tightly closed at +15 to +25° C. Protect from light.

After first opening of the bottle the content can be used up to the expiry date when stored dry and tightly closed at +15 to +25° C.

Suspend 36 g/litre, autoclave (15 min at 121 °C), pour plates.

pH: 7.1 ± 0.2 at 25 °C.

The plates are clear and reddish-brown to violet-brown.

Specimen

e.g. Stool. Urine.

Clinical specimen collection, handling and processing, see general instructions of use.

Experimental Procedure and Evaluation

Inoculate by spreading the sample material thinly on the surface of the plates.

Incubation: 24 hours at 35 °C aerobically.

Appearance of Colonies	Microorganisms
Translucent, amber coloured	Salmonella, Shigella
Greenish, metallic sheen in reflected light, blue-black centre in transmitted light	Escherichia coli
Colonies are larger than those of E. coli, mucoid, confluent, gray-brown centre in transmitted light	Enterobacter, Klebsiella and others

Literature

HOLT-HARRIS, J.E., a. TEAGUE, O.A.: A new culture medium for the isolation of Bacillus typhosus from stools. **- J. Infect. Dis., 18** ; 596-600 (1916).

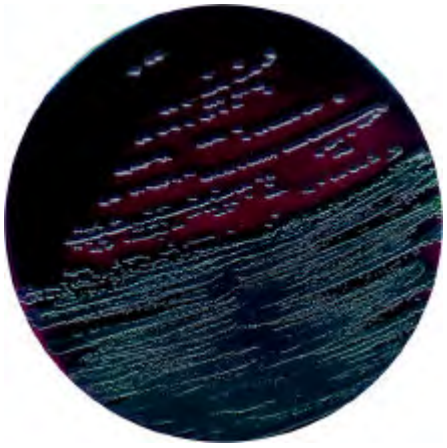
Ordering Information

Product	Merck Cat. No.	Pack size
EMB Agar (Eosin Methylene-blue Lactose Sucrose Agar)	1.01347.0500	500 g

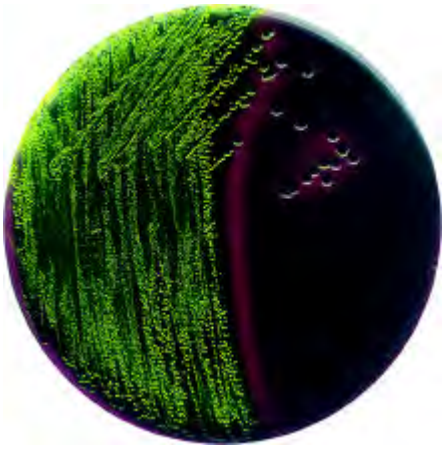
EMB Agar (Eosin Methylene-blue Lactose Sucrose Agar)

Quality control

Test strains	Growth	Colony colour	Metallic sheen
Escherichia coli ATCC 25922	good / very good	violet	+
Escherichia coli ATC 11775	good / very good	violet	+
Escherichia coli 194	good / very good	violet	+
Escherichia coli ATCC 23716	good / very good	violet	+
Escherichia coli ATCC 8739	good / very good	violet	+
Enterobacter cloacae ATCC 13047	fair / very good	pink, dark centre	+ / -
Salmonella typhimurium ATCC 14028	good / very good	colourless, transparent	-
Shigella flexneri ATCC 12022	good / very good	colourless, transparent	-
Bacillus cereus ATCC 11778	none / poor		-
Klebsiella pneumoniae ATCC 13883	fair / very good	pink, dark centre	+ / -



Enterobacter cloacae ATCC 13047



Escherichia coli ATCC 25922

ENDO Agar

Selective culture medium for the detection and isolation of E. coli and coliform bacteria in various materials according to ENDO (1904)

This medium complies with the "Standard Methods for the Examination of Water and Wastewater" (1992).

Mode of Action

Sodium sulfite and fuchsin inhibit the growth of gram-positive bacteria. E. Coli and coliform bacteria metabolize lactose with the production of aldehyde and acid. The aldehyde liberates fuchsin from the fuchsin-sulfite compound, the fuchsin then colours the colonies red. In the case of E. coli, this reaction is so intense that the fuchsin crystallizes out giving the colonies a permanent greenish metallic sheen (fuchsin sheen). Lactose-negative and wealkly lactose-positive E. coli do not show any fuchsin sheen.

Typical Composition (g/litre)

Peptones 10.0; di-potassium hydrogen phosphate 2.5; lactose 10.0; sodium sulfite, anhydrous 3.3; pararosanilin (fuchsin) 0.3; agar-agar 12.5.

Preparation

Suspend 39 g/litre, autoclave (15 min at 121 °C), pour plates.

The plates are clear and pale pink.

If the culture medium is somewhat too red after it has solidified, the red colouration can be removed by adding a few drops (max. 1 ml/litre) of a freshly prepared 10 % sodium sulfite solution and then boiling.

pH: 7.4 ± 0.2 at 25 °C.

On exposure to oxygen the plated culture medium gradually becomes red due to the oxidation of sulfite and can thus no longer be used. It can only be kept for a few days even if it is stored in the dark and at refrigerator temperature.

Experimental Procedure and Evaluation

Inoculate the plates by the streak-plate method.

Incubation: 24 hours at 35 °C aerobically.

Appearance of Colonies	Microorganisms
Red	Lactose-positive:
Red with a permanent metallic sheen	Escherichia coli
Red to reddish, hemispherical, mucoid	Enterobacter aerogenes, Klebsiella and others
Colourless, clear	Lactose-negative

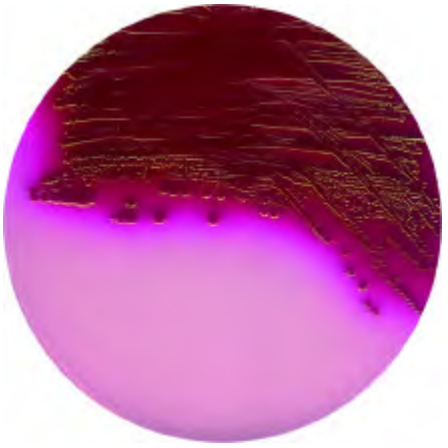
Literature

American Public Health Association, American Water Works Association and Water Pollution Control Federation: Standard Methods for the Examination of Water and Wastewater, 20th ed., Washington, 1998.

ENDO, S.: Über ein Verfahren zum Nachweis von Typhusbacillen. **-Centralbl. Bakt. I. Orig., 35**; 109-110 (1904).

Ordering Information

Product	Merck Cat. No.	Pack size
ENDO Agar	1.04044.0500	500 g
Sodium sulfite	1.06657.0500	500 g



Escherichia coli
194

Shigella flexneri
ATCC 12022

ENDO Agar

Quality control

Test strains	Growth	Colour change to red	Metallic sheen
Escherichia coli ATCC 25922	good / very good	+	+
Escherichia coli 194	good / very good	+	+
Escherichia coli ATCC 11775	good / very good	+	+
Enterobacter cloacae ATCC 13047	good / very good	+ (poor)	±
Klebsiella pneumoniae ATCC 13883	good / very good	+	-
Salmonella typhimurium ATCC 14028	good / very good	-	-
Shigella flexneri ATCC 12022	good / very good	-	-
Proteus mirabilis ATCC 14153	good / very good	-	-
Enterococcus faecalis ATCC 11700	none / fair	-	-

Enterobacteriaceae Enrichment Broth acc. to MOSSEL

Medium proposed by MOSSEL et al. (1963, 1964) for the selective enrichment of all species of Enterobacteriaceae from foodstuffs and other materials.

This medium complies with ISO 21528-1 the specifications of the Eiprodukte-Verordnung (German Egg Product Regulations) (1975) and the European Pharmacopeia II.

Mode of Action

The undesired, accompanying bacterial flora is almost completely inhibited by brilliant green and ox bile. Dextrose favours the growth of all Enterobacteriaceae. The strong buffering capacity of the culture medium prevents the formed acid from killing the culture.

Typical Composition (g/litre)

Peptones 10.0; D(+)glucose 5.0; ox bile, dried 20.0; brilliant green 0.0135; di-sodium hydrogen phosphate-dihydrate 8.0; potassium dihydrogen phosphate 2.0.

Preparation

Suspend 45 g/litre, dispense into test tubes and autoclave under mild conditions (5 min at 121 °C), or heat at 100 °C for 30minutes in a waterbath or flowing steam.

pH: 7.2 ± 0.2 at 25 °C.

The prepared broth is clear and green.

Experimental Procedure and Evaluation

Inoculate the broth with the sample material.

Incubation: 24-48 hours at 35 °C aerobically.

If the medium shows bacterial growth, transfer some of the resulting material to selective culture media.

Literature

Bundesminister für Jugend, Familie und Gesundheit: Verordnung über die gesundheitlichen Anforderungen an Eiprodukte und deren Kennzeichnung (Eiprodukte-Verordnung) (1975).

INTERNATIONAL ORGANIZATION FOR STANDARDIZATION: Microbiology of food and animal feeding stuffs - Horizontal methods for the detection and enumeration of Enterobacteriaceae - Part1: Detection and Enumeration by MPN technique with pre-enrichment. International Standard ISO 21528-1 (2004)

European Pharmacopeia II. Chaptre VIII. 10.

MOSSEL, D.A.A., u. MARTIN, G.: Eine mit dem Salmonella-Nachweis kommensurable Untersuchung von Lebens- und Futtermitteln auf Enterobacteriaceae. **- Arch. f. Lebensmittelhyg., 15**; 169-171 (1964).

MOSSEL, D.A.A., MENGERINCK, W.J.H., a. SCHOLTS, H.H.: Use of a modified MacConkey agar medium for the selective growth and enumeration of all Enterobacteriaaceae. **- J. Bact., 84**; 381 (1962).

MOSSEL, D.A.A., VISSER, M., a. CORNELISSEN, A.M.R.: The examination of foods for Enterobacteriaceae using a test of the type generally adapted for the detection of salmonellae. **- J. Appl. Bact., 24**; 444-452 (1963).

Ordering Information

Product	Merck Cat. No.	Pack size
Enterobacteriaceae Enrichment Broth acc. to MOSSEL	1.05394.0500	500 g
Enterobacteriaceae Enrichment Broth acc. to MOSSEL	1.05394.5000	5 kg

Quality control

Test strains	Growth
Escherichia coli ATCC 8739	good
Escherichia coli ATCC 11775	good
Shigella flexneri ATCC 12022	good
Salmonella typhimurium ATCC 14028	good
Proteus vulgaris ATCC 13315	fair / good
Yersinia enterocolitica ATCC 9610	fair / good
Staphylococcus aureus ATCC 6538	inhibited
Micrococcus luteus ATCC 10240	inhibited
Bacillus cereus ATCC 11778	inhibited

Fluid Thioglycollate Medium

For cultivation and isolation of obligate and facultative anaerobic and microaerophilic bacteria and for sterility tests.

Both culture media comply with the recommendations of United States Pharmacopeia XXVI (2003), the European Pharmacopeia and APHA (1992).

Mode of Action

The reducing agents thioglycollate and cystine ensure an anaerobiosis which is adequate even for fastidious anaerobes. The sulfhydryl groups of these substances also inactivate arsenic, mercury and other heavy metal compounds.

The thioglycollate media are thus suitable for the examination of materials which contain heavy metals or heavy metal preservatives. The higher viscosity of the Fluid Thioglycollate Medium prevents rapid uptake of oxygen. Any increase in the oxygen content is indicated by the redox indicator sodium resazurin which changes its colour to red.

Typical Composition (g/litre)

Peptone from casein 15.0; yeast extract 5.0; D(+)glucose 5.5; L-cystine 0.5; sodium chloride 2.5; sodium thioglycollate 0.5; sodium resazurin 0.001; agar-agar 0.75.

Preparation

Suspend 30 g Fluid Thioglycollate Medium/litre, dispense into tubes, autoclave (15min at 121 °C).

pH: 7.1 ± 0.2 at 25 °C.

The prepared media are clear and yellowish.

- **The culture media should always be freshly prepared. Fluid Thioglycollate Medium cannot be used if more than the upper third of the butt has turned pink due to the presence of oxygen and if this colouration does not disappear after boiling once.**

Experimental Procedure and Evaluation

Inoculate the culture medium with the sample material taking care that the sample reaches the bottom of the tubes. In order to ensure anaerobiosis, the medium can then be overlayed with 1cm of sterile liquid paraffin or agar solution.

Incubation: several days at the optimal incubation temperature (35-37 °C).

Anaerobes grow in the lower part of the culture.

Literature

American Public Health Association: Compendium of methods for the microbiological examination of foods. - 3rd ed. (1992).

European Pharmacopeia II, Chapter VIII. 3.

United States Pharmacopeia XXVI, Chapter "Microbial Limit Tests", 2003.

Ordering Information

Product	Merck Cat. No.	Pack size
Fluid Thioglycollate Medium	1.08191.0500	500 g
Fluid Thioglycollate Medium	1.08191.5000	5 kg
Agar-agar purified	1.01614.1000	1 kg
Paraffin viscous	1.07160.1000	1 l

Quality control

Test strains	Growth
Staphylococcus aureus ATCC 6538	good
Bacillus subtilis ATCC 6633	good
Clostridium sporogenes ATCC 19404	good
Bacteroides vulgatus ATCC 8482	good
Micrococcus luteus ATCC 9341	good
Pseudomonas aeruginosa ATCC 9027	good
Escherichia coli ATCC 25922	good
Clostridium sporogenes ATCC 11437	good

Fluid Thioglycollate Medium G

For the cultivation and isolation of obligate and facultative anaerobic and microaerophilic microorganisms and for sterility tests.

The medium comply with the recommendations of USP, EP and APHA. Formulation is identical to Fluid Thioglycollate Medium with the exception that synthetic agar-agar is used.

Mode of Action

This culture medium is more transparent than the classical Thioglycollate Medium and is therefore especially suitable for performing sterility tests when large volumes and long incubation periods are required. The reducing components thioglycollate and cystine ensure adequate anaerobiosis even in the case of fastidious anaerobes. Possible entry of atmospheric oxygen is indicated by the redox indicator resazurin, which then changes its colour to red. Addition of calcium or magnesium ions to the culture medium increases its solidity.

Typical Composition (g/litre)

Peptone from casein 15.0; yeast extract 5.0; D(+)glucose 5.5; L-cystine 0.5; sodium chloride 2.5; sodium thioglycollate 0.5; sodium resazurin 0.001; gelling agent (synthetic agar-agar) 0.75.

Preparation

Suspend 29 g/litre, dispense into tubes or flasks and autoclave (15 min at 121 °C)

pH: 7.1 ± 0.2 at 25 °C.

The prepared medium is clear and yellow.

- **The culture medium should, if possible, be freshly prepared. After autoclaving, it should not be placed immediately in the refrigerator, but should be allowed to cool at room temperature to minimize entry of atmospheric oxygen. The prepared medium can be stored for up to 3months in an air-tight vessel. It is not for use, if more than one third has turned pink due to the entry of oxygen and if this colouration does not disappear on heating once.**

Experimental Procedure and Evaluation

Inoculate the culture medium with the sample to the bottom of the vessel. In order to ensure anaerobiosis, the medium can then be covered with an approximately 1 cm layer of sterile liquid paraffin.

Incubation: several days at 30 – 35°C aerobically or as otherwise specified. Anaerobes grow in the lower part of the culture tube.

The classical thioglycollate culture medium should be used to test materials that contain large amounts of calcium or magnesium ions.

Ordering Information

Product	Merck Cat. No.	Pack size
Fluid Thioglycollate Medium G	1.16761.0500	500 g
Fluid Thioglycollate Medium G	1.16761.5000	5 kg
Paraffin viscous	1.07162.1000	1 l

Quality control

Test strains	Growth
Staphylococcus aureus ATCC 6538	good
Bacillus subtillis ATCC 6633	good
Clostridium sporogenes ATCC 19404	good (anaerobic)
Bacteroides vulgatus ATCC 8482	good (anaerobic)
Clostridium sporogenes ATCC 11437	good (anaerobic)
Escherichia coli ATCC 25922	good
Micrococcus luteus ATCC 9341	good
Pseudomonas aeruginosa ATCC 9027	good

Fluorocult® dehydrated culture media

Culture media for the rapid detection of E. coli using fluorescence.

Mode of Action

The detection of characteristic bacterial enzymes offers the possibility of a rapid identification of bacteria. Preferably constitutive enzymes are detected here, i.e. enzymes which show considerable activity independent of growth conditions. Apart from a few Salmonella and Shigella strains, E.coli is the only species belonging to the Enterobactericeae which contains the enzyme β-D-glucuronidase. This can split the substrate 4-methylumbelliferyl-β-D-glucuronide (MUG) forming 4-methylumbelliferone which can be identified as it fluorescens under long wave UV light. Thus a strong suggestion for the presence of E.coli can be obtained.

Fluorocult® culture media have the same components as the standard culture media and additionally the substrate MUG. They can therefore be used and evaluated in the usual way and provide the additional possibility of UV light examination for E.coli colonies. Some of the media also contain tryptophan as the substrate for a possible indole reaction to further confirm E.coli presence.

Note: The intensity of fluorescence is reduced with acidic pH. By adding some 1N NaOH-solution, the fluorescence is increased. When continuing with the cultures, the pH-adjustment should be done with a separate aliquot.

Fluorocult® Brillant Green 2 %-Bile (BRILA) Broth

Mode of Action

Bile and brilliant green almost completely inhibit the growth of undesired microbial flora, in particular GRAM-positive microorganisms. E. coli shows a positive fluorescence under UV light (366 nm). A positive indole reaction and, if necessary gas formation due to fermenting lactose, confirm the findings.

Typical Composition (g/litre)

Peptone 10.0; lactose 10.0; ox bile, dried 20.0; brilliant green 0.0133; L-tryptophan 1.0; 4-methylumbelliferyl-β-D-glucuronide 0.1.

Preparation

Suspend 41 g/litre, fill in test tubes, if necessary, fitted with DURHAM tubes; autoclave (15 min at 121 °C), not longer!

pH: 7.2 ± 0.2 at 25 °C.

The prepared broth is clear and green.

Experimental Procedure and Evaluation

The usual procedure is followed. The test tubes are inoculated correctly. For 1 ml of inoculum use at least 10 ml of broth. Incubate 24-48 hours at 35 °C aerobically, E. coli also at 44 °C.

Check the tubes under UV light (ca. 366 nm), e.g. using UV lamp: light blue fluorescence indicates the presence of E. coli in the culture. If fluorescence is negative after 24 hours of incubation do not add KOVACS reagent to check indole reaction (this alcoholic reagent destroys the growth conditions in the medium). Continue incubation for another 24 hours. Then check for fluorescence and indole reaction.

To confirm detection, cover the culture with a 5 mm layer of KOVACS indole reagent. If after 1-2 minutes a red ring shows up, the presence of E. coli is confirmed. Gas formation in the DURHAM tube signifies that the culture contains E. coli and/or other coliform organisms.

Ordering Information

Product	Merck Cat. No.	Pack size
Fluorocult® Brillant Green 2%-Bile (BRILA) Broth	1.12587.0500	500 g
Bactident® Indole (dropper bottle)	1.11350.0001	1 x 30 ml
KOVÁCS Indole Reagent	1.09293.0100	100 ml
UV Lamp (366 nm)	1.13203.0001	1 ea

Quality control

Test strains	Growth		Gas formation		MUG	Indole
	at 35 °C	at 44 °C	at 35 °C	at 44 °C		
Escherichia coli ATCC 25922	+	+	+	+	+	+
Escherichia coli ATCC 11775	+	+	+	+	+	+
Citrobacter freundii ATCC 8090	+		+ / -		-	-
Staphylocccus aureus ATCC 6538-P	-		-			
Micrococcus luteus ATCC 10240	-		-			
Bacillus cereus ATCC 11778	-		-			
Lactobacillus plantarum ATCC 8014	-		-			

Fluorocult® DEV Lactose Peptone Broth

Mode of Action

This medium is particularly suited for the enrichment and determination of the titre of coliform bacteria in the bacteriological analysis of water. E. coli shows a positive fluorescence under UV light (366 nm). A positive indole reaction is made for confirmation.

Typical Composition (g/litre)

Peptone from casein 17.0; peptone from soya 3.0; lactose 10.0; sodium chloride 5.0; bromocresol purple 0.02; tryptophan 1.0; 4-methylumbelliferyl-β-D-glucuronidase 0.01.

Preparation

Suspend 36.1 g/litre or 72.2 g/litre, dispense into test tubes fittted with DURHAM tubes, autoclave (15 min at 121 °C).

pH: 7.2 ± 0.2 at 25 °C.

The prepared broth is clear and purple.

Experimental Procedure and Evaluation

This depends on the various methods of water analysis.

Gas in the DURHAM tubes after incubation (24-48 hours at 35°C aerobically) denotes the presence of E. coli and/or other coliform bacteria.

Check the tubes under UV light: light blue fluorescence indicates the presence of E. coli in the culture. If fluorescence is negative after 24 hours of incubation **do not** add KOVACS reagent to check indole reaction (this alcoholic reagent destroys the growth conditions in the medium). Continue incubation for another 24hours. Then check for fluorescence and indole reaction.

To confirm detection, cover the culture with a 5 mm layer of KOVACS indole reagent. If after 1-2 minutes a red ring shows up, the presence of E. coli is confirmed.

Ordering Information

Product	Merck Cat. No.	Pack size
Fluorocult® DEV Lactose Peptone Broth	1.04037.0500	500 g
Bactident® Indole (dropper bottle)	1.11350.0001	1 x 30 ml
KOVÁCS Indole Reagent	1.09293.0100	100 ml
UV Lamp (366 nm)	1.13203.0001	1 ea

Quality control

Test strains	Growth	Colour change to yellow	Gas	MUG	Indole
Escherichia coli ATCC 25922	good / very good	+	+	+	+
Enterobacter aerogenes ATCC 13048	good / very good	+	+ / -	-	-
Klebsiella pneumoniae ATCC 13883	good / very good	+	+		
Salmonella typhimurium ATCC 14028	good / very good	-	-		
Aeromonas hydrophila ATCC 7966	fair / very good	-	-		
Enterococcus faecalis ATCC 11700	fair / very good	±	-		

Fluorocult® ECD Agar

E. coli Direct Agar

The medium complies with the German-DIN-Norm 10110 for the examination of meat, with the regulations acc. to § 35 LMBG (06.00/36) for the examination of food and with ISO Standard 6391 (1996) for the enumeration of E. coli in meat and meat products.

Mode of Action

The bile salt mixture of this E. coli Direct Agar largely inhibits the accompanying flora not usually found in the intestines. Using fluorescence under UV light and a positive indole reaction, E. coli colonies can be identified among the grown colonies.

Typical Composition (g/litre)

Peptone from casein 20.0; lactose 5.0; sodium chloride 5.0; bile salt mixture 1.5; di-potassium hydrogen phosphate 4.0; potassium dihydrogen phosphate 1.5; agar-agar 15.0; tryptophan 1.0; 4-methylumbelliferyl-β-D-glucuronide 0.07.

Preparation

Suspend 53.1 g/litre, autoclave (15 min at 121 °C), pour plates.

pH: 7.0 ± 0.2 at 25 °C.

The prepared broth is clear and yellowish brown.

Experimental Procedure and Evaluation

The culture medium is inoculated in the usual way by streaking on the surface and uncubated for 18-24 hours at 44 °C aerobically.

Fluorescence is noted with a UV lamp: light blue fluorescing colonies identify E. coli.

For confirmation cover the colonies with 10-20 µl KOVACS indole reagent, e.g. using Bactident® Indole. A reddening after 2-10 seconds shows indole formation.

Literature

Bundesgesundheitsamt: Amtliche Sammlung von Untersuchungsverfahren nach § 35 LMBG. - Beuth Verlag Berlin, Köln

DIN Deutsches Institut für Normung e.V.: Mikrobiologische Fleischuntersuchung. Bestimmung der Escherichia coli. Fluoreszenzoptisches Koloniezählverfahren unter Verwendung von Membranfiltern/ Spatelverfahren (Referenzverfahren). **DIN 10110**.

Draft International Standard ISO/DIS 6391: Meat and meat products - Enumeration of Escherichia coli-colony-count technique at 44 °C using membranes (1996).

Ordering Information

Product	Merck Cat. No.	Pack size
Fluorocult® ECD Agar	1.04038.0500	500 g
Bactident® Indole (dropper bottle)	1.11350.0001	1 x 30 ml
KOVÁCS Indole Reagent	1.09293.0100	100 ml
UV Lamp (366 nm)	1.13203.0001	1 ea

Escherichia coli ATCC 25922

Quality control

Test strains	Growth / Reovery rate %	MUG	Indole
Escherichia coli ATCC 8739	> 70	+	+
Escherichia coli ATCC 25922	> 70	+	+
Enterobacter aerogenes ATCC 13048	good / very good	-	-
Klebsiella pneumoniae ATCC 13883	good / very good	-	
Citrobacter freundii ATCC 8090	good / very good	-	
Proteus mirabilis ATCC 14153	good / very good	-	
Pseudomonas aeruginosa ATCC 27853	good / very good		
Clostridium perfringens ATCC 10543	none / poor (anaerobic)		

Fluorocult® E. coli 0157:H7 Agar

Selective agar for the isolation and differentiation of enterohemorrhagic (EHEC) Escherichia coli 0157:H7-strains from foodstuffs and clinical specimen material.

Description

Four different intestinal-pathogenic E. coli types are presently known: besides the infant-pathogenic (EPEC), the enterotoxin-forming (ETEC), and the entero-invasive (EIEC) E. coli types, in 1982 the so-called enterohemorraghic (EHEC) 0157:H7 E. coli strains were first detected following the ingestion of hamburgers in the United States. Enterohemorrhagic E. coli lead to the formation of toxins - following their passage from the intestine into the blood circulation - resulting in life-threatening extraintestinal complications in the form of the hemolytic uremic syndrome (HUS) and thrombotic-thrombocytopenic purpura (TTP) in 3-20 % of all cases. Due to the in many cases severe nature of the clinical symptoms and the high contagiousness of the pathogens, the detection of EHEC is constantly gaining more and more clinical relevance.

In contrast to most other E. coli strains, E. coli 0157:H7 shows the following characteristics:

- **No sorbitol-cleavage capacity within 48 h.**
- **No formation of glucuronidase (MUG-negative/no fluorescence).**

Mode of Action

Sodium deoxycholate inhibits the growth of the Gram-positive accompanying flora for the greater part. Sorbitol serves, together with the pH indicator bromothymol blue, to determine the degradation of sorbitol which, in the case of sorbitol-positive microorganisms, results in the colonies turning yellow in colour. Sorbitol-negative strains, on the other hand, do not lead to any change in the colour of the culture medium and thus proliferate as greenish colonies. Sodium thiosulfate and ammonium iron(III) citrate result in black-brown discolouration of the agar for colonies, in the presence of hydrogen-sulfide-forming pathogens, precipitating iron sulfide.

Proteus mirabilis in particular, which displays biochemical properties similar to those of E. coli 0157:H7, can thus be very easily differentiated from E. coli 0157:H7 on account of the brownish discolouration. 4-methylumbelliferyl-β-D-glucuronide (MUG) is converted into 4-methylumbelliferone by β–D-glucuronidase- forming pathogens; 4-methylumbelliferone fluoresces under UV light. The activity ofβ-D-glucuronidase is a highly specific characteristic of E. coli. In contrast to most E. coli strains, E. coli 0157:H7 is not capable of forming β-D-glucoronidase. When irradiated with long-wave UV light, no fluorescence is formed.

Typical Composition (g/litre)

Peptone from casein 20.0; meat extract 2.0; yeast extract 1.0; sorbitol 10.0; ammonium iron(III) citrate 0.5;

4-methylumbelliferyl-β-D-glucuronide 0.1; sodium chloride 5.0; bromothymol blue 0.025; sodium thiosulfate 2.0; sodium deoxycholate 1.12; agar-agar 13.0.

Preparation

Suspend 55 g in 1 litre of demin. water and autoclave (15 min at 121 °C).

pH: 7.4 ± 0.2 at 25 °C.

The plates are clear and blue-green.

Incubation: 24 h at 35 °C aerobically.

Literature

SZABO, R.A., TODD, E.C., EAN, A.: Method to isolate E. coli 0157:H7 from food. **- J. Food Prot., 10** ; 768-772 (1986).

Ordering Information

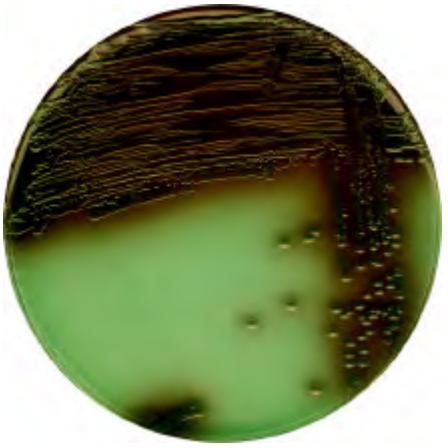
Product	Merck Cat. No.	Pack size
Fluorocult® E. coli 0157:H7 Agar	1.04036.0500	500 g
Laurylsulfate Broth	1.10266.0500	500 g
UV Lamp (366 nm)	1.13203.0001	1 ea

Fluorocult® E. coli 0157:H7 Agar

Quality control

Test strains	Growth	Colony colour	MUG	Sorbitol
Escherichia coli 0157:H7 (427 – 36/89)	good / very good	colourless	-	-
Escherichia coli ATCC 25922	fair / good	yellow	+	+
Proteus mirabilis ATCC 14273	good / very good	brown	-	-
Shigella sonnei ATCC 11060	good / very good	colourless	+	-
Enterobacter aerogenes ATCC 13048	good / very good	yellow	-	+
Salmonella typhimurium ATCC 14028	good / very good	yellow with black centre	-	+
Enterococcus faecalis ATCC 19433	none			

Escherichia coli ATCC 25922



Proteus mirabilis ATCC 14273

Fluorocult® Lauryl Sulfate Broth

LST-MUG Medium

The medium complies with the German-DIN-Norm 10183 for the examination of milk, with the regulations acc. to § 35 LMBG (01.00/54) for the examination of food, and acc. to ISO/DIS 11886 - 2.2 (1994) for milk and milk products. Furthermore to the German Badegewässerverordnung (regulations for bathing water) 76/1604 EWG (1995).

Mode of Action

The lauryl sulfate largely inhibits the growth of undesirable microbial flora. The presence of E. coli is indicated by fluorescence under a long wavelength UV lamp. A positive indole reaction and gas formation due to fermentation of lactose confirm the results.

SCHINDLER (1991) recommended the use of this medium in the quality control of bathing water.

Typical Composition (g/litre)

Tryptose 20.0; lactose 5.0; sodium chloride 5.0; sodium lauryl sulfate 0.1; di-potassium hydrogen phosphate 2.75; potassuim dihydrogen phosphate 2.75; L-tryptophan 1.0; 4-methylumbelliferyl-β-D-glucuronide 0.1.

Preparation

Suspend 36.5 g/litre, fill in test tubes fitted with DURHAM tubes; autoclave (15 min at 121 °C).

pH: 6.8 ± 0.2 at 25 °C.

The prepared broth is clear and yellowish-brown.

Experimental Procedure and Evaluation

The culture medium is used in the usual manner. Inoculate the tubes using at least 1 ml of broth.

Incubation: 16-24 hours at 35 °C aerobically according to instructions.

Check the tubes under UV light (366 nm). Light blue fluorescence indicates the presence of E. coli.

If fluorescence is negative after 24 hours of incubation do not add KOVACS reagent to check indole reaction (this alcoholic reagent destroys the growth conditions in the medium). Continue incubation for another 24 hours. Then check for fluorescence and indole reaction.

To confirm detection, cover the culture with a layer of KOVACS indole reagent of about 5 mm. If the reagent layer becomes cherry red after 1-2 minutes, the presence of E. coli is confirmed.

Gas formation in the DURHAM tube signifies that the culture contains E. coli and/or other coliform organisms.

Literature

SCHINDLER, P.R.G.: MUG-Laurylsulfat-Bouillon - ein optimales Nachweismedium für gesamtcoliforme und fäkalcoliforme Bakterien im Rahmen der hygienischen Überprüfung von Badegewässer gemäß der EG-Richtlinie 76/160 EWG. **- Zbl. Hyg., 191** ; 438-444 (1991).

Bundesgesundheitsamt: Amtliche Sammlung von Untersuchungsverfahren nach § 35 LMBG. - Beuth Verlag Berlin, Köln.

DIN Deutsches Institut für Normung e.V.: Mikrobiologische Milchuntersuchung. Bestimmung der Escherichia coli. Fluoreszenzoptisches Verfahren mit paralleler Bestimmung coliformer Keime. **DIN 10183**.

ISO/DIS 11886 - 2 (1997): Milk and milk products; Enumeration of presumptive E. coli-MPN technique using MUG.

New Zealand Dairy Industry: Microbiological Methods Manual, Section 48: Product Test Methods-Enteric Indicator Organisms. - NZTM2; 48.5.1-48.5.10 (1998).

Mikrobiologische Untersuchungsverfahren von Badegewässern nach Badegewässerrichtlinie 76/160/EWG: Nachweismethoden für fäkalcoliforme (E. coli) und gesamtcoliforme Bakterien. - Bundesgesundheitsblatt, 10; 385-396 (1995).

Ordering Information

Product	Merck Cat. No.	Pack size
Fluorocult® Lauryl Sulfate Broth	1.12588.0500	500 g
Bactident® Indole (dropper bottle)	1.11350.0001	1 x 30 ml
KOVÁCS Indole Reagent	1.09293.0100	100 ml
UV Lamp (366 nm)	1.13203.0001	1 ea

Quality control

Test strains	Growth	Fluorescence	Indole
Escherichia coli ATCC 25922	good / very good	+	+
Klebsiella pneumoniae ATCC 13883	good / very good	-	-
Enterobacter aerogenes ATCC 13048	good / very good	-	-
mixture of Escherichia coli ATCC 25922 and Enterobacter aerogenes ATCC 13048	good / very good	+	+
mixture of Escherichia coli ATCC 25922 and Klebsiella pneumoniae ATCC 13883	good / very good	+	+
Staphylococcus aureus ATCC 6538	none / poor		
Bacillus cereus ATCC 11778	none / poor		
Micrococcus luteus ATCC 10240	none / poor		

A

Fluorocult® LMX Broth Modified

Enrichment for the simultaneous detection of total coliforms and E.coli in water, food and dairy products by the fluorogenic procedure.

Mode of Action

LMX Broth first described by MANAFI and KNEIFEL (1989) was modified by MANAFI and OSSMER (1993) to improve the substrate utilization, to increase sensitivity and at the same time reduce the overall incubation time to 24 hours.

Fluorocult® LMX Broth Modified contains phosphate buffer to guarantee a high growth rate for total coliforms. Lauryl sulfate largely inhibits the accompanying Gram-positive flora. By adding the chromogenic substrate 5-bromo-4-chloro-3-indolyl-ß-D-galactopyranoside, which is cleaved by coliforms and the fluorogenic substrate 4-methylumbelliferyl-ß-D-glucuronide, which is highly specific for E.coli, the simultaneous detection of total coliforms and E.coli is possible. A color change of the broth from yellow to blue-green indicates the presence of coliforms. In addition a blue fluorescence under long-wave UV light permits the rapid detection of E.coli. As tryptophan is added to the broth, the indole reaction is easily done by adding Kocavs reagent. The formation of a red ring additionally confirms the presence of E.coli. The enzyme synthesis is amplified by 1-isopropyl-ß-D-1-thio-galactopyranoside and increases the ß-D-galactosidase activity.

Typical Composition (g/litre)

Tryptose 5.0; sodium chloride 5.0; sorbitol 1.0; tryptophan 1.0; dipotassium hydrogen phosphate 2.7; potassium dihydrogen phosphate 2.0; lauryl sulfate sodium salt 0.1; 5-bromo-4-chloro-3-indolyl-ß-D-galactopyranoside (X-GAL) 0.08; 4-methylum-belliferyl-ß-D-glucuronide (MUG) 0.05; 1-isopropyl-ß-D-1-thio-galactopyranoside (IPTG) 0.1.

Preparation

Food testing:

Suspend 17 g (single strength) in 1 liter of purified water. Heat to boiling to dissolve completely. Fill up to 20 ml aliquots into tubes. Autoclave for 15 min. at 121°C.

Water testing:

If 100 ml water samples (e.g. drinking water) are to be tested, suspend 34 g (double strength) in 1 liter of purified water. Heat to boiling to dissolve completely. Transfer 100 ml aliquots into bottles (250 ml capacity). Autoclave for 15 min. at 121°C.

pH: 6.8 ± 0.2 at 25 °C.

The prepared broth is clear and yellowish-brown.

Experimental Procedure and Evaluation

Application varies with the method/samples used for water or food testing.

Incubation: 24 hours at 35 ± 0.5°C aerobically.

Interpretation of results

Total coliforms: broth color has changed to blue-green.

E.coli: blue-green color of the broth and blue fluorescence using long-wave UV light source (366 nm). Overlay with Kovacs reagent for the indole reaction - a red ring additionally confirms the presence of E.coli.

Note: if the fluorescence is negative after 24 hours of incubation **do not** add Kovacs reagent to check the indole reaction at this point. Kovacs reagent is an alcoholic solution which destroys the growth conditions in the broth. **Continue incubation for another 24 hours** followed by checking fluorescence and indole reaction.

Literature

HAHN, G., a. WITTROCK, E.: Comparison of chromogenic and fluorogenic substances for differentiation of Coliforms and Escherichia coli in soft cheeses. **- Acta Microbiologic Hungarica 38 (3-4)**; 265-271 (1991).

MANAFI, M.: Schnellnachweis von Bakterien mittels fluorogener und chromogener Substrate. **- Forum Städte-Hygiene 41**; 181-184 (1990).

MANAFI, M.: Diagnostik von Mikroorganismen mittels fluorogener und chromogener Substrate. **- Ernährung/Nutrition 15**; Nr.10 (1991).

MANAFI, M., KNEIFEL, W.: Fluorogenic and chromogenic substrates. - A promising tool in Microbiology. **- Acta Microbiologica Hungarica 38 (3-4)**; 293-304 (1991).

MANAFI, M., KNEIFEL, W.: Ein kombiniertes Chromogen-Fluorogen-Medium zum simultanen Nachweis der Coliformengruppe und von E.coli in Wasser. **- Zbl. Hygiene und Umweltmedizin 189**; 225-234 (1989).

MANAFI, M., KNEIFEL, F., BASCON, S.: Fluorogenic and chromogenic substrates used in bacterial diagnosis. **- Microbiol. Rev. 55**; 335-348 (1991).

OSSMER, R.: Simultaneous Detection of Total Coliforms and E.coli - Fluorocult LMX-Broth. - 15th International Symposium/FOOD MICRO 1993. The International Committee on Food Microbiology and Hygiene, Bingen/Rhine (1993).

Ordering Information

Product	Merck Cat. No.	Pack size
Fluorocult® LMX Broth modified	1.10620.0500	500 g
Bactident® Indole (dropper bottle)	1.11350.0001	1 x 30 ml
KOVÁCS Indole Reagent	1.09293.0100	100 ml
UV Lamp (366 nm)	1.13203.0001	1 ea

Fluorocult® LMX Broth Modified

Quality control

Test strains	Colour change to blue-green	Fluorescence	Indole reaction
Escherichia coli ATCC 25922	+	+	+
Klebsiella pneumoniae ATCC 13883	+	-	
Enterobacter cloacae ATCC 13047	+	-	-
Citrobacter brakii ATCC 6750	+	-	
Citrobacter freundii ATCC 8090	+	-	
Shigella flexneri ATCC 12022	-	-	
Salmonella typhimurium ATCC 14028	-	-	
Aeromonas hydrophila ATCC 7966	-	-	

Fluorocult® MacCONKEY Agar

Mode of Action

The culture medium serves to isolate Salmonella, Shigella and coliform bacteria, in particular Escherichia coli, from various materials. The bile salts and crystal violet largely inhibit the growth of Gram-positive microbial flora. Lactose together with the pH indicator neutral red are used to detect lactose-positive colonies and E. coli can be seen among these because of fluorescence under UV light.

Typical Composition (g/litre)

Peptone from casein 17.0; peptone from meat 3.0; sodium chloride 5.0; lactose 10.0; bile salt mixture 1.5; neutral red 0.03; crystal violet 0.001; agar-agar 13.5; 4-methylumbelliferyl-β-D-glucuronide 0.1.

Preparation

Suspend 50.1 g/litre, autoclave (15 min at 121 °C) pour plates.

pH: 7.1 ± 0.2 at 25 °C.

The plates are clear and red to red-brown.

Experimental Procedure and Evaluation

Inoculate plates in the usual way and incubate for 18 to 24 hours at 35 °C aerobically.

Lactose-negative colonies are colourless. Lactose-positive colonies are red and often surrounded by a turbid zone due to the precipitation of bile acids.

Inspect the culture with UV light: light blue fluorescence denotes E. coli.

Ordering Information

Product	Merck Cat. No.	Pack size
Fluorocult® MacCONKEY Agar	1.04029.0500	500 g
UV Lamp (366 nm)	1.13203.0001	1 ea

Quality control

Test strains	Growth	Colour of		Precipitate	MUG
		colony	medium		
Escherichia coli ATCC 11775	good / very good	red	red	+	
Escherichia coli ATCC 25922	good / very good	red	red	+	+
Salmonella typhimurium ATCC 13311	good / very good	colourless	yellowish	-	
Salmonella dublin ATCC 15480	good / very good	colourless	yellowish	-	
Shigella sonnei ATCC 11060	good / very good	colourless	yellowish	-	
Proteus mirabilis ATCC 29906	good / very good	colourless	yellowish	-	
Bacillus cereus ATCC 11778	none				
Staphylococcus aureus ATCC 6538	none				
Enterococcus hirae ATCC 8043	none				

Fluorocult® VRB Agar

VRB-MUG Agar

Mode of Action

This medium is used for the detection and enumeration of coliform bacteria, in particular E. coli. Crystal violet and bile salts largely inhibit the growth of Gram-positive accompanying bacterial flora. Lactose-postitive colonies show a colour change to red of the pH indicator. E. coli colonies schow a fluorescence under UV light.

Typical Composition (g/litre)

Peptone from meat 7.0; yeast extract 3.0; sodum chloride 5.0; lactose 10.0; neutral red 0.03; bile salt mixture 1.5; crystal violet 0.002; agar-agar 13.0; 4-methylumbelliferyl-β-D-glucuronide 0.1.

Preparation

Suspend 39.6 g in 1 litre of demin. water and heat in a boilling waterbath or in free flowing steam with frequent stirring until completely dissolved. Afterwards do not boil for more than 2 minutes.

■ **Do not autoclave! Do not overheat!**

pH: 7.4 ± 0.2 at 25 °C.

The plates are clear and dark red.

Experimental Procedure and Evaluation

Inoculate the medium in the usual way and incubate for 18-24hours at 35 °C aerobically.

Lactose-negative Enterobacteriaceae are colourless. Lactose-positive colonies are red and often surrounded by a turbid zone due to the precipitation of bile acids.

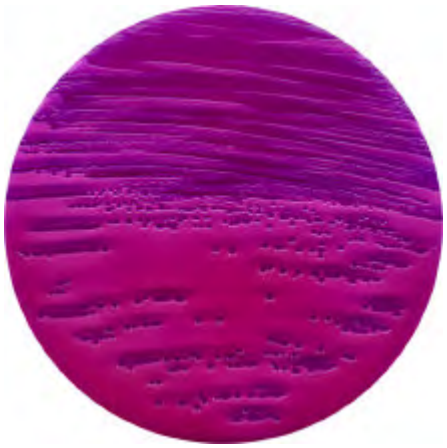
Fluorescence is noted with a UV lamp: light blue fluorescing colonies denote E. coli.

Ordering Information

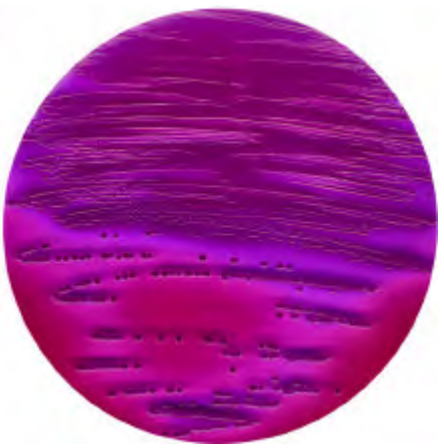
Product	Merck Cat. No.	Pack size
Fluorocult® VRB Agar	1.04030.0500	500 g
UV Lamp (366 nm)	1.13203.0001	1 ea

Quality control

Test strains	Growth	Colony Colour	Precipitate	MUG
Escherichia coli ATCC 11775	good / very good	red	+	+
Enterobacter aerogenes ATCC 13048	good / very good	red	+ / -	-
Salmonella gallinarum NCTC 9240	good / very good	colourless		-
Shigella flexneri ATCC 29903	good / very good	colourless		-
Yersinia enterocolitica ATCC 9610	fair / very good	colourless		-
Staphylococcus aureus ATCC 6538	none			
Micrococcus luteus ATCC 9341	none			
Lactococcus lactis spp. lactis ATCC 19435	none			
Bacillus cereus ATCC 11778	none			
Lactobacillus plantarum ATCC 14917	none / poor			



Enterobacter aerogenes ATCC 13048



Escherichia coli ATCC 11775

FRASER Listeria Selective Enrichment Broth (base)

For the selective enrichment of Listeria in the 2-step method acc. to D.G.AL. and ISO 11290-1 (1996).

Mode of Action

Optimum growth conditions are created for Listeria due to the high nutrient content and the large buffer capacity. The growth of accompanying bacteria is largely inhibited by lithium chloride, nalidixic acid and acriflavine hydrochloride. The detection of the

β-D-glucosidase activity of Listeria is possible by the addition of esculin and amonium iron(III) citrate. The glucose esculin is cleaved by β-D-glucosidase into esculetin and glucose. The esculetin then forms an olive-green to black complex with the iron(III) ions. Therefore, during the growth of Listeria in FRASER broth, usually a blackening of the broth is observed. An improved enrichment of Listeria in comparison with the standard method can be attained using the two-step enrichment method with an initially halved concentration of nalidixic acid and acriflavine hydrochloride.

Typical Composition (g/litre)

Proteose peptone 5.0; peptone from casein 5.0; yeast extract 5.0; meat extract 5.0; sodium chloride 20.0; disodium hydrogen phosphate 9.6; potassium dihydrogen phosphate 1.35; esculin 1.0; lithium chloride 3.0.

Preparation

Suspend 55.0 g in 1 litre demin. water and autoclave (15 min at 121 °C). To prepare half-concentraded FRASER broth, dissolve the contents of 1 vial amonium iron(III) citrate and 1 vial of selective supplement (Cat. No. 1.10399.0001 FRASER Supplement) in 1 ml of sterile distilled water each and add to the broth after it has cooled below 50 °C. FRASER broth is made by adding a further bottle of selective supplement to the half-concentrated FRASER broth. The supplements are homogeneously distributed in the broth by carefully swirling.

pH: 7.2 ± 0.2 at 25 °C.

The prepared broth is clear to almost clear and yellowish-brown.

Application

1. Enrichment step

The half-concentrated FRASER broth is inoculated with sample material and incubated at 30 °C for 24 ± 2 hours. From this culture, a selective growth medium such as OXFORD or PALCAM Agar is inoculated.

2. Enrichment step

From the first enrichment step, 0.1 ml is inoculated on to 10 ml FRASER broth for two incubations of 48 ± 2 hours at 35 °C or 37°C. After each 24 hours period selective growth media such as OXFORD and/or PALCAM agar are inoculated.

Literature

Direction General de l'Alimentation: D.G.AL./SDHA/N93/No 8105 du 24-06-1993.

ISO 11290-1: Microbiology of food and animal feeding stuffs - Horizontal method for the detection and enumeration of Listeria monocytogenes - Part 1: Detection method (1996).

FRASER, J.A., a. SPERBER, W.H.: Rapid detection of Listeria spp. in food and environmental samples by esculin hydrolysis. **- J. Food Prot. 51** ; 762-765 (1988).

Ordering Information

Product	Merck Cat. No.	Pack size
FRASER Listeria Selective Enrichment Broth (base)	1.10398.0500	500 g
FRASER Listeria Supplement (antibiotic mixture + ammonium iron (III) citrate)	1.10399.0001	2 x 8 vials
OXFORD Listeria Selective Agar (Base)	1.07004.0500	500 g
OXFORD Listeria Selective Supplement	1.07006.0001	1 x 13 vials
PALCAM Listeria Selective Agar (Base)	1.11755.0500	500 g
PALCAM Listeria Selective Supplement acc. to VANNETTEN et al.	1.12122.0001	1 x 16 vials
Singlepath® Listeria	1.04142.0001	25 tests

Quality control

Test strains	1. Enrichment step Growth	2. Enrichment step Blackening	Singlepath® Listeria
Listeria monocytogenes ATCC 19111	> 1 x 10^4	+	+
Listeria monocytogenes (NCTC 7973) ATCC 35152	> 1 x 10^4	+	+
Listeria monocytogenes ATCC 13932	> 1 x 10^4	+	+
Listeria innocua ATCC 33090	> 1 x 10^4	+	+
Enterococcus faecalis ATCC 19433	> 1 x 10^3		-
Staphylococcus aureus ATCC 25923	> 1 x 10^3		-

FRASER Listeria Supplement

Additive for the preparation of FRASER Listeria Enrichment Broth acc. to D.G.AL.

Mode of Action

FRASER Listeria Supplement constits of 8 vials with ammonium iron(III) citrate and 8 vials with a selective supplement. The ammonium iron(III) citrate promotes the growth of the Listeria and, together wth esculin, permits the β-D-glucosidase detection in Listeria.

The selective supplement is a mixture of acriflavine and nalidixic acid in a lyophilised form. It largely inhibits the growth of accompanying bacteria by a selective enrichment of Listeria.

Composition (per vial)

Ammonium iron(III) citrate Supplement:

Ammonium iron(III) citrate 500 mg

Selective Supplement:

Acriflavine 12.5 mg; nalidixic acid 10 mg.

Preparation

The content is dissolved in each original vial by adding sterile distilled water (about 1 ml).

To prepare half-concentrated FRASER broth, the contents of 1vial of ammonium iron(III) citrate and 1 vial of selective supplement are evenly mixed into 1 litre of sterile FRASER broth base after it has cooled to about 45-50 °C.

FRASER broth is made by adding a further bottle of selective supplement to the half-concentrated FRASER broth.

Ordering Information

Product	Merck Cat. No.	Pack size
FRASER Listeria Supplement	1.10399.0001	2 x 8 vials
FRASER Broth (base)	1.10398.0500	500 g

Fungi Agar Base acc. to KIMMIG, modified

Medium proposed by KIMMIG and RIETH (1953) for the cultivation, isolation, identification and strain preservation of fungi.

IVD *in vitro diagnosticum –*
For professional use only

See also General Instruction of Use
Warnings and precautions see ChemDAT®
(www.chemdat.info)

This culture medium respresents an improved version of the "Grütz II Agar" which is obtained by mixing it with MERCK Standard II Nutrient broth. According to RIETH (1969), it promotes the development of growth forms, which are used as important characteristic criteria for identification. KIMMIG agar can also be used as a base for preparing selective agars.

Principle

Microbiological method

Typical Composition (g/litre)

Peptone 15.0; sodium chloride 1.0; D(+)glucose 19.0; agar-agar 15.0.

Also to be added:

Glycerol 5.0.

Preparation and Storage

Usable up to the expiry date when stored dry and tightly closed at +15 to +25° C. Protect from light.

After first opening of the bottle the content can be used up to the expiry date when stored dry and tightly closed at +15 to +25° C.

Suspend 50 g/litre together with 5 ml glycerol/litre, autoclave (15 min at 121 °C), pour plates.

pH: 6.5 ± 0.2 at 25 °C.

The plates are clear and yellowish-brown.

Preparation of selective agar:
Cool to approximately 50 °C, add 0.4 g cycloheximide/litre and, as recommended by GEORG et al. (1954), 40.000 IU penicillin/ litre and 40 µg streptomycin/litre or, according to HANTSCHKE (1968), 80 mg colistin/litre and 100 mg novobiocin/litre and mix.

These compounds should be added in the form of filter-sterilized solutions. Pour plates.

Specimen

e.g. Nails, hair, skin.

Clinical specimen collection, handling and processing, see general instructions of use.

Experimental Procedure and Evaluation

Inoculate the plates with the material, which should be obtained by an appropriate method. In the case of heavily contaminated material, use the selective agar described above or another one, for example Selective Agar for Pathogenic Fungi.

Incubation: up to 3 weeks at 25-28 °C.Identify the colonies. Identify the colonies.

Manufacturer	Product
Warner-Chilcott, USA	Colistin

Literature

GEORG, L.K., AJELLO, L., a. PAPAGEORGE, C.: Use of cycloheximide in the selective isolation of fungi pathogenic to man. **- J. Lab. Clin. Med., 44**; 422-428 (1954). (1968).

HANTSCHKE, D.: Ein Colistin-Novobiocin-Actidion-Agar als Anzuchtmedium für humanpathogene Pilze. **- Mykosen, 11**; 769-778.

KIMMIG, J., u. RIETH, H.: Antimykotika in Experiment und Klinik. **-Arzneimittelforsch., 3**; 267-276 (1953).

RIETH, H.: Dermatophyten, Hefen und Schimmelpilze auf Kimmig-Agar. **-Mykosen, 12**; 73-74 (1969).

Ordering Information

Product	Merck Cat. No.	Pack size
Fungi Agar Base acc. to KIMMIG, modified	1.05414.0500	500 g
Merckoplate® Agar for fungi acc. to KIMMIG modified	1.10421.0001	1 x 20 plates
Glycerol (about 87 %)	1.04094.0500	500 ml
Selective Agar for Pathogenic fungi	1.05467.0500	500 g
Novobiocin monosodium salt	CN Biosciences	
Penicillin G potassium salt	CN Biosciences	
Streptomycin sulfate	CN Biosciences	

Quality control

Test strains	Growth
Microsporum gallinae ATCC 12108	good / very good
Trichophyton ajelloi ATCC 28454	good / very good
Trichophyton mentagro-phytes ATCC 18748	good / very good
Microsporum canis ATCC 36299	good / very good
Penicillium spp. ATCC 10428	good / very good
Aspergillus niger ATCC 16404	good / very good
Candida albicans ATCC 10231	good / very good
Geotrichum candidum DSMZ 1240	good / very good

GASSNER Agar (Water-blue Metachrome-yellow Lactose Agar acc. to GASSNER)

Selective agar proposed by GASSNER (1918) for the detection and isolation of pathogenic Enterobacteriaceae in foodstuffs and other materials.

GASSNER agar is one of the culture media prescribed in the regulations for the execution of the German Meat Inspection Law (Deutsches Fleischbeschaugesetz).

Mode of Action

This culture medium contains metachrome yellow, which primarily inhibits the accompanying Gram-positive microbial flora. It also contains lactose, which, when degraded to acid is shown by the indicator water blue, which is deep blue in the acidic range and colourless in the alkaline range. The prepared culture medium is green, in the acidic pH range it becomes blue-green to blue. At alkaline pH's, however, the yellow colour of the metachrome yellow becomes incresingly appearent.

Typical Composition (g/litre)

Peptones 14.0; sodium chloride 5.0; lactose 43.0; water blue 0.62; metachrome yellow 1.25; agar-agar 13.0.

Preparation

Suspend 77 g/litre, autoclave (15 min at 121 °C), pour plates

pH: 7.2 ± 0.2 at 25 °C.

The plates are clear and dark green.

Experimental Procedure and Evaluation

Inoculate the plates by the streak-plate method.

Incubation: 24 hours at 35 °C aerobically.

Literature

Deutsches Fleischbeschaugesetz: Ausführungsbestimmungen A über die Untersuchung und gesundheitspolizeiliche Behandlung der Schlachttiere und des Fleisches bei Schlachtungen im Inland. Anlage 1 zu § 20 Abs. 4: Vorschriften über die bakteriologische Fleischuntersuchung..

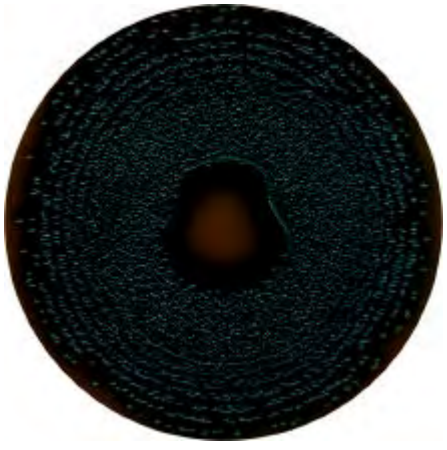
GASSNER, G.: Ein neuer Dreifarbennährboden zur Tyhus-Ruhr-Diagnose. **-Centralbl. f. Bakt. I. Orig., 80** ; 219-222 (1918).

Ordering Information

Product	Merck Cat. No.	Pack size
GASSNER Agar (Water-blue Metachrome-yellow Lactose Agar acc. to GASSNER)	1.01282.0500	500 g
GASSNER Agar (Water-blue Metachrome-yellow Lactose Agar acc. to GASSNER)	1.01282.5000	5 kg

Quality control

Test strains	Inoculum (cfu/ml)	Recovery rate (%)	Colour change of medium
Escherichia coli ATCC 25922	10^3-10^5	≥ 40	blue
Escherichia coli ATCC 11775	10^3-10^5	≥ 40	blue
Enterobacter cloacae ATCC 13047	10^3-10^5	≥ 40	light blue to blue
Salmonella typhimurium ATCC 14028	10^3-10^5	≥ 40	yellowish-brown
Shigella flexneri ATCC 12022	10^3-10^5	≥ 40	yellowish-brown
Salmonella enteritidis ATCC 13076	10^3-10^5	≥ 20	yellowish-brown
Proteus mirabilis ATCC 14153	10^3-10^5	≥ 40	yellowish-brown
Enterococcus faecalis ATCC 11700	> 10^5	≤ 0.01	-
Staphylococcus aureus ATCC 25923	> 10^5	≤ 0.01	-



Enterobacter cloacae ATCC 13047

Salmonella enteritidis ATCC 13076

Gentamicin Solution

For the preparation of culture media containing gentamicin to prevent bacterial contamination of tissue cultures and for the decontamination of virological sample material.

Mode of Action

The aminoglycoside gentamicin is a broad-band antibiotic - it displays an antibacterial action towards a wide variety of pathogenic bacteria.

It acts on Gram-negative and Gram-positive bacteria including those species which are resistant to other antibiotics. On account of these properties gentamicin is used as an antibacterial inhibitor in microbiology.

TAPLIN (1965) recommends addition of gentamicin to culture media to suppress accompanying bacterial flora when isolating fungi from clinical material (see Dermatophytes Selective Agar acc. to TAPLIN, Merck, Cat. No. 1.10896.). CASEMORE (1967), PERLMAN et al. (1967) and FISCHER (1975) have reported that gentamicin is of value as an antibacterial agent for tissue cultures and for the decontamination of sample material containing viruses.

Gentamicin solution is highly stable and can be autoclaved for 15 minutes at 121°C without any loss of activity.

The concentration of the solution is 5% based on the gentamicin base.

Typical Composition (per package)

Gentamicin sulfate 0.83g (equivalent to 0.5 g gentamicin base); sterile distilled water 10.0ml.

Experimental Procedure

1. **Selective culture media**
   Withdraw the required volume of gentamicin should aseptically - it is best to use a syringe - and mix with the sterilized culture medium under sterile conditions. In case of non-sterile investigations add the gentamicin solution to the fully dissolved culture medium before it is sterilized.
2. **Media for tissue culture**
   A concentration range of 50-100 µg gentamicin/ml (i.e. an 1:1000 - 1:500 fold dilution) has a satisfactory bactericidal action. In order to prepare a culture medium with 50µg gentamicin/ml withdraw 1ml gentamicin under aseptic conditions and add to 1 litre sterile culture medium. If the solution is withdrawn under non-sterile conditions the culture medium must be sterilized after addition of the gentamicin.
3. **Pre-treatment of virological samples**
   When decontaminating sample material from which viruses are to be isolated, add 100µg gentamicin/ml either by itself or together with other antibiotics prior to inoculation.

Stability

The gentamicin solution is stable up to the expiry date stated when stored at room temperature. It may become yellow in colour but this does not affect its antibiotic activity. Opened packages should be stored in the refrigerator (+2°C to +8°C).

Literature

CASEMORE, D.P.: Gentamicin as a bactericidal agent in virological tissue cultures. **- J. Clin. Path., 20**; 98-299 (1967).

FISCHER, A.B.: Gentamicin as a bactericidal antibiotic in tissue culture. **-Med. Microbiol. Immunol., 161**; 23-39 (1975).

PERLMAN, D., RAHMAN, S.B., a. SEMAR, J.B.: Antibiotic control of Mycoplasma in tissue culture. **- Appl. Microbiol., 15**; 82-65 (1967).

TAPLIN, D.: The use of gentamicin in mycology media. **- J. Invest Dermatol, 45**; 549-55 (1965).

Ordering Information

Product	Merck Cat. No.	Pack contents
Gentamicin solution	1.11977.0001	1 x 10 ml

GIOLITTI-CANTONI Broth (Staphylococcus Enrichment Broth Base acc. to GIOLITTI and CANTONI)

Medium proposed by GIOLITTI and CANTONI (1966) for the enumeration (MPN method) and selective enrichment of staphylococci from foodstuffs.

This culture medium complies with the recommendations of the International Organization for Standardization (ISO) (1977), the International Dairy Federation (Internationaler Milchwirtschaftsverband, FIL/IDF) (1990) and the DIN Norm 10178 for the examination of milk.

Mode of Action

The growth of staphylococci is promoted by pyruvate, glycine and above all by a high concentration of mannitol. Gram-negative contaminants are inhibited by lithium chloride (LAMBIN and GERMAN 1961) while Gram-positive contaminants are inhibited by tellurite. Micrococci are suppressed to a certain degree because of anaerobiosis. Growth of staphylococci can be recognized by a black colouration of the culture medium due to reduction of tellurite to metallic tellurium.

Typical Composition (g/litre)

Peptone from casein 10.0; meat extract 5.0; yeast extract 5.0; lithium chloride 5.0; D(-)mannitol 20.0; sodium chloride 5.0; glycine 1.2; sodium pyruvate 3.0; Tween® 80 1.0.

Also to be added:

potassium tellurite trihydrate 0.052 g/litre.

Preparation

Suspend 55 g/litre. In accordance with the ISO recommendations, dispense 19 ml aliquots into test tubes, autoclave (20 min at 121 °C), cool, add 0.1 ml of a 1 % potassium tellurite solution to each tube.

pH: 6.9 ± 0.2 at 25 °C.

The prepared broth is clear and yellowish-brown.

- **The prepared culture medium base can be stored for about 2 weeks in the refrigerator. The ready-to-use medium must be used the day it is prepared.**

Experimental Procedure and Evaluation

Homogenize the sample material and prepare dilution series (dilution factor 1 in 10). Inoculate each tube containing the broth with a 1 ml aliquot, overlay with sterilized paraffin viscous.

Incubation: 18-24 hours at 35 °C aerobically.

Streak material from tubes that exhibit a black colouration onto selective culture media (e.g. BAIRD-PARKER Agar). When determining the bacterial count by the MPN method, tubes are considered positive for Staphylococcus, if they produce a positive result in the coagulase test.

Literature

DIN Deutsches Institut für Normung e.V.: Mikrobiologische Milchuntersuchung. Nachweis Koagulase-positiver Staphylokokken. Referenzverfahren für Milchpulver. **- DIN 10178**.

GIOLITTI, G., a. CANTONI, C.: A medium for the isolation of staphylococci from foodstuffs. **- J. Appl. Bacteriol., 29**; 395-398 (1966).

Internationaler Milchwirtschaftsverband FIL/IDF: Nachweis Koagulase-positiver Staphylokokken in Milchpulver. **- Internationaler Standard 60 A** (1990).

International Organization for Standardization: Meat and meat products. -Detection and enumeration of Staphylococcus aureus (Reference methods). **-Draft International Standard ISO/DIS 5551** (1977).

LAMBIN, S., et GERMAN, A.: Précis des microbiologie, p. 63, Paris: Masson; 1961.

Ordering Information

Product	Merck Cat. No.	Pack size
GIOLITTI-CANTONI Broth (Staphylococcus Enrichment Broth Base acc. to GIOLITTI and CANTONI)	1.10675.0500	500 g
BAIRD-PARKER Agar	1.05406.0500	500 g
Paraffin viscous	1.07160.1000	1 l
Potassium tellurite trihydrate	1.05164.0100	100 g

GIOLITTI-CANTONI Broth (Staphylococcus Enrichment Broth Base acc. to GIOLITTI and CANTONI)

Quality control

Test strains	Growth	Blacking
Staphylococcus aureus ATCC 25923	good / very good	+
Staphylococcus aureus ATCC 6538	good / very good	+
Staphylococcus epidermis ATCC 12228	poor / good	±
Micrococcus luteus ATCC 10240	none / fair	-
Bacillus cereus ATCC 11778	none / fair	
E. coli ATCC 25922	none / fair	
Pseudomonas aeruginosa ATCC 27853	none	

BAIRD Broth

Left tube: Pseudomonas aeruginosa ATCC 27853
Middle tube: Staphylococcus epidermidis ATCC 12228
Right tube: Staphylococcus aureus ATCC 25923

GIOLITTI-CANTONI Broth

Left tube: Pseudomonas aeruginosa ATCC 17853
Middle tube: Staphylococcus epidermidis ATCC 12228
Right tube: Staphylococcus aureus ATCC 25923

DA
MPF

GN Enrichment Broth acc. to HAJNA

Medium proposed by HAJNA (1955) for the selective cultivation of Gram-negative intestinal bacteria (especially of Shigella) from all types of materials.

IVD *in vitro diagnosticum –*
For professional use only

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Warnings and precautions see ChemDAT®
(www.chemdat.info)

The yields of shigellae achieved by previous enrichment with GN enrichment broth are higher than those obtained by smearing directly onto selective or elective plates (CROFT and MILLER 1956). The yields of salmonellae and shigellae are considerably improved by using this medium, combined with XLD Agar (TAYLOR and SCHELHART 1967, 1968; DUNN and MARTIN 1971).

Principle

Microbiological method

Mode of Action

Tryptose serves as a nutrient base. Citrate and deoxycholate act as selective agents and suppress the growth of Gram-positive microorganisms (particularly fecal streptococci), all types of spore-forming bacilli and some coliform bacteria.

Mannitol selectively promotes the growth of mannitol-metabolizing salmonellae and shigellae. Phosphate buffer prevents premature over-acidification of the culture medium by acidic metabolic products. If Proteus and Pseudomonas aerguninosa are present, they usually proliferate more slowly than salmonellae and shigellae during the first 6-8 hours of incubation.

Typical Composition (g/litre)

Tryptose 20.0; D(+)glucose 1.0; D(-)mannitol 2.0; di-potassium hydrogen phosphate 4.0; potassium dihydrogen phosphate 1.5; sodium chloride 5.0; sodium citrate 5.0; sodium deoxycholate 0.5.

Preparation and Storage

Usable up to the expiry date when stored dry and tightly closed at +15 to +25° C. Protect from light.

After first opening of the bottle the content can be used up to the expiry date when stored dry and tightly closed at +15 to +25° C.

Suspend 39 g/litre, dispense into suitable containers, autoclave (15 min at 121 °C).

pH: 7.0 ± 0.2 at 25 °C.

The prepared broth is clear and yellowish.

Specimen

e.g. Stool.

Clinical specimen collection, handling and processing, see general instructions of use.

Experimental Procedure and Evaluation

Inoculate the enrichment broth with the sample material.

Incubation: approx. 6 hours at room temperature aerobically.

Spread the resulting culture thinly on the surface of elective plates.

Literature

DUNN, C., a. MARTIN, W.: Comparison of media for isolation of Salmonella and Shigella from fecal specimen. **- Appl. Microbiol., 22**; 17-22 (1971).

HAJNA, A.A.: A new specimen preservative for gram-negative organisms of the intestinal group. **- Publ. Hlth. Lab., 13**; 59-62 (1955).

HAJNA, A.A.: A new enrichment broth medium for gram-negative organisms of the intestinal group. **- Publ. Hlth. Lab., 13**; 83-89 (1955).

CROFT, C.C., a. MILLER, M.J.: Isolation of shigella from rectal swabs with HAJNA "GN" broth. **- Am. J. Clin. Path., 26**; 411-417 (1956).

TAYLOR, W.I., a. SCHELHART, D.: Isolation of shigellae, IV. Comparison of plating media with stools. **- Am. J. Clin. Path., 48** ; 356-362 (1968).

TAYLOR, W.I., a. SCHELHART, D.: Isolation of shigellae, V. Comparison of enrichment broth with stools. **- Appl. Microbiol., 16**; 1383-1386 (1967).

Ordering Information

Product	Merck Cat. No.	Pack size
GN Enrichment Broth acc. to HAJNA	1.10756.0500	500 g

Quality control

Test strains	Growth
Shigella flexneri ATCC 12022	good
Shigella sonnei ATCC 11060	good
Salmonella typhimurium ATCC 14028	good
Salmonella enteritidis NCTC 5188	good
Escherichia coli ATCC 25922	good
Staphylococcus aureus ATCC 25923	none
Enterococcus faecalis ATCC 11700	none
Bacillus cereus ATCC 11778	none

GSP Agar (Pseudomonas Aeromonas Selective Agar Base) acc. to KIELWEIN

Medium proposed by KIELWEIN (1969, 1971) for detecting Pseudomonas and Aeromonas in foodstuffs as well as in wastewater and equipment of the food industry.

Mode of Action

This glutamate starch phenol-red agar contains glutamate and starch as its sole nutrients. Many accompanying microorganisms cannot metabolize these compounds (STANIER et al. 1966). Starch is degraded by Aeromonas with acid production causing phenol red to change the yellow, but not by Pseudomonas. The selective inhibitors penicillin and, if desired, the antimycotic pimaricin are added to the medium to improve its selectivity.

Typical Composition (g/litre)

Sodium L(+)glutamate 10.0; starch, soluble 20.0; potassium dihydrogen phosphate 2.0; magnesium sulfate 0.5; phenol red 0.36; agar-agar 12.0

Also to be added:

penicillin G 100,000 IU; if required pimaricin 0.01.

Preparation

Suspend 45 g/litre, autoclave (15 min at 121 °C), cool to 45-50 °C. Add 100,000 IU sodium penicillin g/litre and, if required, 0.01 g pimaricin/litre, mix and pour plates.

pH: 7.2 ± 0.2 at 25 °C.

The plates are clear and red.

Experimental Procedure and Evaluation

Inoculate by spreading the sample material on the surface of the plates.

Incubation: up to 3 days at approx. 28 °C aerobically.

Appearance of Colonies	Microorganisms
Large, diameter of 2-3 mm, blue-violet, surrounded by a red-violet zone	Pseudomonas
Large, diameter of 2-3 mm, yellow, surrounded by a yellow zone	Aeromonas
Usually small, delayed growth, sometimes mucoid	Enterobacteriaceae and others

Literature

KIELWEIN, G., GERLACH, R., u. JOHNE, H.: Untersuchungen über das Vorkommen von Aeromonas hydrophila in Rohmilch. **- Arch. f. Lebensmittelhyg., 20**; 34-38 (1969).

KIELWEIN, G.: Ein Nährboden zur selektiven Züchtung von Pseudomonaden und Aeromonaden. **- Arch. f. Lebensmittelhyg., 20**; 131-133 (1969).

KIELWEIN, G.:Pseudomonaden und Aromonaden in Trinkmilch: Ihr Nachweis und ihre Bewertung. **- Arch. f. Lebensmittelhyg., 22**; 15-19 (1971).

KIELWEIN, G.: die Isolierung und Differenzierung von Pseudomonaden aus Lebensmitteln. **- Arch. f. Lebensmittelhyg., 22**; 29-37 (1971).

STANIER, R.Y., PALLERONI, N.J., a. DOUDOROFF, M.: The aerobic Pseudomonas - a taxonomic study. **- J. Gen. Microbiol., 42**; 159-271 (1966).

Ordering Information

Product	Merck Cat. No.	Pack size
GSP Agar (Pseudomonas Aeromonas Selective Agar Base) acc. to KIELWEIN	1.10230.0500	500 g
Pimaricin	1.07360.0001	1 g
Penicillin G potassium salt	CN Biosciences	

Quality control

Test strains	Growth	Colour change to
Pseudomonas aeruginosa ATCC 27853	good / verygood	red-violet
Pseudomonas aeruginosa ATCC 9027	good / verygood	red-violet
Pseudomonas aeruginosa ATCC 10145	fair / good	red-violet
Aeromonas hydrophila ATCC 7966	good	yellow
Aeromonas caviae ATCC 15468	good	yellow
Escherichia coli. ATCC 25922	none / poor	-
Staphylococcus aureus ATCC 25923	none	
Citrobacter freundii ATCC 8090	none / fair	

GRIESS-ILOSVAY's Nitrite Reagent

Reagent solution for detecting microbial nitrite in order to identify nitrate-reducing microorganisms.

Mode of Action

GRIESS-ILOSVAY's reagent reacts with nitrate to form a red diazo dye. If high concentration of nitrate are present, the colour of the dye may change to yellow.

Typical Composition

Sulfanilic acid; 1-naphthylamine; acetic acid.

Experimental Procedure and Evaluation

The pure culture of the microorganism to be tested is inoculated into Nitrate Broth or into Standard II Nutrient Broth to which 1.5g potassium nitrate (Merck, Cat. No. 1.05063.) have been added. Incubate for 12-24hours at the optimal temperature.

After incubation, add several drops of the nitrite reagent to the culture. If nitrite is present an intense red colour appears within one minute, the intensity of the colour is proportional to the quantity of nitrite formed. In case of microorganisms which produce large amounts of nitrite, the initial red coloration changes to yellow.

A negative reaction signifies that nitrate has not undergone any reaction or that is has been reduced to nitrogen of ammonia. The "zinc dust test" must be then performed to verify which of these reactions have been taken place.

Zinc dust test: If the result of GRIESS-ILOSVAY test is negative, add a quantity of zinc dust (Merck, Cat. No. 1.08774.) about the size of a pepper corn for every 5 ml of culture medium and allow to settle without shaking. If nitrate is present, the medium surrounding the zinc dust becomes pink. The nitrate is reduced to nitrite which can then react with the GRIESS-ILOSVAY's reagent. A positive zinc dust test signifies "no nitrate reduction"; a negative zinc dust test signifies "nitrate has been reduced".

Nitrate may not be reduced, if the zinc particles are coated with a layer of patina. The zinc dust should therefore be as fresh as possible.

Reaction	Nitrate Reduction
GRIESS-ILSOVAY: positive negative → Zinc dust test: negative: positive:	positive positive negative

Ordering Information

Product	Merck Cat. No.	Pack contents
GRIESS-ILOSVAY's Nitritre Reagent	1.09023.0500	500 ml

AC
M
MPF
D

HEKTOEN Enteric Agar

Selective agar proposed by KING and METZGER (1968) for detecting and isolating pathogenic intestinal bacteria including Salmonella and Shigella in various materials such as faeces, foodstuffs, etc.

IVD *in vitro diagnosticum –*
For professional use only

See also General Instruction of Use
Warnings and precautions see ChemDAT®
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When compared with other selective culture media (e.g. SS Agar, BPL Agar and Bismuth Sulfite Agar), HEKTOEN* enteric agar has the advantage that it only slightly inhibits the growth of Salmonella and Shigella thus giving high yields of these microorganisms, but at the same time ensures adequate inhibition of accompanying microorganisms (KING and METZGER 1968, TAYLOR and SCHELHART 1971, BISCIELLO and SCHRADE 1974).

Principle

Microbiological method

Mode of Action

Lactose-positive colonies have a clearly different colour from lactose-negative colonies due to the presence of the two indicators bromothymol blue and acidic fuchsin. This colour difference is also observed for colonies, which can only slowly ferment lactose due to the presence of sucrose and salicin. These reactive compounds can be fermented more easily - false-positive pathogenic results are thus avoided. The combination of thiosulfate as a reactive compound with an iron salt as an indicator causes H2S-positive colonies to become black in colour. The mixture of bile salts suppresses the growth of most of the accompanying microorganisms.

HOBEN et al. (1973) recommended addition of 10-20 µg novobiocin/ml to the medium to improve its selectivity i.e. to inhibit Citrobacter and Proteus colonies which resemble those of Salmonella (black centre).

Typical Composition (g/litre)

Peptones 15.0; sodium chloride 5.0; yeast extract 3.0; sucrose 14.0; lactose 14.0; salicin 2.0; sodium thisulfate 5.0; ammonium iron(III) citrate 1.5; bile salt mixture 2.0; bromothymol blue 0.05; acidic fuchsin 0.08; agar-agar 13.5.

Preparation and Storage

Usable up to the expiry date when stored dry and tightly closed at +15 to +25° C. Protect from light.

After first opening of the bottle the content can be used up to the expiry date when stored dry and tightly closed at +15 to +25° C.

Suspend 75 g in 1 litre of demin. water and let soak for 10 minutes.

Gently heat and bring to boil for a few seconds to dissolve the medium completely.

■ **Do not autoclave.**

If desired, add 15 mg novobiocin per litre to the cooled (50 °C) medium in form of a filter-sterilized solution. Pour plates.

pH: 7.7 ± 0.2 at 25 °C.

The plates are clear and blue-green.

Specimen

e.g. Stool.

Clinical specimen collection, handling and processing, see general instructions of use.Experimental Procedure and Evaluation

Inoculate the culture medium with material taken from an enrichment culture by spreading thinly on the surface of the plates.

Incubation: 18-24 hours at 35 °C aerobically.

Colonies of the most important bacteria usually have the appearance described below. Colonies which are suspected to be pathogenic should be subjected to further tests to confirm their identity.

Appearance of Colonies	Microorganisms
Green, moist, flat, transparent	Shigella, Providencia
Blue-green, with or without a black centre	Salmonella, Paracolobactum, Proteus
Green to bluish, flat, irregular edge	Pseudomonas
Orange-red surrounded by a zone of precipitate	Coliform bacteria

Literature

BISCIELLO, N.B. jr. a. SCHRADE, J.: Evaluation of Hektoen Enteric Agar for the detection of Salmonella in foods and feeds. **- Journ. of AOAC, 57**; 992-996 (1974).

HOBEN, D.A., ASHTON, D.H., a. PETERSEN, A.C.: Some observations on the incorporation of novobiocin into Hektoen Enteric Agar for improved Salmonella isolation. **- Appl. Microbiol., 26**; 126-127 (1973).

KING, S. a. METZGER, W.J.: A new plating medium for the isolation of enteric pathogens. I. Hektoen Enteric Agar. **- Appl. Mikrobiol., 16**; 557-578 (1968).

KING, S. a. METZGER, W.J.: A new plating medium for the isolation of enteric pathogens. II. Comparison of Hektoen Enteric Agar with SS- and EMB-Agar. **- Appl. Microbiol., 16**; 579-581 (1968).

TAYLOR, W.I., a. SCHELHART, D.: Isolation of Shigellae, VII. Comparison of Xylose Lysine Deoxycholate Agar, Hektoen Enteric Agar, Salmonella-Shigella Agar and Eosin Methylene Blue Agar with stool specimen. **- Appl. Microbiol., 21**; 32-37 (1971).

HEKTOEN Enteric Agar

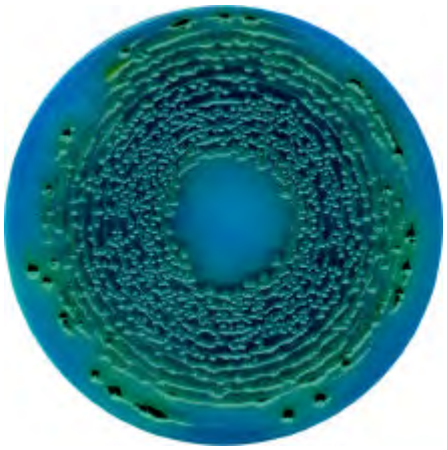
Ordering Information

Product	Merck Cat. No.	Pack size
HEKTOEN Enteric Agar	1.11681.0500	500 g
Novobiocin monosodium salt	CN Biosciences	

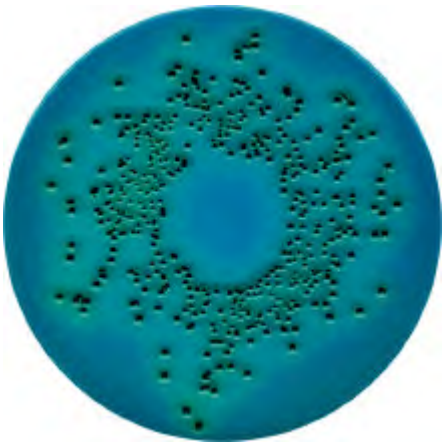
* Hektoen Institute for Medical Research, Chicago, USA

Quality control (spiral plating method)

Test strains	Inoculum (cfu/ml)	Rovery rate (%)	Colour	Colonies Black centre	Recipitate
Escherichia coli ATCC 25922	$< 10^5$	Not limited	orange-red	-	±
Enterobacter cloacae ATCC 13047	10^3-10^5	≥ 30	orange-red	-	±
Klebsiella pneumoniae ATCC 13883	10^3-10^5	≥ 30	orange-red	-	+
Salmonella typhimurium ATCC 14028	10^3-10^5	≥ 20	blue-green	+	-
Salmonella enteritidis ATCC 13076	10^3-10^5	≥ 20	blue-green	+	-
Shigella flexneri ATCC 12022	10^3-10^5	≥ 5	green to blue-green	-	-
Shigella sonnei ATCC 11060	10^3-10^5	≥ 20	green to blue-green	-	-
Proteus mirabilis ATCC 14273	10^3-10^5	≥ 30	green to blue-green	±	-
Enterococcus faecalis ATCC 11700	$> 10^5$	≤ 0.01			
Staphylococcus aureus ATCC 25923	$> 10^5$	≤ 0.01			
Yersinia enterocolitica ATCC 9610	10^3-10^5	≥ 30	orange-yellow	-	±



Proteus mirabilis ATCC 14273



Salmonella enteritidis ATCC13076

Kanamycin Esculine Azide Agar

For the isolation, differentiation and enumeration of enterococci in foodstuffs, water and other materials according to MOSSEL et al. (1978).

Kanamycin esculin azide agar is, unlike culture media containing bile which sometimes exhibit a fluctuating selectivity towards D-streptococci, always highly selective for this group of bacteria.

Mode of Action

Kanamycin and azide largely inhibit the accompanying bacterial flora. D-streptococci are, however, only slightly sensitive to these substances, so they can grow almost normal and hydrolyse the glucoside esculin to give glucose and esculetin. Esculetin forms an olive green to black complex with iron(III) ions.

Typical Composition (g/litre)

Peptones from casein 20.0; yeast extract 5.0; sodium chloride 5.0; sodium citrate 1.0; sodium azide 0.15; kanamycin sulfate 002; esculin 1.0; ammonium iron(III) citrate 0.5; agar-agar 15.0.

Preparation

Suspend 47.5 g/litre, autoclave (15 min at 121 °C), and pour plates.

■ **Do not overheat.**

pH: 7.1 ± 0.2 at 25 °C.

The plates are clear and brown-bluish.

Experimental Procedure and Evaluation

Inoculate by spreading the samples on the surface of the plates.

Incubation: up to 3 days at 35 °C or 42 °C aerobically. The higher temperature increases the selectivity of the medium.

Enterococci colonies are surrounded by a dark zone. Confirmatory tests, e.g. catalase test, glucose utilisation and growth at 45°C ± 1 °C, may be carried out.

Literature

BRANDL, E., ASPERGER, H., PFLEGER, F., u. IBEN, CH.: Zum Vorkommen von D-Streptokokken in Käse. **- Arch. Lebensmittelhyg., 36**; 18-22 (1985).

MOSSEL, D.A.A., BIJKER, P.G.H., a. EELDERING, J.: Streptokokken der Lancefield-Gruppe D in Lebensmitteln und Trinkwasser - Ihre Bedeutung, Erfassung und Bekämpfung. **- Arch. f. Lebensmittelhyg., 29**; 121-127 (1978).

Ordering Information

Product	Merck Cat. No.	Pack size
Kanamycin Esculine Azide Agar	1.05222.0500	500 g

Quality control (spiral plating method)

Test strains	Inoculum (cfu/ml)	Recovery rate (%)	Colour change to olivegreen-black
Enterococcus faecalis ATCC 11700	10^3-10^5	≥ 70	+
Enterococcus hirae ATCC 8043 8043	10^3-10^5	≥ 70	+
Enterococcus durans BFM* 11507	10^3-10^5	≥ 70	+
Staphylococcus aureus ATCC 6538	10^3-10^5	-	-
Bacillus cereus ATCC 11778	> 10^5	≤ 0.01	-
Escherichia coli ATCC 11775	> 10^5	≤ 0.01	-

* Bundesanstalt f. Milchforschung, Kiel, Germany

Enterococcus faecalis ATCC 29212

Streptococcus pyrogenes ATCC 19615

A
MPF

KF Streptococcus Agar Base

For the detection and enumeration of enterococci (faecal streptococci) in water, foodstuffs and other materials according to KENNER, CLARK and KABLER (1960, 1961).

KF Streptococcus agar complies with the recommendations given by APHA for the examination of water (1998) and foodstuffs (1992).

Mode of Action

Maltose and lactose are metabolized by most enterococci with the production of acid and thus promote the growth of these bacteria; undesired microorganisms are largely supressed by sodium azide. Acid formation is detected by bromocresol purple which changes its colour to yellow. Enterococci reduce TTC to give a red formazan and thus appear as red colonies.

Typical Composition (g/litre)

Proteose peptone 10.0; yeast extract 10.0; sodium chloride 5.0; sodium glycerophosphate 10.0; maltose 20.0; lactose 1.0; sodium azide 0.4; bromocresol purple 0.015; agar-agar 15.0.

Also to be added:

2,3,5-triphenyltetrazolium chloride 0.1.

Preparation

Suspend 71.5 g in 1 litre of demin. water. Bring to the boil with frequent agitation. Boil for 5 minutes (or autoclave 10 min at 121°C, if total selectivity is required).

■ **Do not overheat.**

Cool to approx. 50 °C, add 10 ml of a 1 % TTC solution (2,3,5-triphenyltetrazolium chloride), mix, pour plates.

pH: 7.2 ± 0.2 at 25 °C.

The plates are clear and purple.

Experimental Procedure and Evaluation

The membrane filtration method should be used for detection and enumeration if only small numbers of enterococci are suspected to be present; the pour plate method should be employed for larger numbers. The inoculated membrance filters are placed on the agar surface.

Incubation: 48 hours at 35 °C aerobically.

Tropical marine water samples should be incubated anaerobically, due to high incidence of false-positive presumptive counts for enterococci.

The red or pink colonies should be counted, the bacterial count can then be calculated.

Appearance of Colonies	Microorganisms
Abundant growth, red colonies, mostly surrounded by a yellow zone	Enterococci (E. faecalis, E.faecalis var. liquefaciens, E.faecalis var. zymogenes), Str. mitis, Str. bovinus, E. equinus, Str. salivarius and others
Usually scanty growth and no colour change	Lact. plantarum, Pediococcus cerevisiae and others

Literature

American Public Health Association: Compendium of methods for the microbiological examination of foods. - 3rd. ed., 1992.

American Public Health Association: American Water Works Association and Water Pollution Control Federation: Standard Methods for the Examination of Water and Wastewater 20th ed., Washington, 1998.

KENNER, B.A., CLARK, H.F., a. KABLER F.W.: Faecal streptococci. II. Quantification of streptococci in faeces. **- Am. J. Publ. Health., 50**; 1553-1559 (1960).

KENNER, B.A., CLARK, H.F., a. KABLER F.W.: Faecal streptococci. I. Cultivation and enumeration of streptococci in surface waters. **- Appl. Microbiol., 9**; 15-20 (1961)

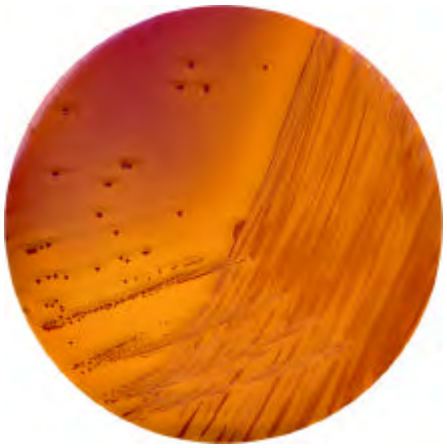
Ordering Information

Product	Merck Cat. No.	Pack size
KF Streptococcus Agar Base	1.10707.0500	500 g
2,3,5-Triphenyltetrazolium chloride	1.08380.0010	10 g

KF Streptococcus Agar Base

Quality control

Test strains	Growth	Red colonies	Yellow zone
Enterococcus faecalis ATCC 11700	good / very good	+	+
Enterococcus hirae ATCC 8043	good / very good	+ (poor)	+
Enterococcus faecalis ATCC 19433	good / very good	+	+
Streptococcus pyogenes ATCC 12344	none / fair	-	-
Streptococcus agalactiae ATCC 13813	none / fair	-	-
Lactobacillus plantarum ATCC 8014	none / fair	-	-
Escherichia coli ATCC 25922	none		
Enterobacter cloacae ATCC 13047	none		
Pseudomonas aeruginosa ATCC 27853	none		



Enterococcus faecalis ATCC 11700

Streptococcus agalactiae ATCC 13813

KING Agar B, Base (Dansk Standard)

Medium proposed by KING et al. (1954) for the detection and enumeration of fluorescing bacteria in water, especially of Pseudomonas fluorescens in drinking water.

This culture medium complies with the Dansk Standard (BONDE 1962, 1965, 1972). FORMIGA (1985) successfully used KING Agar B to identify Corynebacterium diphtheriae with the UV fluorescene test.

Mode of Action

See Pseudomonas Agar F Base (MERCK, Cat. No. 1.10989.). Substitution of di-potassium hydrogen phosphate (which is recommended in the Dansk Standard) by tri-potassium phosphate 3-hydrate prevents decrease in pH after autoclaving and resulting decrease in the development of fluorescein.

Typical Composition (g/litre)

Proteose peptone 20.0; magnesium sulfate 1.5; tri-potassium phosphate 3-hydrate 1.8; agar-agar 10.0.

Also to be added:

Glycerol 10.0 g/litre.

Preparation

Suspend 33.5 g/litre together with 10 g glycerol/litre, autoclave (15 min at 121 °C).

pH: 7.1 ± 0.2 at 25 °C.

The plates are clear and yellowish-brown.

Experimental Procedure and Evaluation

In accordance with the Dansk Standard, prepare dilution series of the sample material (dilution factor 1 in 10), take two 1 ml aliquots from each dilution step and inoculate the plate using pour plate method.

Incubation: up to 72 hours at 20-25 °C aerobically.

Determine the count of the fluorescing bacteria (UV lamp) and the total microbial. count.

For the identification see Pseudomonas Agar F Base (MERCK, Cat. No. 1.10989.).

Literature

BONDE, G.J.: Bacterial Indicators of Water Pollution. (1962).

BONDE, G.J.: øresunds-Vandkomiteens undersøgelser, 288-291 (1965-70).

BONDE, G.J.: Medlemsblad for Den danske Dyrlaegeforening. 55, 671 (1972).

FORMIGA, L.C.D.: New possibilies for the laboratory diagnosis of diphtheria. **- Brazilian J. Med. Biol. Res., 18**; 401-402 (1985).

KING, E.O., WARD, M.K.., a. RANEY, D.E.: Two simple media for the demonstratoin of pyocyanin and fluorescein. **- J. Lab. Clin. Med., 44**; 301-307 (1954).

Ordering Information

Product	Merck Cat. No.	Pack size
KING Agar B, Base (Dansk Standard)	1.10991.0500	500 g
Glycerol (about 87 %)	1.04094.0500	500 ml
Pseudomonas Agar F, Base	1.10989.0500	500 g
UV Lamp (366 nm)	1.13203.0001	1 ea

Quality control

Test strains	Growth	Yellow-green pigment in daylight	Fluorescense at 366 nm
Pseudomonas aeruginosa ATCC 27853	good / very good	+	+
Pseudomonas fluorescens ATCC 13525	good / very good	+	+
Pseudomonas fluorescens ATCC 17397	good / very good	+	+
Aeromonas hydrophila ATCC 7966	good / very good	-	-
Escherichia coli ATCC 25922	good / very good	-	-
Enterobacter cloacae ATCC 13047	good / very good	-	-

KLIGLER Agar (Double sugar iron agar acc. to KLIGLER)

KLIGLER Iron Agar

Test culture medium proposed by KLIGLER (1917, 1918) for identifying Gram-negative intestinal bacteria.

IVD *in vitro diagnosticum –*
For professional use only

See also General Instruction of Use
Warnings and precautions see ChemDAT®
(www.chemdat.info)

KLIGLER agar can be modified as proposed by BADER and HOTZ (1951) by adding 0.2 % urea to give iron-urea agar.

Principle

Microbiological method

Mode of Action

See Triple Sugar Iron Agar.

Typical Composition (g/litre)

Peptone from casein 15.0; peptone from meat 5.0; meat extract 3.0; yeast extract 3.0; sodium chloride 5.0; lactose 10.0; D(+)glucose 1.0; ammonium iron(III) citrate 0.5; sodium thiosulfate 0.5; phenol red 0.024; agar-agar 12.0.

Preparation and Storage

Usable up to the expiry date when stored dry and tightly closed at +15 to +25° C. Protect from light.

After first opening of the bottle the content can be used up to the expiry date when stored dry and tightly closed at +15 to +25° C.

Suspend 55 g/litre, dispense into test tubes, autoclave (15 min at 121 °C). Allow to solidify to give sugar slants.

pH: 7.4 ± 0.2 at 25 °C.

The plates are clear and red

Specimen

e.g. Isolated bacteria from stool,

Clinical specimen collection, handling and processing, see general instructions of use.

Experimental Procedure and Evaluation

See Triple Sugar Iron Agar.

Literature

BADER, R.E., u. HOTZ, G.: Eisen-Harnstoff-Agar, eine Modifikation des Eisen-Agars nach KLIGLER. **- Z. Hyg. Infekt.-Kr., 133**; 20-25 (1951).

KLIGLER, I.J.: A simple medium for the differentiation of members of typhoid-paratyphoid group. **- Am. J. Publ. Health, 7**; 1042-1044 (1917).

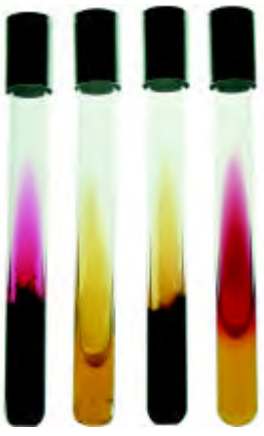
KLIGLER, I.J.: Modification of culture media used in the isolation and differentiation of typhoid, dysentery and allied bacilli. **- J. Exper. Med., 28**; 318-322 (1918).

Ordering Information

Product	Merck Cat. No.	Pack size
KLIGLER Agar (Double sugar iron agar acc. to KLIGLER)	1.03913.0500	500 g
Urea	1.08487.0500	500 g

ohne

mirabilis
ATC 14153



Schraeg

KLIGLER Agar (Double sugar iron agar acc. to KLIGLER)

KLIGLER Iron Agar

Quality control

Test strains	Growth	Butt	Slant
Escherichia coli ATCC 25922	good / very good	yellow	yellow
Citrobacter freundii ATCC 8090	good / very good	yellow and black	yellow
Enterobacter cloacae ATCC 13047	good / very good	yellow	yellow
Shigella flexneri ATCC 12022	good / very good	yellow	red
Salmonella typhimurium ATCC 14028	good / very good	yellow and black	red
Salmonella enteritidis ATCC 13076	good / very good	yellow and black	red
Proteus mirabilis ATCC 14153	good / very good	yellow	red
Proteus vulgaris ATCC 13315	good / very good	yellow and black	red

KOVÁCS' Indole Reagent

Reagent proposed bei KOVÁCS (1928) for detecting microbial indole in the identification of indole-positive and indole-negative microorganisms.

Mode of Action

Some microorganisms can cleave tryptophan which is especially abundant in trypticalle digested peptone to give pyruvic acid, ammonia and indole. Indole then reacts with 4-dimethyl-aminobenzaldehyde to form a dark red dye. As tryptophan also gives a colour reaction with 4-dimethylaminobenzaldehyde, it must be separated from the indole. This is achieved by selectively extracting indole with butanol.

Typical Composition

n-Butanol; hydrochloric acid; 4-dimethylaminobenzaldehyde.

Experimental Procedure and Evaluation

The strain purity of the organism to be tested must first be established; it is then inoculated into an appropriate culture medium (e.g. Standard II Nutrient Broth (Merck, Cat. No. 1.07884.), Nitrate Broth (Merck, Cat. No. 1.10204.), DEV Tryptophan Broth (Merck, Cat. No. 1.10694.), SIM Medium (Merck, Cat. No. 1.05470.), etc.) and incubated for 18-24hours at the optimal incubation temperature. The medium is then covered with a layer of KOVÁCS' indole reagent of about 0.5cm. If indole is present the reagent layer turns cherry red in colour after a few minutes.

- **The reagent solution must be stored in the dark in the refrigerator, otherwise it may turn brown and cannot be used.**

Literature

KOVÁCS, N.: Eine vereinfachte Methode zum Nachweis der Indolbildung durch Bakterien. **- Z. Immunitätsforsch., 55**; 311-315 (1928).

Ordering Information

Product	Merck Cat. No.	Pack size
KOVÁCS' Indole Reagent	1.09293.0100	100 ml

Lactose Broth

Inhibitor-free culture medium used as a preliminary test for coliform bacteria, especially E. coli.

The composition of this medium complies with the recommendations of the American Public Health Association for the examination of water (1998) and foodstuffs (1992), and with the recommendations of the United States Pharmacopeia XXVI (2003) and the European Pharmacopeia II for the examination of pharmaceutical products and raw materials.

Mode of Action

Lactose utilization is indicated by gas production. The gas liberated is collected in DURHAM tubes.

Typical Composition (g/litre)

Peptone 5.0; meat (beef) extract 3.0; lactose 5.0.

Preparation

Suspend 13 g or more/litre (see Table), dispense into test tubes fitted with DURHAM tubes, autoclave (15 min at 121 °C).

pH: 6.9 ± 0.2 at 25 °C.

The prepared broth is clear and yellowish.

Experimental Procedure and Evaluation

Mix 1, 10 or 100 ml samples with the specified volumes of lactose broth. The initial concentaton of the lactose broth must be increased so that the final concentration of the components is maintained at a constant level (13 g/l). See table.

Inoculum (ml)	Amount of Medium in Tube ml	Volume of Medium + Inoculum ml	Dehydrated Lactose Broth Required g/L	Broth concentration
1	10 or more	11 or more	13	1-fold
10	10	20	26	2-fold
10	20	30	19.5	1.5-fold
100	20	120	78	6-fold
100	50	150	39	3-fold

Incubation: 24-48 hours at 35 °C aerobically.

Check the DURHAM tubes for gas production.

Literature

American Public Health Association: Compendium of methods for the microbiological examination of foods. - 3rd ed., 1992.

American Public Health Association: American Water Works Association and Water Pollution Control Federation: Standard Methods for the Examination of Water and Wastewater 20th ed., Washington, 1998.

European Pharmacopeia II, Chapter VIII, 10.

United States Pharmacopeia XXIII, Chaptre "Microbiol. Limit Test", 1995.

Ordering Information

Product	Merck Cat. No.	Pack size
Lactose Broth	1.07661.0500	500 g

Quality control

Test strains	Growth	Gas formation
Escherichia coli ATCC 8739	good / very good	+
Klebsiella pneumoniae ATCC 13883	good / very good	+
Salmonella typhimurium ATCC 14028	good / very good	-
Proteus vulgaris ATCC 13315	poor / fair	-

Lactose TTC Agar with Tergitol® 7

Selective differential medium for the detection and enumeration of E. coli und coliform bacteria in water using the membrane filtration method.

The medium complies with the recommendations of the ISO 9308-1 (1988) and the AFNOR norm NF 90-414 (1985) Water quality – Detection and enumeration of E. coli and coliform bacteria – Membrane filtration method.

Mode of Action

Degradation of lactose to acid is indicated by the pH indicator bromothymol blue, which changes the colour of the medium under the membrane to yellow. Selectivity is achieved by the use of sodium heptadecylsulfate (Tergitol®7) and 2,3,5-Triphenyltetrazoliumchloride (TTC) to inhibit most Gram-positive bacteria. TTC is also part of the differential system. The reduction of TTC by lactose-negative bacteria produces dark red colonies. Lactose-positive E. coli and coliform bacteria reduce TTC weakly; hence their colonies are yellow-orange.

Typical Composition (g/litre)

Lactose 20.0; peptone 10.0; yeast extract 6.0; meat extract 5.0; bromothymol blue 0.05; Tergitol®7 0.1; agar-agar 12.7.

Additive: TTC 0.025.

Preparation

Suspend 53.9 g in 1 litre of demin. water, dissolve and autoclave (121 °C, 15 min). Cool the medium in a water bath to 45-50 °C, add 5 ml of a sterile filtrated 0.05 % aqueous solution of TTC to 100 ml basal medium. Mix homogeneously and pour the medium into Petridishes. The agar layer should have a height of at least 5 mm.

pH: 7.2 ± 0.2 at 25 °C.

The prepared medium is clear and green.

The TTC-solution and the medium is stable for 4 week when stored at +2° - +8°C and protected from light.

Experimental Procedure

Detailed instructions on titer determination are contained in APHA: Standard Methods for the examination of Water and Wastewater (1998).

The type of membrane filter affects the performance of the medium. The best results were obtained using cellulose-nitrate filters, e.g. from Sartorius (order no. 13906-47-ACN).

After filtration the filter is transferred, under aseptical conditions, to the agar surface.

Incubation: 21 ± 3 hours at 36 ± 2 °C.

Evaluation

Lactose-positive bacteria produce yellow-orange colonies and under the membrane yellow-orange halos. The count of these typical colonies is considered to be presumptive coliform bacteria count.

Confirmation of coliform and E. coli count requires further subculture of typical colonies on a non selective agar (e.g. CASO agar) and Tryptophan broth, respectively.

Colonies that are oxidase negative are considered to be **coliform bacteria**. Coliform bacteria that form indole from tryptophane at 44 ± 0.5 °C within 21 ± 3 hours are considered to be **E. coli**.

Literature

CHAPMAN, G.H. 1947. A superior culture medium for the enumeration and differentiation of coliforms. **- J. Bact. 53**: 504 T (1947).

KULP, W., MASCOLI, C., TAVSHANJIAN, O. 1953. Use of tergitol-7 triphenyl tetrazolium chloride agar as the coliform confirmatory medium in routine sanitary water analysis. **- Am. J. Publ. Hlth. 43**: 1111-1113 (1953).

POLLARD, A.L. 1946. A useful selective bactericidal property of Tergitol-7. Science 103: 758-759.AE.

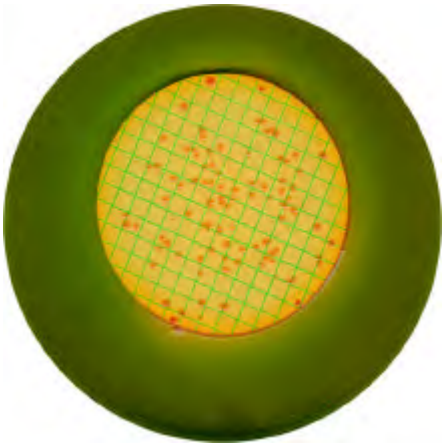
Ordering Information

Product	Merck Cat. No.	Pack size
Lactose TTC Agar with Tergitol® 7	1.07680.0500	500 g
2,3,5-Triphenyltetra-zolium chloride	1.08380.0010	10 g
Bactident® Indole (dropper bottle)	1.11350.0001	1 x 30 ml
Bactident® Oxidase	1.13300.0001	50 test strips
CASO Agar (Casein Peptone Soymeal Peptone Agar)	1.05458.0500	500 g
CASO Agar (Casein Peptone Soymeal Peptone Agar)	1.05458.5000	5 kg
DEV-Tryptophan-Broth	1.10694.0500	500 g
KOVÁCS Indole Reagent	1.09293.0100	100 ml

Lactose TTC Agar with Tergitol® 7

Quality control with the membrane filtration method

Test strains	Growth	Colour of medium (under membrane)	Colony colour	Oxidase	Indole (44 °C)
Escherichia coli ATCC 25922	+	yellow	yellow-orange	-	+
Citrobacter freundii ATCC 8090	+	yellow	yellow-orange	-	-
Pseudomonas aeruginosa ATCC 27853	+	blue	red	+	-
Bacillus cereus ATCC 11778	-				



Citrobachter freundii ATCC 8090 von oben

Escherichia coli ATCC 25922 von oben

Lauryl Sulfate Broth

Selective culture medium used as a presumptive test for coliform bacteria and for the selective enrichment of coliform organisms in the analysis of water, according to MALLMANN and DARBY (1941).

This medium complies with the APHA Recommendations for Water Examination (1998) and the ISO 5541-2 (1996) for milk and milk products.

Mode of Action

The high nutrient quality and the presence of phosphate buffer in this medium ensure rapid growth and increased gas production of even "slowly lactose-fermenting" colifirm bacteria. Gas formation can be detected by using fermentation tubes. The lauryl sulfate largely inhibits the growth of undesired bacteria.

Typical Composition (g/litre)

Tryptose 20.0; lactose 5.0; sodium chloride 5.0; sodium lauryl sulfate 0.1; di-potassium hydrogen phoshate 2.75; potassium dihydrogen phosphate 2.75.

Preparation

Suspend 35.6 g/litre or more, dispense into test tubes fitted with DURHAM tubes, autoclave (15 min at 121 °C).

pH: 6.8 ± 0.2 at 25 °C.

The prepared broth is clear and yellowish-brown.

Inoculum ml	Amount of Medium in Tube ml	Volume of Inoculum ml	Dehydrated Lauryl Sulfate Broth Required g/L	Broth Concentration
1	10 or more	11 or more	35.6	1-fold
10	10	20	71.2	2-fold
10	20	30	53.4	1.5-fold
10	10	30	106.8	3-fold
100	50	150	106.8	3-fold
100	35	135	137.1	3.85-fold
100	20	120	213.6	6-fold

Experimental Procedure and Evaluation

See 7661 Lactose Broth.

Incubation: up to 48 h at 35 °C (or 30 °C) aerobically.

Literature

American Public Health Association, American Water Works Association and Water Pollution Control Federation: Standard Methods for the Examination of Water and Wastewater, 20th ed., Washington, 1998.

HAJNA, A.A., a. PERRY, C.A. Comparative study of presumptive and confirmative media for bacteria of the coliform group and for fecal Streptococci. **– Am. J. Publ. Health** , 33; 550-556 (1943).

MALLMANN, W.L., a. DARBY, C.W.: Use of a lauryl sulfate tryptose broth for the detection of coliform organisms. **– Am. J. Publ. Health, 31**; 127-134 (1941).

International Standardization Organization: Milk and milk products – Enumeration of coliforms. Part 2: Most probable number technique at 30 °C. **ISO/CD 5541-2 (1996)**.

Ordering Information

Product	Merck Cat. No.	Pack size
Lauryl Sulfate Broth	1.10266.0500	500 g

Quality control

Test strains	Growth	Gas formation
Escherichia coli ATCC 25922	good	+
Escherichia coli ATCC 8739	good	+
Citrobacter freundii ATCC 43864	good	+
Staphylococcus aureus ATCC 25923	none / poor	-
Enterococcus faecalis ATCC 19433	fair / very good	-
Aeromonas hydrophila ATCC 7966	unlimited	-
Aeromonas sobria Linx 16	unlimited	- / poor

LB-Agar (Miller)

For the cultivation of E. coli in fermentation and molecular genetic studies.

Mode of Action

LB-Agar is based on the formulation of MILLER (1972) supporting growth of E. coli.

Casein peptone and yeast extract supply essential growth factors, such as nitrogen, carbon, sulfur, minerals and vitamins.

Typical Composition (g/litre)

LB-Agar:

Yeast extract 5.0; peptone from casein 10.0; sodium chloride 10.0; agar-agar 12.0.

Preparation

Suspend 37 g LB-Agar in 1 litre of demin. water and autoclave for 15 min at 121 °C.

pH: 7.0 ± 0.2 at 25 °C.

The prepared agar is clear and yellowish-brown.

Experimental Procedure

According to appropriate use or purpose.

Incubation: 24 h at 35-37 °C aerobically.

Literature

MILLER J.H.: Experiments in Molecular Genetics, Cold Spring Harbor Laboratory (1972).

Ordering Information

Product	Merck Cat. No.	Pack size
LB-Agar (Miller)	1.10283.0500	500 g

Quality control

Test strains	Growth
Escherichia coli ATCC 25922	good
Escherichia coli ATCC 11775	good

Escherichia coli ATCC 11775

Escherichia coli ATCC 25922

LB-Broth (Miller)

For the cultivation of E. coli in fermentation and molecular genetic studies.

Mode of Action

LB-Broth is based on the formulation of MILLER (1972) supporting growth of E. coli.

Casein peptone and yeast extract supply essential growth factors, such as nitrogen, carbon, sulfur, minerals and vitamins.

Typical Composition (g/litre)

LB-Broth:

Yeast extract 5.0; peptone from casein 10.0; sodium chloride 10.0.

Preparation

Suspend 25 g LB-Broth in 1 litre of demin. water and autoclave for 15 min at 121 °C.

pH: 7.0 ± 0.2 at 25 °C.

The prepared broth is clear and yellowish-brown.

Experimental Procedure

According to appropriate use or purpose.

Incubation: 24 h at 35-37 °C aerobically.

Literature

MILLER J.H.: Experiments in Molecular Genetics, Cold Spring Harbor Laboratory (1972).

Ordering Information

Product	Merck Cat. No.	Pack size
LB-Broth (Miller)	1.10285.0500	500 g
LB-Broth (Miller)	1.10285.5000	5 kg

Quality control

Test strains	Growth
Escherichia coli ATCC 25922	good
Escherichia coli ATCC 11775	good

D

Legionella Combi Pack

Selective culture medium for the growth and isolation of Legionella spp. from biological sample material. In combination with sample preparation by heat or acid, this culture medium is acknowledged as the best method of isolating Legionella pneumophila from natural water (DENNIS et al., 1984).

IVD

in vitro diagnosticum –
For professional use only

See also General Instruction of Use
Warnings and precautions see ChemDAT®
(www.chemdat.info)

CE

In 1977 McDADE et al. isolated a bacterium, which was first described in connection with an epidemic, which occured after a meeting of the "American Legion" in Philadelphia. For this reason the disease was called legionellosis ("Legionnaires' Disease"). The most important pathogen of legionellosis, among a total of 33 species, is Legionella pneumophila.

Principle

Microbiological method

Mode of Action

The growth of Legionella is improved by the following components: the activated charcoal binds CO2, changes surface tension and neutralises growth-inhibiting substances. L-cysteine HCl and a-ketoglutarate are directly used to form amino acid and chelate respectively. Ferric pyrophosphate serves as a source of iron and the optimal pH value for growth is adjusted by the ACES buffer. The accompanying flora is largely inhibited by the addition of glycine and the use of the antibiotic mixture of vancomycin, Polymyxin B and cycloheximide.

Typical Composition

1. Legionella CYE-Agar Base (g/liter):activated charcoal 2.0; yeast extract 10.0; agar-agar 12.0
2. Legionella BCYE a-growth supplement (composition of one vial; for 500 ml of culture medium) ACES buffer 5.0 g; ferric pyrophosphate 0.125 g; cysteine HCl 0.2 g; a-ketoglutarate 0.5
3. Legionella GVPC selective supplement (composition of one vial; for 500 ml of culture medium) glycine 1.5 g, vancomycin-HCl 0.5mg; Polymyxin B-sulfate 40,000 I.E.; cycloheximide 40 mg

Preparation and Storage

Usable up to the expiry date when stored dry and tightly closed at +15 to +25° C. Protect from light.

After first opening of the bottle the content can be used up to the expiry date when stored dry and tightly closed at +15 to +25° C.

Suspend 12.0 g CYE-Agar Base (contents of one vial No. 1) in 440 ml demineralized water. Autoclave for 15 min at 121 °C. Dissolve contents of one vial of BCYE a-growth supplement (vial No. 2) aseptically in 50 ml sterile demin. water. Pour contents of one vial of GVPC selective supplement (vial No. 3) aseptically in 10 ml sterile demin. water. To make the Legionella GVPC selective agar, pour the contents of both vials into the CYE Agar Base after it has cooled to about 45-50 °C. Mix well and pour plates.

pH: 6.9 ± 0.2 at 25 °C.

Specimen

e.g. Pleural fluid, human lung tissue, respiratory tract samples. Clinical specimen collection, handling and processing, see general instructions of use.

Experimental Procedure and Evaluation

Sample Preparation

It is recommended that three plates should be prepared for every sample: one after heat treatment, one after acid treatment and one without pre-treatment.

Heat treatment:

10 ml of concentrated examination material are incubated in a water bath at 50 °C for 30 minutes.

Acid treatment:

1. Centrifuge 10 ml of concentrated examination material at 2,500R/min for 20 minutes in screw capped centrifuge vessels.
2. Pour off the supernatant to about 1 ml.
3. Add 9 ml HCl-KCl buffer*, shake gently and leave to stand for about 5 minutes.

   * HCl-KCl buffer:
   3.9 ml of 0.2 M HCl
   25.0 ml of 0.2 M KCl
   adjust pH to 2.2 ± 0.2 by adding 1 M KOH

Application

1. Spread 0.1 ml of pre-treated sample onto GVPC-Selective Agar.
2. Incubate for up to 7 days at 35 °C under microaerophilic conditions (see Anaerocult® C).

Evaluation

Legionella grows as a 2-3 mm, hour-glass shaped, grey-white colony. A few strains have a slightly blue colouring. Suspicious colonies are subcultured on to CASO Agar (Cat. No. 1.05458) with 5 % sheep-blood and B.C.Y.E.-Agar. Isolates that fail to grow on Blood Agar and poorly staining Gram-negative rods are presumptively identified as Legionella. These presumptive colonies should be serologically typified for further identification.

Literature

BOPP, C.A., SUMMER, J.W., MORRIS, G.K., a. WELLS, J.G.: Isolation of Legionella spp. from environmental water samples by low-pH treatment and use of a selective medium. **- J. Clin. Microbiol., 13**; 714-719 (1981).

DENNIS, P.J., BARTLETT, C.L.R., a. WRIGHT, A.E.: Comparison of isolation methods for Legionella spp. in Thornsbury, C. et al. (eds) Legionella: Proceedings of the 2nd International Symposium Washington D.C. **- Am. Soc. Microbiol.**, pp. 294-296 (1984).

Legionella Combi Pack

EDELSTEIN, P.H.: Improved semiselective medium for isolation of Legionella pneumophila from contaminated clinical and environmental specimens. **- J. Clin. Microbiol., 14**; 298-303 (1981).

EDELSTEIN, P.H.: Comparative study of selective media for isolation of Legionella pneumophila from potable water. **- J. Clin. Microbiol., 16**; 697-699 (1982).

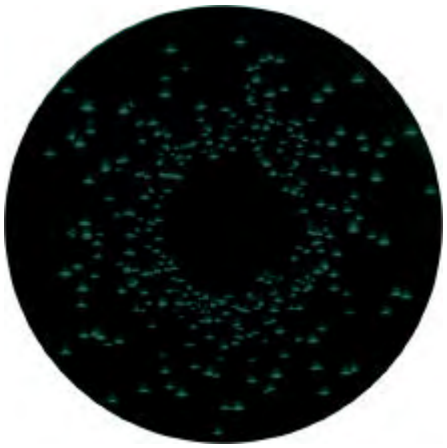
FEELEY, J.C., GORMAN, G.W., WEAVER, R.E., Mackel, D.C., a. SMITH, H.W.: Primary isolation media for legionnaires disease bacterium. **- J. Clin. Microbiol., 8**; 320-325 (1978).

FEELEY, J.C., GIBSON, R.J., GORMAN, G.W., LANGFORD, N.C., RASHEED, J.W., MACKEL, D.C., a. BAINE, W.B.: Charcoal Yeast extract agar: Primary isolation medium for Legionella pneumophila. **- J. Clin. Microbiol., 10**; 437-441 (1979).

McDADE, J.E., SHEPARD, C.C., FRASER, D.W., et al.: Legionnaires' disease. Isolation of bacterium and demonstration of its role in other respiratory disease. **- N. Engl. J. Med., 297**; 1197-1203 (1977).

PASCULLE, A.W., FEELEY, J.C., GIBSON, R.J., CORDES, L.G., Myerowitz, R.L., PATTON, C.M., GORMAN, G.W., CARMACK, L.L., EZZELL, J.W. a. DOWLING, J.N.: Pittsburgh pneumonia agent: direct isolation from human lung tissue. **- J. Infect. Dis., 141**; 727-732 (1980).

WEAVER, R.E.: Cultural and staining characteristics. In Jones, G.L., and Herbert, G.A. (eds). "Legionairs" the disease, the bacterium and methodology. October Ed. U. S. Dept. Health, Education, and Welfare, Public Health Service, Center for Disease Control, Atlanta, GA. pp. 39-43 (1978).



Legionella pneumophila spp. fraseri
ATCC 33216

Ordering Information

Product	Merck Cat. No.	Pack size
Legionella Combi Pack	1.10425.0001	6 x 500 ml
Legionella BCYE α-Growth-Supplement	1.10240.0001	1 x 5 vials
Legionella GVPC Selective Supplement	1.10241.0001	1 x 4 vials
Legionella CYE Agar Base	1.10242.0001	12 x 500 ml
Anaerobic jar	1.16387.0001	1 ea
Anaeroclip®	1.14226.0001	1 x 25
Anaerocult® C	1.16275.0001	1 x 10
Anaerocult® C mini	1.13682.0001	1 x 25
BCYE α-Growth Supplement	1.10240.0001	5 x 50 ml
CASO Agar (Casein Peptone Soymeal Peptone Agar)	1.05458.0500	500 g, 5 kg
CYE Agar Base	1.10242.0001	12 x 12.0 g
CYE Agar Base	1.10242.0500	500 g
GVPC Selective Supplement	1.10241.0001	4 x 10 ml
Legionella Combi Pack contents: CYE Agar Base (No. 1) BCYE α-Growth Supplement (No. 2) GVPC Selective Supplement (No.3)	1.10425.0001	6 x 12.0 g 6 vials à 50 ml 6 vials à 10 ml
Plate basket	1.07040.0001	1ea

Quality control

Test strains	Growth single colonies	Growth 3-loop smear
Legionella pneumophila spp. fraseri ATCC 33216	good	good
Legionella pneumophila ATCC 33823	good	good
Legionella pneumophila subsp. pneumophila ATCC 33152	good	good
Staphylococcus epidermidis ATCC 12228	none / fair	medium
Escherichia coli ATCC 25922	none / fair	medium
Pseudomonas aeruginosa ATCC 27853	none / fair	medium
Bacillus cereus ATCC 11778	none / fair	medium

LEIFSON Agar
(Deoxycholate Citrate Agar acc. to LEIFSON, modified)

Medium proposed by LEIFSON (1935) and modified by HYNES (1942) for the isolation of salmonellae and shigellae.

The culture medium complies with the European PharmacopeiaII.

Mode of Action

The concentrations of deoxycholate and citrate contained in this medium are so high that they completely suppress the gram-positive microbial flora and inhibit the coliform bacteria more or less. Salmonellae grow normally; some species of Shigella are slightly inhibited (e.g. Shig. shigae).

The degradation of lactose causes an acidification of the medium surrounding the relevant colonies and the pH indicator neutral red changes its colour to red. These colonies usually are also surrounded by a turbid zone of precipitated deoxycholic acid due to acidification of the medium. Colonies of lactose-negative microorganisms are colourless. The reduction of thiosulfate to sulfide is indicated by the formation of black iron sulfide.

Typical Composition (g/litre)

Meat extract 5.0; peptone from meat 5.0; lactose 10.0; sodium thiosulfate 5.4; ammonium iron(III) citrate 1.0; sodium citrate 6.0; sodium deoxycholate 3.0; neutral red 0.02; agar-agar 12.0:

Preparation

Suspend 47.5 g/litre, cool quickly, pour plates.

The medium is heat sensitive. Bring to boil with frequent agitation. Do not remelt.

- **Do not autoclave.**

pH: 7.5 ± 0.2 at 25 °C.

The plates are clear and reddish-brown.

- **The prepared culture medium can be stored for 1 week in the refrigerator.**

Experimental Procedure and Evaluation

Inoculate by spreading the sample or material from an enrichment culture on the surface of the culture medium.

In view of the strong inhibitory action of LEIFSON Agar, it is advisable to use a less inhibitory selective medium as well, e.g. MaxCONKEY Agar or Deoxycholate Lactose Agar.

Incubation: 24-48 hours at 35 °C aerobically.

Appearance of Colonies	Microorganisms
After 18 hours: pale pink to colourless, diameter 1 mm. After 48 hours: slightly opaque, often with a central gray dot, diameter approx. 2 mm	Salmonella typhosa
After 18 hours: pale pinkt to colourless, diameter approx. 1mm After 48 hours: slightly opaque, convex, with a central black dot	Salmonella paratyphi B and other H_2S-positive salmonellae
At first colourless, then pale pink (slight lactose degradation). After 18 hours: flat, diameter approx. 1 mm. After 38 hours: diameter approx 2 mm	Shigella sonnei
As S. sonnei but with a convex centre, often with flat edges	Shigella flexneri
Similar to Salmonella and Shigella, characteristic sweet smell	Pseudomonas
Similar to Salmonella and Shigella, black central dot	Proteus vulgaris, most strains of Proteus mirabilis
Inhibited growth, pink colonies surrounded by turbid precipitation zone, diameter approx. 1-2 mm	Escherichia coli
Inhibited growth, colourless or pink centre, convex, mucoid, opaque, diameter approx. 1-2mm	Enterobacter, Klebsiella

Literature

European Pharmacopeia II, Chapter VIII, 10.

LEIFSON, E.: New culture media based on sodium deoxycholate for the isolation of intestinal pathogens and for the enumeration of colon bacilli in milk and water. **- J. Path. Bact., 40**; 581-599 (1935).

HYNES, M.: The isolation of intestinal pathogens by selective media. **-J.Path. Bact., 54**; 193-207 (1942).

Ordering Information

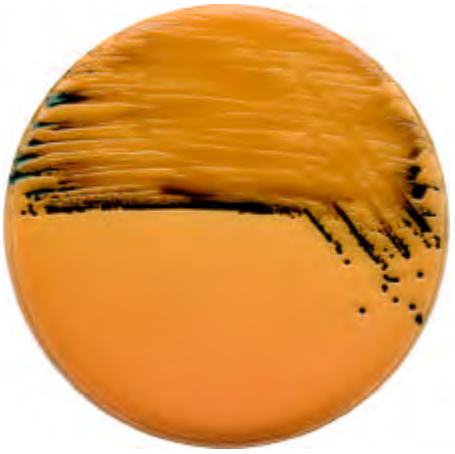
Product	Merck Cat. No.	Pack size
LEIFSON Agar (Deoxycholate Citrate Agar acc. to LEIFSON, modified)	1.02896.0500	500 g
MacCONKEY Agar	1.05465.0500	500 g
XLD Agar	1.05287.0500	500 g

LEIFSON Agar
(Deoxycholate Citrate Agar acc. to LEIFSON, modified)

Quality control

Test strains	Growth	Colour	Colonies Precipitate	Black centre
Escherichia coli ATCC 25922	poor / fair	red / pink	+	-
Klebsiella pneumoniae ATCC 13883	good / very good	red / pink	-	-
Shigella flexneri ATCC 12022	fair / very good	colourless	-	-
Shigella sonnei ATCC 11060	fair / very good	colourless	-	-
Salmonella typhimurium ATCC 14028	good / very good	colourless	-	+
Salmonella enteritidis NCTC 5188	good / very good	colourless	-	+
Proteus mirabilis ATCC 14273	good / very good	colourless	-	±
Staphylococcus aureus ATCC 25923	none			
Bacillus cereus ATCC 11778	none			

Proteus mirabilis ATCC 14273



Salmonella enteritidis NCTC 5188

A

Letheen Agar Base, modified

Letheen Agar is special formulations for the determination of bacterial activity of quaternary ammonium compounds.

The media formulations comply with the recommendations of FDA/BAM (1995).

Mode of Action

Letheen media are highly nutritious containing Lecithin and Tween® 80 for neutralizing quaternary ammonium compounds. These media are modifications of the AOAC formulae.

Letheen Agar Base is used for the microbiological sampling of environmental surfaces that have been treated with disinfectants.

Typical Composition (g/litre)

Letheen Agar Base:

Peptone from meat 10.0; peptone from casein 10.0; meat extract 3.0; yeast extract 2.0; sodium chloride 5.0; D(+) glucose 1.0; lecithin 1.0; sodium bisulfite 0.1; agar-agar 20.0

Preparation of Letheen Agar

Suspend 52.1 g and 7 ml of Tween® 80 in 1 litre of distilled or demin. water until evenly dispersed. Heat, if necessary, with repeated stirring and boil for 1 minute; autoclave at 121 °C for 15 min. Pour into plates.

pH: 7.2 ± 0.2 at 25 °C.

The plates are turbid and brownish.

Incubation: 24 - 48 hours at 35°C aerobically.

Experimental Procedure

Depending on the purpose for which the media are used.

Literature

FDA Bacteriological Analytical Manual (BAM), 8th ed. (1995), chapter 23: Microbiological Methods for Cosmetics, Letheen Agar (modified) = M 78, Letheen Broth (modified) = M 79.

Ordering Information

Product	Merck Cat. No.	Pack size
Letheen Agar Base, modified	1.10404.0500	500 g
Tween® 80	8.22187.0500	500 ml

Staphylococcus epidermidis ATCC 12228

Quality control

Test strains	Growth
Escherichia coli ATCC 25922	good / very good
Enterococcus faecalis ATCC 29212	good / very good
Pseudomonas aeruginosa ATCC 27853	good / very good
Staphylococcus aureus ATCC 25923	good / very good
Staphylococcus epidermidis ATCC 12228	good / very good
Salmonella typhimurium ATCC 14028	good / very good

A

Letheen Broth Base, modified

Letheen Broth is special formulations for the determination of bacterial activity of quaternary ammonium compounds.

The media formulations comply with the recommendations of FDA/BAM (1995).

Mode of Action

Letheen media are highly nutritious containing Lecithin and Tween® 80 for neutralizing quaternary ammonium compounds. These media are modifications of the AOAC formulae.

Letheen Broth Base is used for determining the phenol coefficient of quaternary compounds.

Typical Composition (g/litre)

Letheen Broth Base:

Peptone from meat 20.0; peptone from casein 5.0; meat extract 5.0; yeast extract 2.0; sodium chloride 5.0; lecithin 0.7; sodium bisulfite 0.1.

Preparation of Letheen Broth

Suspend 37.8 g and 5 ml of Tween® 80 in 1 litre of distilled or demin. water until evenly dispersed. Heat, if necessary, with repeated stirring to dissolve completely and autoclave at 121 °C for 15 min.

pH: 7.2 ± 0.2 at 25 °C.

The prepared broth is turbid and yellowish-brown.

Incubation: 24 - 48 hours at 35 °C aerobically.

Experimental Procedure

Depending on the purpose for which the media are used.

Literature

FDA Bacteriological Analytical Manual (BAM), 8th ed. (1995), chapter 23: Microbiological Methods for Cosmetics, Letheen Agar (modified) = M 78, Letheen Broth (modified) = M 79.

Ordering Information

Product	Merck Cat. No.	Pack size
Letheen Broth Base, modified	1.10405.0500	500 g
Tween® 80	8.22187.0500	500 ml

Quality control

Test strains	Growth
Escherichia coli ATCC 25922	good / very good
Enterococcus faecalis ATCC 29212	good / very good
Pseudomonas aeruginosa ATCC 27853	good / very good
Staphylococcus aureus ATCC 25923	good / very good
Staphylococcus epidermidis ATCC 12228	good / very good
Salmonella typhimurium ATCC 14028	good / very good

LEVINE EMB Agar
(Eosin Methylene-blue Lactose Agar acc. to LEVINE)

For the isolation and differentiation of Escherichia coli and Enterobacter and for the rapid identification of Candida albicans according to LEVINE (1918, 1921).

IVD *in vitro diagnosticum –*
For professional use only

See also General Instruction of Use
Warnings and precautions see ChemDAT®
(www.chemdat.info)

The culture medium complies with the recommendations of the APHA Standard Methods for the Examination of Water and Wastewater (1998) and the United States Pharmacopeia XXVI (2003).

Principle
Microbiological method

Mode of Action
The dyes contained in this medium inhibit the growth of many accompanying Gram-positive microorganisms. According to WELD (1952, 1953) and VOGEL and MOSES (1957), LEVINE EMB Agar can be used to identify Candida albicans in clinical specimens, if chlorotetracycline hydrochloride is added to inhibit the entire accompanying bacterial flora. LEVINE EMB Agar can also be utilized for the identification of coagulase-positive staphylococci which grow characteristically as colourless "pin-point" colonies and which show good agreement with the results of the coagulase test (MENOLASINO et al. 1960).

Typical Composition (g/litre)
Peptone 10.0; lactose 10.0; di-potassium hydrogen phosphate 2.0; eosin, yellowish 0.4; methylene blue 0.065; agar-agar 13.5.

Preparation and Storage
Usable up to the expiry date when stored dry and tightly closed at +15 to +25° C. Protect from light.

After first opening of the bottle the content can be used up to the expiry date when stored dry and tightly closed at +15 to +25° C.

Suspend 36 g/litre, autoclave (15 min at 121 °C), and pour plates.

pH: 7.0 ± 0.2 at 25 °C.

The plates are clear and red-brown.

If cultivating Candida, add 0,1 mg tetracycline hydrochloride/ litre after autoclaving and mix homogeneously. The culture medium then is blue.

Specimen
e.g. Stool.
Clinical specimen collection, handling and processing, see general instruction of use.

Experimental Procedure and Evaluation
Inoculate by thinly spreading the sample material on the surface of the culture medium.

Incubation: 1-2 days at 35 °C aerobically.

To obtain a primary culture of Candida, incubate the plates containing chlorotetracycline in a 10 % carbon dioxide atmosphere (e.g. with Anaerocult® C or C mini).

Appearance of Colonies	Microorganisms
Diameter 2-3 mm, greenish metallic sheen in reflected light, dark or even black centre in transmitted light	Escherichia coli
Diameter 4-6 mm, graybrown centre in transmitted light, no metallic sheen	Enterobacter
Transparent, amber-coloured	Salmonella and Shigella
Colourless, "pin-point" colonies	Coagulase-positive staphylococci
"Spidery" - or "feathery"	Candida albicans
Yeast-like, round, smooth	Other Candida species. Sometimes Nocardia

Literature

American Public Health Association, American Water Works Association and Water Pollution Control Federation: Standard Methods for the Examination of Water and Wastewater, 20th ed., Washington 1998.

LEVINE, M.: Differentation of E. coli and A. aerogenes on a simplified eosin-methylene blue agar. **- J. Infect. Dis., 23**; 43-47 (1918).

LEVINE, M.: Bacteria fermenting lactose and the significance in water analysis. **- Bull., 62**; Iowa State College Engr. Exp. Station (1921).

MENOLASINO, N.I., GRIEVES, B., a PAYNE, P.: Isolation and Identification of coagulase-positive staphylococci on Levine's eosin-methylene blue agar. **- J. Lab. Clin. Med., 56 (6)**; 908-910 (1960).

VOGEL, R.A., a. MOSES, M.R.: Welds method for the rapid identification of Candida albicans in clinical materials. **- Am. J. Clin. Path., 28 (1)**; 103-106 (1957).

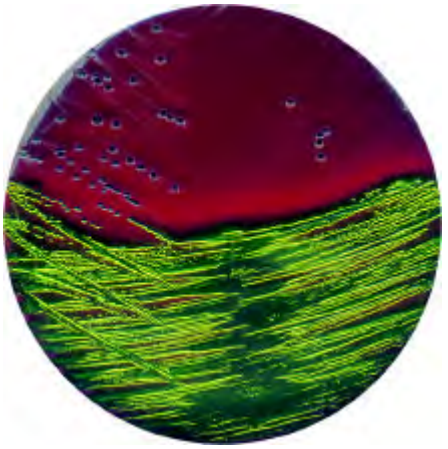
WELD, J.T.: Candida albicans. Rapid identification in pure cultures with carbon dioxyde on modified eosin-methylene blue medium. **- Arch. Dermat. Syph., 66** ; 691-694 (1952).

WELD, J.T.: Candida albicans. Rapid identification in cultures made directly from human materials. **- Arch. Dermat. Syph., 67 (5)**; 473-478 (1953).
United States Pharmacopeia XXVI, Chapter "Microbial Limit Tests", 1985.

LEVINE EMB Agar
(Eosin Methylene-blue Lactose Agar acc. to LEVINE)

Ordering Information

Product	Merck Cat. No.	Pack size
LEVINE EMB Agar (Eosin Methylene-blue Lactose Agar acc. to LEVINE)	1.01342.0500	500 g
Anaerobic jar	1.16387.0001	1 ea
Anaeroclip®	1.14226.0001	1 x 25
Anaerocult® C	1.16275.0001	1 x 10
Anaerocult® C mini	1.13682.0001	1 x 25
Plate basket	1.07040.0001	1ea
Tetracycline hydrochloride	CN Biosciences	



Escherichia coli ATCC 11775

Quality control

Test strains	Growth	Colonies	
		Blue	Metallic sheen
Escherichia coli ATCC 25922	good / very good	+	+
Escherichia coli ATCC 11775	good / very good	+	+
Escherichia coli 194	good / very good	+	+
Enterobacter cloacae ATCC 13047	good / very good	pale blue	-
Shigella sonnei ATCC 11060	good / very good	-	-
Salmonella typhimurium ATCC 14028	good / very good	-	-
Proteus mirabilis ATCC 14273	good / very good	-	-
Staphylococcus aureus ATCC 25923	none / poor	-	-

Listeria Enrichment Broth (LEB) acc. to FDA/IDF-FIL

For the selective enrichment of Listeria.

Mode of Action

The formulation of the broth is a modified Tryptic Soy (CASO) Broth with additional 6 g/litre of yeast extract.

The inhibition of undesired accompanying flora is achieved by the addition of acriflavin HCl, cycloheximide and nalidixic acid.

In contrary to Listeria Enrichment Broth (Base) (Merck Cat. No. 1.11951.) this broth already contains the antibiotic substances.

Typical Composition (g/litre)

Peptone from casein 17.0; peptone from soymeal 3.0; D(+) glucose 2.5; sodium chloride 5.0; di-potassium hydrogen phosphate 2.5; yeast extract 6.0; acriflavine 0.010; cycloheximide 0.05; nalidixic acid 0.04.

Preparation

Suspend 36.1 g in 1 litre of demin. water and autoclave (15 min at 121 °C).

pH: 7.3 ± 0.2 at 25 °C.

The prepared broth is clear and yellowish-brown.

Experimental Procedure

The broth is inoculated with the sample (usually 25 g or 25 ml sample into 225 ml broth).

Incubation: up to 48 hours at 30 °C aerobically.

Afterwards 0.1 ml of the broth are streaked on a Listeria Selective Agar, e.g. PALCAM Agar and/or OXFORD Agar, for separated colonies.

Literature

LOVETT J., FRANCIS D.W., a HUNT J.M.: **- J. Food Protection, 50**; 188-192 (1987)

LOVETT J., HITCHINS, A.D.: FDA Federal Register, 53;X 44148-44153 (1988)

AJELLO, G., HAYES, P., a FEELEY, J.: Abstracts of the Annual Meeting, A.S.M., Washington DC, P 5 (1986)

FDA Bacteriological Analytical Manual; 8th ed. (1995), chapter 10.

Ordering Information

Product	Merck Cat. No.	Pack size
Listeria Enrichment Broth (LEB) acc. to FDA/IDF-FIL	1.10549.0500	500 g
Listeria Enrichment Broth (LEB) acc. to FDA/IDF-FIL	1.10549.5000	5 kg
OXFORD Listeria Selective Agar (Base)	1.07004.0500	500 g
OXFORD Listeria Selective Supplement	1.07006.0001	1 x 13 vials
PALCAM Listeria Selective Agar (Base)	1.11755.0500	500 g
PALCAM Listeria Selective Supplement acc. to VAN NETTEN et al.	1.12122.0001	1 x 16 vials

Quality control

Test strains	cfu/ml after 24 hours	Growth
Listeria monocytogenes ATCC 19114	$\geq 10^4$	good
Listeria monocytogenes ATCC 13932	$> 10^4$	good
Listeria monocytogenes ATCC 35152	$> 10^4$	good
Listeria innocua ATCC 33090	$> 10^4$	good
Staphylococcus aureus ATCC 25923	$< 10^3$	fair
Enterococcus faecalis ATCC 19433	$< 10^3$	fair

Listeria Enrichment Broth (LEB) Base acc. to FDA (IDF-FIL)

For the selective enrichment of Listeria according to FIL/IDF Standard 143 (1990) and FDA/BAM (1992).

Mode of Action

The Enrichment Broth is based on the formulation of Casein-peptone Soymeal-peptone Broth (**k** CASO Broth) with additional 6 g/litre yeast extract. The inhibition of the common bacteria is achieved by the addition of selective supplemnts, e.g. Listeria Selective Supplement acc. to IDF-FIL (Cat. No. 1.11781.0001) or Listeria Selective Supplement acc. to FDA (Cat. No. 1.11883.0001).

Typical Composition (g/litre)

Peptone from casein 17.0; peptone from soymeal 3.0; D(+)glucose 2.5; sodium chloride 5.0; potassium dihydrogen phosphate 2.5 (CASO-broth); yeast extract 6.0.

Preparation

Suspend 18 g in 500 ml of distilled water, autoclave (15 min at 121 °C). Dissolve the lyophilisate of 1 vial Listeria-Enrichment-Supplement (Cat. No. 1.11781.0001 or 1.11883.0001) by adding of 1 ml sterile distilled water. Mix gently and add the contents to the Enrichment Broth cooled to under 50 °C.

pH: 7.3 ± 0.2 at 25 °C.

The prepared broth is clear and brown.

Experimental Procedure and Evaluation

Inoculate Listeria-Enrichment Broth (usually add 25 g test portion of the sample to 225 ml broth) and incubate 48 h in total at 30 °C aerobically.

Streak about 0.1 ml Enrichment Broth on a Listeria-Selective-Agar plate, e.g. PALCAM-Agar (Cat. No. 1.11755.0500) or Oxford-Agar (Cat. No. 1.07004.0500), in a way that single colonies are well isolated.

Literature

LOVETT, J., FRANCIS, D.W., a. HUNT, J.M.: **- J. Food Protection, 50**; 188-192 (1987).

LOVETT, J., HITCHINS, A.D.: **- FDA Federal Register, 53**; 44148-44153 (1988).

IDF Standard 143: Milk and Milk products; detection of Listeria monocytogenes (1990).

AJELLO, G., HAYES, P., a. FEELEY, J.: Abstracts of the Annual Meeting, A.S.M., Washington DC, P 5 (1986).

Ordering Information

Product	Merck Cat. No.	Pack size
Listeria Enrichment Broth (LEB) Base acc. to FDA (IDF-FIL)	1.11951.0500	500 g
Listeria Selective Enrichment Supplement acc. to FDA-BAM	1.11781.0001	1 x 16 vials
Listeria Selective Enrichment Supplement acc. to FDA-BAM 1992	1.11883.0001	1 x 16 vials

Quality control

Test strains	Growth
Listeria monocytogenes ATCC 19118	good / very good
Listeria monocytogenes ATCC 13932	good / very good
Listeria monocytogenes ATCC 7973	good / very good
Listeria innocua ATCC 33090	good / very good
Staphylococcus aureus ATCC 25923	good / very good
Escherichia coli ATCC 25922	none
Enterococcus faecalis ATCC 19433	good / very good

M

Listeria Selective Enrichment Supplement acc. to FDA-BAM 1992

Additive for the preparation of Listeria-Enrichment-Broth (Base) acc. to FDA/IDF-FIL (Merck Cat. No. 1.11951.0500).

Mode of Action

Listeria-Selective-Enrichment-Supplement acc. to FDA is a mixture of two antibiotics and a dye in lyophilzed form. It largely inhibits the growth of accompanying bacteria for the selective enrichment of Listeria monocytogenes.

Composition (per vial)

Acriflvin HCl 7.5 mg; cycloheximide 25.0 mg; nalidixic acid (sodium salt) 20.0 mg.

Experimental Procedure and Evaluation

The lyophilisate is to dissolve in the original vial by adding about 1 ml of sterile distilled water. the vial contents is mixed evenly into 500 ml of sterile medium base cooled below 50 °C.

Literature

FDA Bacteriological Analytical Manual; 7th ed. (1992), chapter 10.

Ordering Information

Product	Merck Cat. No.	Pack size
Listeria Selective Enrichment Supplement acc. to FDA-BAM 1992	1.11883.0001	1 x 16 vials

Listeria Selective Enrichment Supplement acc. to FDA-BAM 1995/IDF-FIL

Additive for the preparation of Listeria-Enrichment-Broth (Base) acc. to FDA/IDF-FIL (Merck Cat. No. 1.11951.0500) and Buffered Listeria-Enrichment-Broth acc. to FDA-BAM 8th Edition 1995 (Merck Cat. No. 1.09628.0500).

Mode of Action

Listeria-Selective-Enrichment-Supplement acc. to FDA/IDF is a mixture of two antibiotics and a dye in lyophilzed form. It largely inhibits the growth of accompanying bacteria for the selective enrichment of Listeria monocytogenes. In accordance with the standard 143: 1990 of the IDF-FIL (milk and milk products) and FDA-BAM 1995 for the detection of Listeria monocytogenes, the concentration of Acriflavine HCl in this supplement is reduced from 15 mg to 10 mg/litre.

Composition (per vial)

Acriflvin HCl 5.0 mg; cycloheximide 25.0 mg; nalidixic acid (sodium salt) 20.0 mg.

Experimental Procedure and Evaluation

The lyophilisate is to dissolve in the original vial by adding about 1 ml of sterile distilled water. the vial contents is mixed evenly into 500 ml of sterile medium base cooled to under 50 °C.

Literature

FDA Bacteriological Analytical Manual; 8th ed. (1995), chapter 10.

Ordering Information

Product	Merck Cat. No.	Pack size
Listeria Selective Enrichment Supplement acc. to FDA-BAM 1995/IDF-FIL	1.11781.0001	1 x 16 vials

Lysine Iron Agar

Test agar introduced by EDWARDS and FIFE (1961) for the simultaneous detection of lysine decarboxylase (LDC) and hydrogen sulfide (H_2S) production for the identification of Enterobacteriaceae, especially for Salmonella and Arizona.

JOHNSON et al. (1966) and TIMMS (1971) obtained good results with Lysine Iron Agar. Identification is improved by using the medium in combination with Triple Sugar Iron Agar (THATCHER and CLARK 1968). HENNER et al. (1982) reported that Lysine Iron Agar is superior to other comparable culture media for differentiating between Proteus and Salmonella.

Mode of Action

Lysine is decarboxylated by LDC-positive microorganisms to give the amine cadaverine which causes the pH indicator bromocresol purple to change its colour to violet. As decarboxylation only occurs in an acidic medium (below pH 6.0), the culture medium must first be acidified by glucose fermentation. This medium can therefore only be used for the differentiation of glucose-fermenting microoganisms.

LDC-negative, glucose-fermenting microoganisms cause the entire culture medium to turn yellow. On prolonged incubation alkalinisation of the culture medium surface may occur, resulting in a colour change to violet. H_2S production causes a blackening of the culture medium due to the formation of iron sulfide.

Species of the Proteus-Providencia group, with the exception of a few Proteus morganii strains, deaminate lysine to give α-ketocarboxylic acid; this compound reacts with the iron salt near the surface of the medium, under the influence of oxygen, to form reddish-brown compounds.

Typical Composition (g/litre)

Peptone from meat 5.0; yeast extract 3.0; D(+)glucose 1.0; L-lysine monohydrochloride 10.0; sodium thiosulfate 0.04; ammonium iron(III) citrate 0.5; bromocresol purple 0.02; agar-agar 12.5.

Preparation

Suspend 32 g/litre, dispense into test tubes, autoclave (15 min at 121 °C). Allow to solidify to give agar slants.

pH: 6.7 ± 0.2 at 25 °C.

The prepared culture medium is clear and violet.

Experimental Procedure and Evaluation

Inoculate the medium with the pure culture under investigation by streaking it onto the slant surface and by a central stab into the butt.

Incubation: 16-24 hours at 35 °C aerobically.

Characteristic reactions of some Enterobacteriaceae cultured on Lysine Iron Agar:

Microorganisms	Butt	Slant surface	H_2S production
Arizona	violet	violet	+
Salmonella*	violet	violet	+
Proteus mirabilis Proteus vulgaris	yellow	red-brown	+
Proteus morganii Proteus rettgeri	yellow	red-brown	-
Providencia	yellow	red-brown	-
Citrobacter	yellow	violet	+
Escherichia	yellow	violet	-
Shigella	yellow	violet	-
Klebsiella	violet	violet	-

* Exception: Salm. paratyphi A (no lysine decarboxyloase production, butt = yellow, slant surface violet)

Literature

EDWARDS, P.R., a. FIFE, M.A.: Lysine iron agar in the detection of Arizona cultures. **- Appl. Microbiol., 9**; 478-480 (1961).

EWING, W.H., DAVIN, B.R., a. EDWARDS, P.R.: The decarboxylase reactions of Enterobacteriaceae and their value in taxonomy. **- Publ. Hlth. Lab., 18**; 77-83 (1960).

HENNER, S., KLEIH, W., SCHNEIDERHAN, M., BUROW, H., FRIESS, H., GRANDJEAN, C.: Reihenuntersuchungen an Rind- und Schweinefleisch auf Salmonellen. **- Fleischwirtsch., 62**; 322-323 (1982).

JOHNSON, J.G., KUNZ, L.J., BARRON, W., a. EWING, W.H.: Biochemical differentiation of the Enterobacteriaceae with the aid of Lysine-iron-Agar. **-Appl. Microbiol., 14**; 212-217 (1966).

RAPPOLD, H., a. BOLDERDIJK, R.F.: Modified lysine iron agar for isolation of Salmonella from food. **- Appl. Environ. Microbiol., 38**; 162-163 (1979).

THATCHER, F.S., a. CLARK, D.S.: Microorganisms in FOOD (University of Toronto Press. 1968).

TIMMS, L.: Arizona infection in turkeys in Great Britain. **- Med. Lab. Techn., 28**; 150-156 (1971).

Ordering Information

Product	Merck Cat. No.	Pack size
Lysine Iron Agar	1.11640.0500	500 g

Lysine Iron Agar

Quality control

Test strains	Growth	Butt	Slant
Shigella flexneri ATCC 12022	good / very good	yellow	violet
Escherichia coli ATCC 25922	good / very good	yellow	violet
Salmonella typhimurium ATCC 14028	good / very good	violet and black	violet
Salmonella enteritidis NCTC 5188	good / very good	violet and black	violet
Citrobacter freundii ATCC 8090	good / very good	yellow and black	violet
Proteus mirabilis ATCC 29906	good / very good	yellow and black	reddish-brown
Morganella morganii ATCC 25830	good / very good	yellow	reddish-brown / violet

Citrobacter freundii ATCC 8090

Morganella morganii ATCC 25830

Salmonella enteritidis NCTC 5188

Shigella flexneri ATCC 12022

AC
MPF

M-(Mannose) Broth

For the accelerated detection of Salmonella in dried foods and feeds within the enrichment serology (ES) procedure.

Mode of Action

M-(Mannose) Broth is based on the formulation of SPERBER and DEIBEL (1969), eliminating dextrose of APT-Broth to allow citrate to serve as an energy source. Mannose was added to the medium to prevent fibrial agglutination in Salmonella in the serological procedure. Inorganic salt ions stimulate Salmonella growth. Tween® is a source for fatty acids.

Typical Composition (g/litre)

Yeast extract 5.0; peptone from casein 12.5; D-mannose 2.0; sodium citrate 5.0; sodium chloride 5.0; di-potassium hydrogen phosphate 5.0; manganese chloride 0.14; mangesium sulfate 0.8; ferrous(II) sulfate 0.04; Tween® 80 0.75.

Preparation

Suspend 36.2 g in 1 litre of demin. water and autoclave (15min at 121 °C).

pH: 7.0 ± 0.2 at 25 °C.

The prepared broth is clear and yellowish-brown.

Experimental Procedure and Evaluation

1. Suspend sample in Buffered Peptone Water, incubate for 18-24hours at 35 °C aerobically.
2. Transfer 1 ml each to 9 ml RAPPAPORT-VASSILIADIS (RVS) Broth and 9 ml Selenite Cystine Broth, incubate for 24 hours at 35 °C.
3. Transfer 1 drop of each selective broth in 10 ml M-Broth; incubate for 6-8 hours at 35 °C.
4. Perform a modified H-agglutination test according to SPERBER and DEIBEL literature.

Literature

SPERBER, W.H., a. DEIBEL, R.H.: Accelerated procedure for Salmonella detection in dried foods and feeds involving only broth culture and serological reaction. - **Appl. Microbiol. 17** ; 533-539 (1969).

Ordering Information

Product	Merck Cat. No.	Pack size
M-(Mannose) Broth	1.10658.0500	500 g
Peptone Water (buffered)	1.07228.0500	500 g
RVS Broth	1.07700.0500	500 g
Selenite Cystine Broth	1.07709.0500	500 g

Quality control

Test strains	Growth
Salmonella choleraesuis ATCC 12011	good / very good
Salmonella typhimurium ATCC 14028	good / very good
Salmonella enteritidis ATCC13076	good / very good

MPF

M 17 Agar acc. to TERZAGHI

Media proposed by TERZAGHI and SANDINE (1975) for the cultivaiton and enumeration of lactic streptococci in milk and dairy products and for the differentiation of bacteriophages infecting lactic streptococci.

The M 17 media are superior to other comparable culture media for the cultivation of the fastidious species Strept. cremoris, Strept. diacetilactis and Strept. lactis. Mutants which are incapable of metabolizing lactose can also be isolated on these media.

Mode of Action

Addition of sodium β-glycerophosphate increases the buffering capacity of the medium; this promotes the growth of lactic streptococci and the development of large bacteriophage plaques.

Typical Composition (g/litre)

Peptone from soymeal 5.0; peptone from meat 2.5; peptone from casein 2.5; yeast extract 2.5, meat extract 5.0; lactose monohydrate 5.0; ascorbic acid 0.5; sodium β-glycerophosphate 19.0; magnesium sulfate 0.25; agar-agar 12.75.

Preparation

Suspend 55 g M 17 agar/litre, autoclave (15 min at 121 °C).

pH: 7.2 ± 0.2 at 25 °C.

The prepared media are clear and brown.

- **Prepared plates can be stored in the refrigerator (approx. 6-8 °C) for up to 10 days.**

Experimental Procedure and Evaluation

Inoculate the plates.

Incubation: 24-48 hours at 28 °C aerobically.

Colonies of lactose-positive streptococci are clearly visible after 15 hours. Colonies of lactose-positive lactic streptococci have a diameter of 3-4 mm after 5 days while those of lactose-negative mutants have a diameter of less than 1 mm.

Phage infection can be recognized by the presence of large, distinct plaques in the opaque growth of the host bacteria grown on M 17 agar. The phage detection technique is described by TERZAGHI and SANDINE (1975), TERZAGHI (1976), KEOGH (1980), BRINCHMANN et al. (1983) and other authors.

Literature

BRINCHMANN, E., NAMORK, E., JOHANSEN, B.V., a. LANGSRUD, T.: A morphological study of lactic streptococcal bacteriophages isolated from Norwegian cultured milk. - **Milchwirtschaft., 38**; 1-4 (1983).

KEOGH, B.P.: Appraisal of media and methods for assay of bacteriophages of lactic streptococci. - **Appl. Environ. Microbiol., 40**; 798-802 (1980).

TERZAGHI, B.E.: Morphologics and host sensitives of lactic streptococcal phages from cheese factories. - **N.Z.J. Dairy Sci. Technol., 11**; 155-163 (1976).

TERZAGHI, B.E., a. SANDINE, W.E.: Improved medium for lactic streptococci and their bacteriophages. - **Appl. Microbiol., 29**; 807-813 (1975).

Ordering Information

Product	Merck Cat. No.	Pack size
M 17 Agar acc. to TERZAGHI	1.15108.0500	500 g

Quality control

Test strains	Growth
Streptococcus agalactiae ATCC 13813	good / very good
Lactococcus lactis spp. cremoris ATCC 19257	good / very good
Lactococcus lactis spp. lactis ATCC 19435	good / very good
Enterococcus faecalis ATCC 11700	good / very good
Escherichia coli ATCC 25922	good / very good
Staphylococcus aureus ATCC 25923	good / very good
Lactobacillus acidophilus ATCC 4356	fair / good
Lactobacillus casei ATCC 393	fair / good
Lactobacillus fermentum ATCC 9338	fair / good

MPF

M 17 Broth acc. to TERZAGHI

Media proposed by TERZAGHI and SANDINE (1975) for the cultivaiton and enumeration of lactic streptococci in milk and dairy products and for the differentiation of bacteriophages infecting lactic streptococci.

The M 17 media are superior to other comparable culture media for the cultivation of the fastidious species Strept. cremoris, Strept. diacetilactis and Strept. lactis. Mutants which are incapable of metabolizing lactose can also be isolated on these media.

Mode of Action

Addition of sodium β-glycerophosphate increases the buffering capacity of the medium; this promotes the growth of lactic streptococci and the development of large bacteriophage plaques.

Typical Composition (g/litre)

Peptone from soymeal 5.0; peptone from meat 2.5; peptone from casein 2.5; yeast extract 2.5, meat extract 5.0; lactose monohydrate 5.0; ascorbic acid 0.5; sodium β-glycerophosphate 19.0; magnesium sulfate 0.25.

Preparation

Suspend 42.5 g M 17 broth/litre; dispense the broth into test tubes, autoclave (15 min at 121 °C).

pH: 7.2 ± 0.2 at 25 °C.

The prepared media are clear and brown.

Experimental Procedure and Evaluation

Inoculate the tubes.

Incubation: 24-48 hours at 28 °C aerobically.

Literature

BRINCHMANN, E., NAMORK, E., JOHANSEN, B.V., a. LANGSRUD, T.: A morphological study of lactic streptococcal bacteriophages isolated from Norwegian cultured milk. - **Milchwirtschaft., 38**; 1-4 (1983).

KEOGH, B.P.: Appraisal of media and methods for assay of bacteriophages of lactic streptococci. - **Appl. Environ. Microbiol., 40**; 798-802 (1980).

TERZAGHI, B.E.: Morphologics and host sensitives of lactic streptococcal phages from cheese factories. - **N.Z.J. Dairy Sci. Technol., 11**; 155-163 (1976).

TERZAGHI, B.E., a. SANDINE, W.E.: Improved medium for lactic streptococci and their bacteriophages. - **Appl. Microbiol., 29**; 807-813 (1975).

Ordering Information

Product	Merck Cat. No.	Pack size
M 17 Broth acc. to TERZAGHI	1.15029.0500	500 g

Quality control

Test strains	Growth
Streptococcus agalactiae ATCC 13813	good / very good
Lactococcus lactis spp. cremoris ATCC 19257	good / very good
Lactococcus lactis spp. lactis ATCC 19435	good / very good
Enterococcus faecalis ATCC 11700	good / very good
Escherichia coli ATCC 25922	good / very good
Staphylococcus aureus ATCC 25923	good / very good
Lactobacillus acidophilus ATCC 4356	good / very good
Lactobacillus casei ATCC 393	good / very good
Lactobacillus fermentum ATCC 9338	fair / very good

A

m-Aeromonas Selective Agar Base (HAVELAAR)

Medium for the detection and counting of Aeromonas species in water.

The medium complies with the recommendations of USEPA method 1605 (2001) which describes the Ampicillin Dextrin Agar with Vancomycin (ADA-V). This method describes a membrane filter technique for the detectin and count of Aeromonades.

Mode of Action

m-Aeromonas Selective Agar enhances growth of nearly all Aeromonades. The supplements Ampicillin and Vancomycin partly inhibits growth of accompanying Gram-positive and Gram-negative organisms.

Aeromonades form acid from dextrin indicated by a color change from blue to yellow of the pH indicator bromothymol-blue.

Typical composition (g/liter)

Tryptose 5.0; dextrin 11.4; yeast extract 2.0; sodium chloride 3.0; potassium chloride 2.0; magnesium sulfate 0.1; iron chloride 0.06; bromothymolblue 0.08; sodium desoxycholate 0.1; agar-agar 13.0.

Preparation

Suspend 18.4 g in 500 ml of purified water and heat to boiling to dissolve completely.

Autoclave (15 min. at 121 °C).

Cool the medium to 45 – 50 °C and aseptically add the contents of one vial m-Aeromonas Selective Supplement (Cat.No.1.07625.0001). Mix. Pour plates.

pH: 8.0 ± 0.2 at 25 °C.

The prepared medium is clear and blue.

Plates can be stored for up to 2 weeks at 2-8 °C. Protect from light and drying.

Experimental procedure and evaluation

Use the membrane filter technique for inoculation.

The filter material impacts results. Good results were achieved when using Cellulose-Mixester membranes (e.g. Gelman GN.-6).

Incubation: 24 ± 2 hours at 35 ± 0.5 °C.

All colonies growing with a yellow color on the membrane filter are suspected aeromonades and counted as such.

The suspect colonies have to be confirmed. Typical confirmation reactions for Aeromonades: oxidase positive, trehalose positive and indole positive.

Literature

Havelaar, A.H., M. During, and J. F. M. Versteegh. 1987. Ampicillin-dextrin agar medium for the enumeration of Aeromonas species in water by membrane filtration. **J. Appl. Microbio. 62**: 279 – 287. (1987).

United States Environmental Protection Agency (USEPA), Method 1605: Aeromonas in Finished Water by Membrane Filtration using Ampicillin-Dextrin Agar with Vancomycin (ADA-V). October 2001.

Ordering Information

Product	Merck Cat. No.	Pack size
m-Aeromonas Selective Agar Base (HAVELAAR)	1.07621.0500	500 g
m-Aeromonas Selective Supplement (Ampicillin, Vancomycin)	1.07625.0001	1 x 16 vials

Quality control

Test strains	Recovery rate	Colony color
Aeromonas hydrophila ATCC 7966	> 70 %	yellow
Aeromonas veronii ATCC 9071	> 70 %	yellow
Escherichia coli ATCC 11775	< 0.10 %	milky
Enterococcus faecalis ATCC 19433	< 0.01 %	milky
Pseudomonas aeruginosa ATCC 27853	not limited	milky

m-Aeromonas Selective Supplement

Additive for the preparation of m-Aeromonas Selective Agar

Mode of Action

m-Aeromonas Selective Supplement is a mixture of two different inhibitors in lyophilized format. Growth of Gram-positive as well as Gram-negative accompanying flora is inhibited whereas most of aeromonades grow.

Composition (mg per vial)

Ampicillin 5.0; Vancomycin 1.0.

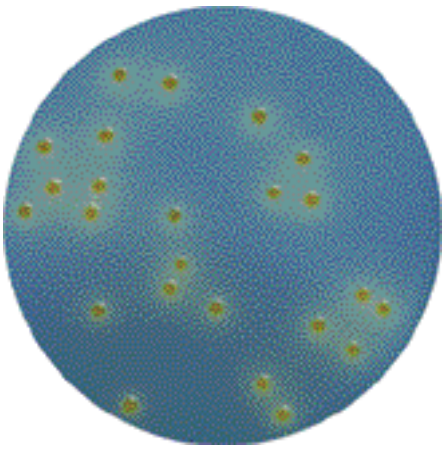
Preparation

Aseptically add 1 ml of purified water to the contents of one vial. Mix. The solution is clear.

Add the contents of one vial to 500 ml m-Aeromonas Selective Agar Base cooled to 45 – 50 °C. Mix to suspend evenly.

Ordering Information

Product	Merck Cat. No.	Pack size
m-Aeromonas Selective Supplement	1.07625.0001	1 x 16 vials
m-Aeromonas Selective Agar Base	1.07621.0500	500 g



Aeromonas hydrophilla ATCC 7966

m-Endo Agar LES

m-ENDO Agar LES is a medium for the enumeration of coliforms in water used in the Standard Total Coliform Membrane Filter Procedure in the Standard Methods for the Examination of Water and Wastewater. It follows the two-step membrane filter procedure using Lauryl Sulfate Broth as a preliminary enrichment, resulting in higher coliform counts.

Mode of Action

Growth of coliform bacteria is promoted by the selection of versatile nutrient bases. The accompanying flora is inhibited by lauryl sulfate and deoxychlolate. Lactose-positive colonies are coloured red due to the liberation of fuchsin from fuchsin-sulfite compound; E. coli colonies have a metallic sheen.

Typical Composition (g/litre)

Yeast extract 1.2; casein hydrolysate 3.7; peptone from meat 3.7; tryptose 7.5; lactose 9.4; di-potassium hydrogen phosphate 3.3; potassium hydrogen phosphate 1.0; sodium chloride 3.7; sodium deoxycholate 0.1; sodium lauryl sulfate 0.05; sodium sulfite 1.6; pararosanilin (fuchsin) 0.8; agar-agar 15.0.

Preparation

Suspend 51 g in 1 litre of distilled or deionized water containing 20 ml of ethanol 96 % and heat to boiling to dissolve completely. Do not autoclave! Cool to 45-50 °C. Dispense 4 ml amounts into Petridishes (∅ 50-60 mm) and allow to solidify. Cool to 50°C and pour plates.

pH: 7.2 ± 0.2 at 25 °C.

The plates are opalescent and red.

Experimental Procedure

1. Prepare Lauryl Sulfate Broth according to label instructions.
2. Prepare m-ENDO Agar LES according to label instructions in 50-60 mm Petridishes and allow to solidify.
3. Invert plate and place membrane filter pad in the lid and add 1.8-2.0 ml Lauryl Sulfate Broth to each pad. Remove any excess liquid.
4. Using a rolling motion apply membrane filter, through which a water sample has been filtered, top side up on the pad. Avoid air bubbles.
5. Incubate at 35 °C for 1.5 to 2.0 hours in a humid atmosphere.
6. Transfer the filter - again top side up - to the surface of the agar. Avoid entrapment of air.
7. Incubate inverted plates at 35 °C ± 0.5 °C for 20 to 24 hours aerobically.

Evaluation

Count all red colonies on the filtre having the characteristic metallic sheen.

Literature

American Public Health Association, American Water Works Association and Water Pollution Control Federation: Standard Methods for the Examination of Water and Wastewater, 20th Ed., Washington, 1998.

Ordering Information

Product	Merck Cat. No.	Pack size
m-Endo Agar LES	1.11277.0500	500 g
Laurylsulfate Broth	1.10266.0500	500 g

Escherichia coli ATCC 25922

Quality control

Test strains	Growth	Colour of colony	Metallic sheen
Escherichia coli ATCC 25922	good / very good	red	+
Enterobacter aerogenes ATCC 13048	good / very good	red	+
Proteus mirabilis ATCC 14273	good / very good	colourless	-
Staphylococcus aureus ATCC 25923	none		
Enterococcus faecalis ATCC 19433	none / poor		

m-FC Agar

m-FC Agar is used for the detection of faecal coliforms by the membrane filtration technique.

Mode of Action

In the beginning the faecal coliforms, derived from the intestinal tract of warm-blooded animals, were separated from the non-faecal coliforms by use of elevated temperature tests, which needed confirmatory MPN procedure in addition. GELDREICH et al. published the development of a Faecal Coliform (FC) Medium for the membrance filtration technique using an incubation temperature of 44.5 °C ± 0.2 °C.

m-FC Agar is supplemented with rosolic acid and incubated at 44.5 °C ± 0.2 °C for 24 h.

Peptone and yeast extract serve as nutritious source and bile salts are added to inhibit accompanying Gram-positive flora.

Lactose can be fermented by faecal coliforms at the elevated temperature to form blue colonies on the ready medium (agar base plus rosolic acid), whereas other organisms show grey colonies.

Typical Composition (g/litre)

Proteose peptone 5.0; tryptose 10.0; yeast extract 3.0; sodium chloride 5.0; bile salts 1.5; lactose 12.5; methyl blue (formerly aniline blue) 0.1; agar-agar 15.0.

Preparation

Suspend 52 g in 1 litre of distilled or deionized water and heat to boiling to dissolve completely. Add 10 ml of a 1 % solution of rosolic acid in 0.2 N NaOH. Continue heating for 1 minute with frequent agitation. Do not autoclave! Cool to 45-50 °C. Dispense 4 ml amounts into Petridishes (∅ 50-60 mm) and allow to solidify. Cool to 50 °C and pour plates.

pH: 7.4 ± 0.2 at 25 °C.

The plates are clear and blue to violet.

Experimental Procedure

Place membrane filter, through which the sample has been filtered, on the surface of the agar. Avoid formatoin of air bubbles between the filter and the agar surface.

Incubation: 24 hours at 44.5 °C ± 0.2 °C aerobically.

Evaluation

Blue coloured colonies on the membrane filter are counted as faecal coliforms. Other organisms form grey to cream colonies.

Literature

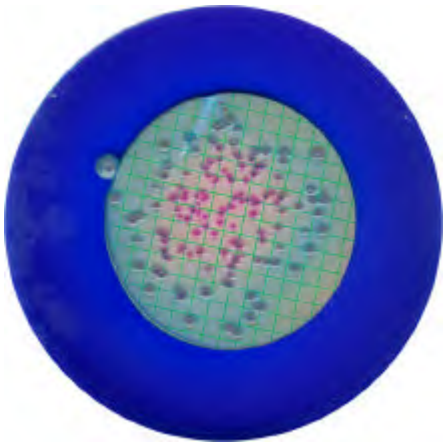
GELDREICH, CLARK, HUFF, a. BERG: - **J. Am. Water Works Assoc., 57**; 208 (1965).

Ordering Information

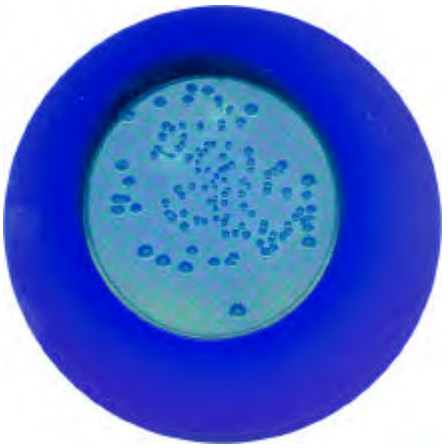
Product	Merck Cat. No.	Pack size
m-FC Agar	1.11278.0500	500 g
0.2 N Sodium hydroxide solution	1.09140.1000	1 l
Rosolic acid	Sigma Chem.	25 g

Quality control

Test strains	Growth at 44.5 °C	Colour of colony
Escherichia coli ATCC 25922	good	blue to dark-blue
Salmonella typhimurium ATCC 14028	good	pink to red
Enterobacter cloacae ATCC 13047	good	blue to dark-blue
Enterococcus faecalis ATCC 19433	none	
Enterobacter aerogenes ATCC 13048	good	grey to grey-blue



Enterobacter cloacae ATCC 13047



Escherichia coli ATCC 25922

MacCONKEY Agar

Selective agar for the isolation of Salmonella, Shigella and coliform bacteria from faeces, urine, foodstuffs, waste water etc. according to MacCONKEY (1950).

IVD *in vitro diagnosticum –*
For professional use only

See also General Instruction of Use
Warnings and precautions see ChemDAT®
(www.chemdat.info)

The composition of this medium largely complies with the United States Pharmacopeia XXVI (2003) and the European Pharmacopeia II.

Principle

Microbiological method

Mode of Action

Bile salts and crystal violet largely inhibit the growth of the Gram-positive microbial flora. Lactose and the pH indicator neutral red are used to detect lactose degradation.

Typical Composition (g/litre)

Peptone from casein 17.0; peptone from meat 3.0; sodium chloride 5.0; lactose 10.0; bile salt mixture 1.5; neutral red 0.03; crystal violet 0.001; agar-agar 13.5.

Preparation and StorageCat. No. 1.05465. MacCONKEY Agar (500 g/5 kg)

Usable up to the expiry date when stored dry and tightly closed at +15 to +25° C. Protect from light.

After first opening of the bottle the content can be used up to the expiry date when stored dry and tightly closed at +15 to +25°C.

Suspend 50 g/litre, autoclave (15 min at 121 °C), pour plates.

pH: 7.1 ± 0.2 at 25 °C.

The plates are clear and red-brown to dark red.

Specimen

e.g. Stool, urine.

Clinical specimen collection, handling and processing, see general instructions of use.

Experimental Procedure and Evaluation

Inoculate by spreading the sample material on the surface of the plates.

Incubation: 18-24 hours at 35 °C aerobically.

Lactose-negative colonies are colourless; lactose-positive colonies are red and surrounded by a turbid zone which is due to the precipitation of bile acids as a result of pH decrease.

Appearance of Colonies	Microorganisms
Appearance of Colonies	Microorganisms
Colourless, translucent	Salmonella, Shigella and others
Large, red, surrounded by turbid zone	Escherichia coli
Large, pink, mucoid	Enterobacter, Klebsiella
Very small, opaque, isolated colonies	Enterococci, Staphylococci and others

Literature

European Pharmacopeia II, Chapter VIII, 10.

MacCONKEY, A.: Lactose-fermenting bacteria in faeces. - **J. Hyg., 8** ; 333-379 (1905).

United States Pharmacopeia XXVI, Chapter "Microbiol. Limit Test", 1995.

Ordering Information

Product	Merck Cat. No.	Pack size
MacCONKEY Agar	1.05465.0500	500 g
MacCONKEY Agar	1.05465.5000	5 kg
Merckoplate® MacCONKEY Agar	1.10748.0001	20 plates
Merckoplate® MacCONKEY Agar	1.15276.0001	480 plates

MacCONKEY Agar

Quality control (spiral plating method)

Test strains	Inoculum (cfu/ml)	Recovery rate (%)	Colour of		Precipitate
			colony	medium	
Escherichia coli ATCC 8739 *	10^3-10^5	≥ 30	red	red	+
Salmonella typhimurium ATCC 14028	10^3-10^5	≥ 30	colourless	yellowish	-
Salmonella dublin ATCC 15480	10^3-10^5	≥ 30	colourless	yellowish	-
Shigella sonnei ATCC 11060	10^3-10^5	≥ 30	colourless	yellowish	-
Proteus mirabilis ATCC 29906	10^3-10^5	≥ 30	colourless	yellowish	-
Bacillus cereus ATCC 11778	> 10^5	≤ 0.01			
Staphylococcus aureus ATCC 6538	> 10^5	≤ 0.01			
Enterococcus hirae ATCC 8043	> 10^5	≤ 0.01			
Enterococcus faecalis ATCC 19433	> 10^5	≤ 0.01			
* (at 37 °C and 43-45 °C)					

MacCONKEY Broth

Selective culture medium used as a presumptive test for E. coli and coliform bacteria and for determining the E. coli or coliform titre of milk, water and other materials according to MacCONKEY and HILL (1901).

This medium largely complies with the European Pharmacopeia II.

Mode of Action

This broth contains lactose which, when degraded, gives acid and gas, according to the definition indicating the presence of E.coli. The gas formed is collected in DURHAM tubes and acid production is detected by the indicator bromocresol purple, which turns yellow. Ox bile promotes the growth of several species of intestinal bacteria and inhibits that of microorganisms, which do not inhabit the intestine.

Typical Composition (g/litre)

Peptone from casein 20.0; lactose 10.0; ox bile, dried 5.0; bromocresol purple 0.01.

Preparation

Suspend 35 g/litre or more (see Table under Lactose Broth), fill into test tubes, if desired insert DURHAM tubes, autoclave

(15 min at 121 °C).

pH: 7.1 + 0.2 at 25 °C.

The prepared broth is clear and purple.

Experimental Procedure and Evaluation

See 1.07661. Lactose Broth.

Incubation: 48 hours at 35 °C.

Gas and acid are produced:	suggests E. coli and possibly other coliform bacteria
Only acid is produced:	suggests coliform bacteria without E. coli

Literature

European Pharmacopeia II, Chapter VIII, 10.

MacCONKEY, A.: Bile salt media and their advantages in some bacteriological examinations. – **J. Hyg., 8**; 322-334 (1908).

MacCONKEY, A.: Lactose-fermenting bacteria in faeces. – **J. Hyg., 8**; 333-379 (1905).

Deutsches Arzneibuch DAB 10.

MacCONKEY, A., a. HILL: Bile salt broth. – **Thompson Yates Lab. Rep., VI/1**; 151 (1901) (zitiert in MacCONKEY, 1905).

Ordering Information

Product	Merck Cat. No.	Pack size
MacCONKEY Broth	1.05396.0500	500 g

Quality control

Test strains	Growth	Colour change to yellow	Gas formation
Escherichia coli ATCC 25922	good	+	+
Escherichia coli ATCC 11775	good	+	+
Determination of E. coli acc. to DAB10	good	+	+
Escherichia coli ATCC 8739 (24 h/43-45 °C)	good	+	+
Enterobacter cloacae ATCC 13047	good	+	+
Klebsiella pneumoniae ATCC 13883	good	+	+
Proteus mirabilis ATCC 14273	good	-	-
Pseudomonas aeruginosa ATCC 27853	fair	-	-
Staphylococcus aureus ATCC 25923	fair	-	-

Malachite-green Broth, Base

For the selective enrichment of Pseudomonas aeruginosa according to HABS and KIRSCHNER (1943).

The culture medium has been recommended by SCHUBERT and BLUM (1974) for water testing and has been accepted by the Deutsches Institut für Normung (German Institute for Standardization, DIN) in the respective DIN-Standard 38411, Part 8, for the examination of ground-, surface-, drinking-, bathing and process-water. In addition, it is suitable for the examination of mineral and spring-water.

Mode of Action

The malachite-green essentially suppresses growth of the accompanying flora while leaving Pseudomonas aeruginosa virtually unaffected. The addition of a small amount of phosphate buffer is favourable for maintaining the correct pH of the broth.

Typical Composition (g/litre)

Peptone from meat 5.0; meat extract 3.0; di-potassium hydrogen phosphate 0.37.

These values are valid for the single-strength broth.

Preparation

Base Broth:

Suspend 8.4 g (single-strength broth) or 25.1 g (triple-strength broth) in 1 litre of demineralized water. Dispense in 50 ml volumes into suitable culture vessels and autoclave (15 min at 121°C).

The broth base is clear and yellow-brown.

Malachite-green solution:

Suspend 0.15 g malachite-green oxalate in 90 ml of demineralized water and sterilize by filtration.

Complete broth:

Add to 50 ml of cooled base broth 0.3 ml (single-strength broth) or 0.9 ml (triple-strength broth) of the malachite-green solution under sterile conditions.

pH: 7.3 ± 0.2 at 25 °C.

The complete broth is clear and green.

Alternative method of preparation:

If preferred, the malachite-green solution may also be added before distributing the base broth into the vessels. In this case it has to be autoclaved first. Then add to 1 litre of base broth 6ml (single-strength broth) or 18 ml (triple-strength broth) of malachite-green solution under sterile conditions and dispense in 50 ml volumes into sterile vessels.

Experimental Procedure and Evaluation

An optimal enrichment of Pseudomonas aeruginosa requires a concentration of 0.01 g /l malachite-green oxalate. Therefore, small sample-volumes (5 ml or less and solid materials such as membrane filters) will be directly inoculated into 50 ml of single-strength broth. In the case of high sample-volumes, 2 parts of sample are added to 1 part of triple-strength broth (e.g. 100ml of water to 50 ml of broth). Thus, final concentration of the inoculated broth will always be single-strength.

Incubation: 24 ± 4 to 44 ± 4 hours at 35 °C ± 1 °C.

Cultures showing turbidity, i.e. growth after the incubation are regarded positive. Growth may, but does not have to, be accompanied by a change of colour. Positive cultures are further examined to confirm the presence of Pseudomonas aeruginosa according to standard procedures.

Literature

DIN Deutsches Institut für Normung e.V.: Deutsches Einheitsverfahren zur Wasser-, Abwasser- und Schlammuntersuchung. Mikrobiologische Verfahren (Gruppe K). Nachweis von Pseudomonas aeruginosa (K 8). – **DIN38411**.

HABS, H., a. KIRSCHNER, K.H.: Der Pyocyaneus-Meerschweinchenhautversuch zur Prüfung von Hautdesinfektionsmitteln. – **Z. Hyg., 124**; 557-578 (1943).

SCHUBERT, R., a. BLUM, U.: Zur Frage der Eignung der Malachitgrün-Bouillon nach HABS und KIRSCHNER als Anreicherungsmedium für Pseudomonas aeruginosa aus dem Wasser. – **Zbl. Bakt. Hyg., I. Orig. B., 158**; 583-587 (1974).

Ordering Information

Product	Merck Cat. No.	Pack size
Malachite-green Broth, Base	1.10329.0500	500 g
BROLACIN Agar	1.01638.0500	500 g
Malachite-green oxalate	1.01398.0025	25 g

Quality control

Test strains	Inoculum	Growth on BROLACIN Agar after	
		20 hours	44 hours
Pseudomonas aeruginosa ATCC27853	approx. 1 %	≥ 50 %	≥ 80 %
Enterobacter aerogenes ATCC15038	approx. 99 %	≤ 50 %	≤ 20 %

AC
M

Malt Extract Agar

For the detection, isolation and enumeration of fungi, particularly yeasts and moulds, in various materials and for the cultivation of test strains for the microbiological vitamin assays.

Mode of Action

If fungal counts are to be performed, the pH value of the culture medium should be adjusted to 3.5 to suppress the growth of the bacterial flora.

REISS (1972) recommends a modified malt extract agar for the selective cultivation of Aspergillus flavus. According to RAPP (1974), addition of certain indicator dyes to malt extract agar allows differentiation of yeast and bacterial colonies.

Typical Composition (g/litre)

Malt extract 30.0; peptone from soymeal 3.0; agar-agar 15.0:

Preparation

Suspend 48 g/litre, autoclave under mild conditions (10 min at 121°C).

■ **Do not overheat.**

pH: 5.6 ± 0.2 at 25 °C.

The plates are clear and yellowish-brown.

If the pH has to be lowered, liquefy the sterile culture medium and adjust the pH with filter-sterilized 10 % lactic acid solution or 5 % tartaric acid solution. Avoid subsequent heating.

Experimental Procedure and Evaluation

Depend on the purpose for which the media are used.

Incubation: 7 days at 28 °C aerobically (yeasts: 3 days)

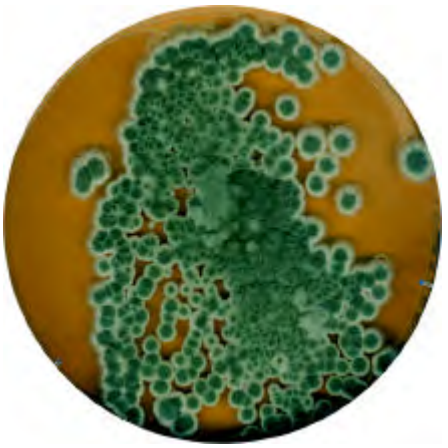
Literature

RAPP, M.: Indikatorzusätze zur Keimdifferenzierung auf Würze- und Malzextrakt-Agar. - **Milchwiss., 29**; 341-344 (1974)

REISS, J.: Ein selektives Kulturmedium für den Nachweis von Aspergillus flavus in verschimmeltem Brot. - **Zbl. Bakt. Hyg. I. Abt. Orig. A 220**; 564-566

Ordering Information

Product	Merck Cat. No.	Pack size
Malt Extract Agar	1.05398.0500	500 g
L(+)-Tartaric acid	1.00804.0250	250 g
Lactic acid about 90 % purified	1.00366.0500	500 ml



Penicillium commune ATCC 10428

Quality control of Malt Extract Agar

Test strains	Growth
Geotrichum candidum DSMZ 1240	good / very good
Penicillium commune ATCC 10428	good / very good
Aspergillus niger ATCC 16404	good / very good
Trichophyton ajelloi ATCC 28454	fair / good

Quality control of Malt Extract Agar (spiral plating method)

Test strains	Inoculum (cfu/ml)	Recovery rate
Candida albicans ATCC 10231	10^3-10^5	≥ 70 %
Saccharomyces cerevisiae ATCC 9763	10^3-10^5	≥ 70 %
Saccharomyces cerevisiae ATCC 9080	10^3-10^5	≥ 70 %
Rhodotorula mucilaginosa DSMZ 70403	10^3-10^5	≥ 70 %

AC
M
MPF
WW

Malt Extract Broth

For the detection, isolation and enumeration of fungi, particularly yeasts and moulds, in various materials and for the cultivation of test strains for the microbiological vitamin assays.

Mode of Action

If fungal counts are to be performed, the pH value of the culture medium should be adjusted to 3.5 to suppress the growth of the bacterial flora.

REISS (1972) recommends a modified malt extract agar for the selective cultivation of Aspergillus flavus. According to RAPP (1974), addition of certain indicator dyes to malt extract agar allows differentiation of yeast and bacterial colonies.

Typical Composition (g/litre)

Malt extract 17.0.

Preparation

Suspend 17.0 g/litre, dispense into suitable containers, autoclave under mild conditions (10 min at 115 °C).

pH: 4.8 ± 0.2 at 25 °C.

The prepared broth is clear and yellow.

Experimental Procedure and Evaluation

Depend on the purpose for which the media are used.

Incubation: 7 days at 28 °C aerobically (yeasts: 3 days)

Literature

RAPP, M.: Indikatorzusätze zur Keimdifferenzierung auf Würze- und Malzextrakt-Agar. - **Milchwiss., 29**; 341-344 (1974)

REISS, J.: Ein selektives Kulturmedium für den Nachweis von Aspergillus flavus in verschimmeltem Brot. - **Zbl. Bakt. Hyg. I. Abt. Orig. A 220**; 564-566.

Ordering Information

Product	Merck Cat. No.	Pack size
Malt Extract Broth	1.05397.0500	500 g
L(+)-Tartaric acid	1.00804.0250	250 g
Lactic acid about 90 % purified	1.00366.0500	500 ml

Quality control of Malt Extract Broth

Test strains	Growth
Candida albicans ATCC 10231	good / very good
Saccharomyces cerevisiae ATCC 9763	good / very good
Saccharomyces cerevisiae ATCC 9080	good / very good
Geotrichum candidum DSMZ 1240	good / very good
Rhodotorula mucilaginosa DSMZ 70403	good / very good
Penicillium commune ATCC 10428	good / very good
Aspergillus niger ATCC 16404	good / very good
Trichophyton ajelloi ATCC 28454	good / very good

Mannitol Salt Phenol-red Agar

A modified version of the selective agar proposed by CHAPMAN (1945) for detecting pathogenic staphylococci in foodstuffs and other materials.

It complies with the recommendations in the United States Pharmacopeia XXVI (2003).

Mode of Action

Only salt-tolerant microorganisms, including staphylococci, can grow on this medium, because of its high salt concentration. Degradation of mannitol to acid correlates, more or less, with the pathogenicity of Staph. aureus and thus serves as an indicator for this species.

Typical Composition (g/litre)

Peptones 10.0; meat extract 1.0; sodium chloride 75.0; D(-)-mannitol 10.0; phenol red 0.025; agar-agar 12.0.

Preparation

Suspend 108 g/litre, autoclave (15 min at 121 °C), pour plates.

pH: 7.4 ± 0.2 at 25 °C.

The plates are clear and red.

Experimental Procedure and Evaluation

Inoculate by spreading the sample on the surface of the medium. Inoculation should be massive on account of the strong inhibitory effect of the medium.

Incubation: up to 3 days at 35 °C aerobically.

Further tests should be performed to confirm the diagnosis.

Appearance of Colonies	Microorganisms
Surrounded by bright yellow zones, abundant growth	Mannitol-positive: Staphylococcus aureus
No colour change, growth is usually poorer	Mannitol-negative: Staphylococcus epidermis and others

Literature

CHAPMAN, G.H.: The significance of sodium chloride in studies of staphylococci. - **J. Bact., 50**; 201-203 (1945).

United States Pharmacopeia XXVI, Chapter "Microbial Limits Tests", 1995.

Ordering Information

Product	Merck Cat. No.	Pack size
Mannitol Salt Phenol-red Agar	1.05404.0500	500 g

Quality control (spiral plating method)

Test strains	Inoculum (cfu/ml)	Recovery rate %	Colour change to yellow
Staphylococcus aureus ATCC 25923	10^3-10^5	≥ 10	+
Staphylococcus aureus ATCC 6538	10^3-10^5	≥ 30	+
Staphylococcus epidermidis ATCC 12228	10^3-10^5	-	-
Staphylococcus epidermidis ATCC 14990	10^3-10^5	-	-
Proteus mirabilis ATCC 12453	10^3-10^5	-	-
Escherichia coli ATCC 25922	> 10^5	< 0.01	

Maximum Recovery Diluent

For the preparation of an isotonic diluent for maximum recovery of organisms, especially in milk and meat testings.

Mode of Action

The diluent complies with the recommendations of ISO 6887 and the German § 35 Lebensmittelgesetz (German food law). This diluent can be used as an alternative to RINGER solution for milk and liquid milk products, dried milk, cheese, butter, meat and meat products, ice cream and chilled food based on milk.

Maximum Recovery Diluent is of isotonic strength to ensure recovery of organisms from various sources and combines the protective effect of peptone in the diluent with the osmotic support of physiological saline.

Within 1-2 hours of dilution of the sample there is no multiplication of organisms due to the low concentration of peptones.

Typical Composition (g/litre)

Peptone 1.0; sodium chloride 8.5.

Preparation

Suspend 9.5 g in 1 litre of demin. water and autoclave (15 min at 121 °C).

pH: 7.0 ± 0.2 at 25 °C.

The prepared diluent is clear and colourless.

Experimental Procedure

According to appropriate examination procedures.

Literature

Amtliche Sammlung von Untersuchungsverfahren nach §35
LM BG 01.00/1; 02.07/1; 03.00/1; 04.00/1; 06.00/16; 42.00/1; 48.01/6.

ISO 6887. Microbiology - General guidance for the preparation of dilutions for microbiological examination; 1st edition (1983).

Ordering Information

Product	Merck Cat. No.	Pack size
Maximum Recovery Diluent	1.12535.0500	500 g

Quality control

Test strains	Colony count (at room temperature) after:
Escherichia coli ATCC 25922	0, 2, 4, 6 hours
Enterococcus faecalis ATCC 11700	0, 2, 4, 6 hours

Meat Liver Agar

For the cultivation of anaerobic microorganisms.

Mode of Action

The nutrient basis of meat and liver tissue maintains an adequate degree of anaerobiosis in the culture medium and also provides a rich supply of nutrients. It thus ensures that even strict and fastidious anaerobes grow well. The sulfite present in the culture medium, is reduced to H_2S by some anaerobes (e.g. many Clostridium species); this is indicated by blackening due to the presence of iron salt.

Typical Composition (g/litre)

Meat-liver base 20.0; D(+)glucose 0.75; starch 0.75; sodium sulfite 1.2; ammonium iron(III) citrate 0.5; agar-agar 11.0.

Preparation

Suspend 34 g in 1 litre of demin. water and autoclave (15 min at 121 °C).

pH: 7.6 ± 0.2 at 25 °C.

The plates are clear and yellowish-brown.

Experimental Procedure and Evaluation

The culture medium can be dispensed into tubes or poured into plates. Inoculation can be performed by the pour plate method or by surface spreading. Inoculated plates must be incubated in an anaerobic environment established by e.g. Anaerocult® A, Anaerocult® A mini or Anaerocult® P.

Incubation temperature and period: as optimal as possible (up to 48 hours at 35 °C aerobically). H_2S-positive anaerobes grow as black colonies.

Ordering Information

Product	Merck Cat. No.	Pack size
Meat Liver Agar	1.15045.0500	500 g
Anaerobic jar	1.16387.0001	1 ea
Anaeroclip®	1.14226.0001	1 x 25
Anaerocult® A	1.13829.0001	1 x 10
Anaerocult® A mini	1.01611.0001	1 x 25
Anaerocult® P	1.13807.0001	1 x 25
Anaerotest®	1.15112.0001	1 x 50
Plate basket	1.07040.0001	1 ea

Quality control

Test strains	Growth	Black colonies
Clostridium perfringens ATCC 10543	good / very good	+
Clostridium sporogenes ATCC 11437	good / very good	+
Clostridium tetani ATCC 19406	good / very good	+ / -
Escherichia coli ATCC 25922	good / very good	-
Proteus mirabilis ATCC 14153	good / very good	- / poor
Bacteroides vulgatus ATCC 8482	medium / very good	+ / -

MPF
A
MD
WW

Membrane-filter ENDO Broth

For the identification and enumeration of coliform bacteria in water, milk and other liquids when using the membrane filtration method as modified by FIFIELD and SCHAUFUS (1958).

This culture media complies with the "APHA recommendations for the examination of water" (1998) and the recommendations for the examination of dairy products (1985).

Mode of Action

This versatile nutrient base allows Lactose-positive coliform bacteria to develop, but growth of accompanying bacteria is inhibited by lauryl sulfate and deoxycholate. Lactose-positive colonies are coloured red by the liberation of fuchsin from the fuchsin-sulfate compound; E. coli and coliform colonies show a metallic sheen in general.

This medium is normally used to impregnate absorbent materials (e.g. cardboard discs) on which the inoculated filters are placed.

Typical Composition (g/litre)

Tryptose 10.0; peptone from meat 5.0; peptone from casein 5.0; yeast extract 1.5; sodium chloride 5.0; di-potassium hydrogen phosphate 4.375; potassium dihydrogen phosphate 1.375; lactose 12.5; sodium deoxycholate 0.1; sodium lauryl sulfate 0.05; fuchsin, basic 1.05; sodium sulfite 2.1.

Preparation

Suspend 48 g in 1 litre of demin. water and heat to boiling (up to 30 min) until completely dissolved.

■ **Do not autoclave.**

If desired, soak sterile cardboard discs with the cooled broth in Petridishes.

pH: 7.2 ± 0.2 at 25 °C.

The prepared broth is clear to opalescent and red.

Preparation of membrane-filter ENDO agar:

Suspend 48 g culture medium and 14 g agar-agar in 1 litre of water and allow to swell for about 10 minutes. Boil until dissolved, pour plates.

Experimental Procedure and Evaluation

One-step procedure: After filtration, the filters are placed on the soaked pieces of cardboard or on the surface of the agar plates.

Incubation: 18-24 hours at 35 °C aerobically.

Two-step procedure: After filtration, the filters are first placed on pieces of cardboard soaked in Lauryl Sulfate Broth.

Incubation: 2-3 hours at 35 °C.

They are then transferred to agar plates.

Incubation: 24 hours at 35 °C aerobically.

According to McCARTHY et al. (1961) and DELANEY et al. (1962), better yields are obtained with this method.

The colonies of coliform bacteria are dark red in colour and usually have a greenish sheen (fuchsin sheen). Counting is performed with the aid of a magnifying glass with a magnification power of 10 (see Standard Methods for the Examination of Water and Wastewater and Standard Methods for the Examination of Dairy Products).

Literature

American Public Health Association: Standard Methods for the Examination of Dairy Products (15th ed., 1985).

American Public Health Association, American Water Works Association and Water Pollution Control Federation: Standard Methods for the Examination of Water and Wastewater, 20th ed., Washington, 1998.

DELANEY, J.E., McCARTHY, J.A., a. GRASSO, R.J.: Measurement of E. coli Type I by the membrane-filter. – **Water a. Sewage Works, 109**; 289-294 (1962).

FIFIELD, C.W., a. SCHAUFUS, C.P.: Improved membrane filter medium for the detection of coliform organisms. – **J. Am. Water Works Assoc., 50**; 193-196 (1958).

McCARTHY, J.A., THOMAS, H.A., a. DELANEY, J.E.: Evaluation of reliability of coliform density tests. – **Am. J. Publ. Hlth., 48**; 12 (1958).

McCARTHY, J.A. DELANEY, J.E., a. GRASSO, R.J.: Measuring coliforms in water. – **Water a. Sewage Works, 108** ; 238-243 (1961).

Ordering Information

Product	Merck Cat. No.	Pack size
Membrane-filter ENDO Broth	1.10750.0500	500 g
Agar-agar purified	1.01614.1000	1 kg
Laurylsulfate Broth	1.10266.0500	500 g

Quality control

Test strains	Growth	Colour change to red	Metallic lustre
Escherichia coli ATCC 25922	good / very good	+	+
Escherichia coli 194	good / very good	+	+
Enterobacter cloacae ATCC 13047	good / very good	+	+
Salmonella typhimurium ATCC 14028	good / very good	-	-
Proteus mirabilis ATCC 14153	good / very good	±	-
Aeromonas hydrophila DSMZ 30187	good / very good	- / poor	-

ww

Membrane-filter Enterococcus Selective Agar acc. to SLANETZ and BARTLEY

For the enumeration of enterococci in water and other liquids by the membrane filtration technique according to SLANETZ und BARTLEY (1957).

Mode of Action

The growth of the entire accompanying Gram-negative microbial flora is inhibited by sodium azide. Enterococci reduce TTC to give a red formazan, their colonies are thus red in colour. According to LACHICA and HARTMAN (1968), the selectivity for enterococci can be improved by adding carbonate and Tween80®.

Typical Composition (g/litre)

Tryptose 20.0; yeast extract 5.0; D(+)glucose 2.0; di-potassium hydrogen phosphate 4.0; sodium azide 0.4; 2,3,5-triphenyltetrazolium chloride 0.1; agar-agar 10.0.

Preparation

Suspend 41.5 g/litre, sterilize by heating for 20 minutes in a current of steam (e.g. autoclave without excess pressure). Afterwards cool rapidly!

■ **Do not autoclave.**

pH: 7.2 ± 0.2 at 25 °C.

The plates are clear and yellowish-brown. A reddish colour with Cat. No. 1.05262.500 might occur.

Experimental Procedure and Evaluation

Place the inoculated membrane filters on the surface of the plates.

Incubation: up to 48 hours at 35 °C aerobically.

Pink to brown colonies with a diameter of 0.5 to 2 mm are usually enterococci.

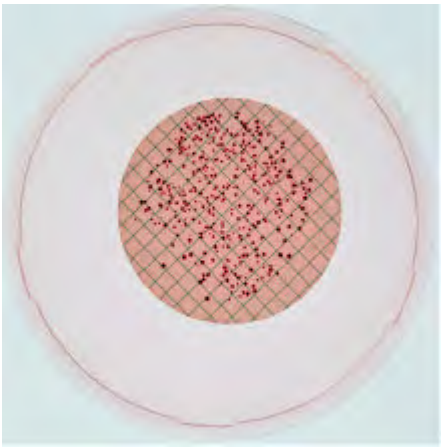
Literature

LACHICA, R.V.F., a. HARTMAN, P.A.: Two improved media for isolating and enumerating enterococci in certain frozen foods. – **J. Appl. Bact., 31**; 151-156 (1968).

SLANETZ, L.W., a. BARTLEY, C.H.: Numbers of enterococci in water, sewage, and faeces determined by the membrane filter technique with an improved medium – **J. Bact., 74**; 591-595 (1957).

Ordering Information

Product	Merck Cat. No.	Pack size
Membrane-filter Enterococcus Selective Agar acc. to SLANETZ and BARTLEY	1.05262.0500	500 g



Enterococcus faecalis ATCC 19433 red/maroon/pink colored colonies m-Enterococcus Selective Agar acc. to SLANETZ and BARTLEY

Quality control

Test strains	Growth	Red colonies
Streptococcus pyogenes ATCC 12344	poor / fair	-
Streptococcus agalactiae ATCC 13813	poor / fair	-
Enterococcus faecalis ATCC 11700	≥ 50 %	+
Enterococcus faecalis ATCC 19433	≥ 50 %	+
Enterococcus hirae ATCC 8043	≥ 50 %	+ (poor)
Staphylococcus aureus ATCC 25923	none	
Escherichia coli ATCC 25922	none	
Pseudomonas aeruginosa ATCC 27853	none	

WW

Membrane-filter Enterococcus Selective Agar Base acc. to SLANETZ and BARTLEY

For the enumeration of enterococci in water and other liquids by the membrane filtration technique according to SLANETZ und BARTLEY (1957).

Mode of Action

The growth of the entire accompanying Gram-negative microbial flora is inhibited by sodium azide. Enterococci reduce TTC to give a red formazan, their colonies are thus red in colour. According to LACHICA and HARTMAN (1968), the selectivity for enterococci can be improved by adding carbonate and Tween80®.

Typical Composition (g/litre)

Tryptose 20.0; yeast extract 5.0; D(+)glucose 2.0; di-potassium hydrogen phosphate 4.0; sodium azide 0.4; agar-agar 10.0.

Preparation

Suspend 41.5 g/litre, sterilize by heating for 20 minutes in a current of steam (e.g. autoclave without excess pressure). Afterwards cool rapidly!

■ **Do not autoclave.**

Add 10 ml of a filtersterilized 1% 2,3,5-triphenyltetrazolium chloride solution/litre to the base medium at a temperature of approximately 50 °C. Pour plates.

pH: 7.2 ± 0.2 at 25 °C.

The plates are clear and yellowish-brown.

Experimental Procedure and Evaluation

Place the inoculated membrane filters on the surface of the plates.

Incubation: up to 48 hours at 35 °C aerobically.

Pink to brown colonies with a diameter of 0.5 to 2 mm are usually enterococci.

Literature

LACHICA, R.V.F., a. HARTMAN, P.A.: Two improved media for isolating and enumerating enterococci in certain frozen foods. – **J. Appl. Bact., 31**; 151-156 (1968).

SLANETZ, L.W., a. BARTLEY, C.H.: Numbers of enterococci in water, sewage, and faeces determined by the membrane filter technique with an improved medium – **J. Bact., 74**; 591-595 (1957).

Ordering Information

Product	Merck Cat. No.	Pack size
Membrane-filter Entero-coccus Selective Agar Base acc. to SLANETZ and BARTLEY	1.05289.0500	500 g
2,3,5-Triphenyltetrazo-lium chloride	1.08380.0010	10 g

Quality control

Test strains	Growth	Red colonies
Streptococcus pyogenes ATCC 12344	poor / fair	-
Streptococcus agalactiae ATCC 13813	poor / fair	-
Enterococcus faecalis ATCC 11700	≥ 50 %	+
Enterococcus faecalis ATCC 19433	≥ 50 %	+
Enterococcus hirae ATCC 8043	≥ 50 %	+ (poor)
Staphylococcus aureus ATCC 25923	none	
Escherichia coli ATCC 25922	none	
Pseudomonas aeruginosa ATCC 27853	none	

Membrane-filter Rinse Fluid (USP)

The broth is used as a rinsing fluid in the membrane filtration procedure.

This medium largely complies with the formulation prescribed in the recommendations of the United States Pharmacopeia XXVI (2003).

Mode of Action

After filtration it is often necessary to rinse the membrane filter in order to remove residues of liquid sample materials. If the sample contains higher hydrocarbons such as vaseline, paraffin, etc. or fats, the use of rinse fluid is recommended.

This fluid contains balanced concentrations of nutrients which prevent the microorganisms, retained by the filter, from being exposed to physiological shock, thus being capable to grow further rapidly. The detergent polysorbate 80 ensures emulsification of carbohydrates and fats without seriously affecting the microorganisms. If the sample contains large quantities of these compounds, additional up to 9.0 g/litre of polysorbate 80 (Tween® 80) can be added in accordance with the USP recommendations before the broth is filtered.

Typical Composition (g/litre)

Peptone from meat 5.0; meat extract 3.0; polysorbate 80 1.0.

Preparation

Suspend 9 g/litre, if desired together with up to 9 g/litre of polysorbate 80 (Tween® 80), filter until clear, autoclave (15 min at 121 °C).

pH: 6.9 ± 0.2 at 25 °C.

The broth is clear and yellow.

Experimental Procedure and Evaluation

After filtering the liquid sample, rinse the filter 3 times with 100 ml portions of the membrane-filter rinse fluid, then complete the test in the usual way.

Incubation for 24 hours at 35 °C aerobically.

Literature

United States Pharmacopeia XXVI, Chapter "Microbial Limit Tests", 1995.

Ordering Information

Product	Merck Cat. No.	Pack size
Membrane-filter Rinse Fluid (USP)	1.05286.0500	500 g
Tween® 80	8.22187.0500	500 ml

Quality control (streaked on Standard I Nutrient Agar 1.07881.)

Test strains	Growth
Test strains	Growth
Staphylococcus aureus ATCC 25923	good
Streptococcus pyogenes ATCC 12344	good
Enterococcus faecalis ATCC 11700	good
Escherichia coli ATCC 25922	good
Citrobacter freundii ATCC 8090	good
Pseudomonas aeruginosa ATCC 27853	good

MPF

mEC Broth with Novobiocin

For the selective enrichment of enterohemorrhagic E. coli (EHEC) in foods.

The culture medium complies with the requirements of the USDA-FSIS method for the isolation and identification of Escherichia coli 0157:H7 from meat.

Mode of Action

The nutrient substrates contained in mEC Broth provide favourable growth conditions. Especially lactose improves proliferation of lactose-positive bacteria. The mixture of bile salts No. 3 and Novobiocin suppresses the growth of Gram-positive microbial flora.

Typical Composition (g/litre)

Peptone 20.0; sodium chloride 5.0; bile salts No. 3 1.12; lactose 5.0; di-potassium hydrogen phosphate 4.0; potassium dihydrogen phosphate 1.5; novobiocin 0.02.

Preparation

Suspend 36.7 g in 1 litre of demin. water; autoclave (15 min at 121 °C).

pH: 6.9 ± 0.2 at 25 °C.

The prepared broth is clear and yellow-orange. The broth is stable for up to 6 months when stored at +2 to +8 °C.

Experimental Procedure and Evaluation

Inoculate mEC Broth with the sample material (usually add 25 g test portion of the sample to 225 ml of broth).

Incubation: 18 to 24 h at 37 °C or 41.5 °C. Incubation temperature depends on standard used. Afterwards about 0.1 ml of the broth is streaked on the dry surface of a E. coli 0157:H7 Selective Agar, e.g. Fluorocult® E. coli 0157:H7 Agar, SMAC Agar or CT-SMAC Agar in a way that single colonies can be well isolated.

Literature

OKREND, A.J.G., ROSE, B.E., a. BENNETT, B.: A research not: A screening method for the isolation of Escherichia coli 0157:H7 from ground beef. **-J.Food Prot., 53**; 249-252 (1990).

USDA-FSIS, Revision 4 of Laboratory Communication #38 Protocol for Isolation and Identification of Escherichia coli 0157:H7. - Amelia K. Sharar and Bonnie E. Rose, (1996).

Ordering Information

Product	Merck Cat. No.	Pack size
mEC Broth with Novobiocin	1.14582.0500	500 g
CT-Supplement	1.09202.0001	1 x 16 vials
Fluorocult® E. coli 0157:H7 Agar	1.04026.0500	500 g
Sorbitol-MacConkey Agar	1.09207.0500	500 g
Singlepath® E.coli 0157	1.04141.0001	25 tests

Quality control

Test strains	Growth	Singlepath® E. coli 0157
E. coli 0157:H7 ATCC 35150 (0157)	good	+
E. coli ATCC 11775	fair / good	-
Proteus vulgaris ATCC 13315	good inhibition	
Pseudomonas aeruginosa ATCC 27853	good inhibition	
Enterococcus faecalis ATCC 33186	good inhibition	

HA
MPF
MD

MRS Agar
(Lactobacillus Agar acc. to DE MAN, ROGOSA and SHARPE)

Media introduced by DE MAN, ROGOSA and SHARPE (1960) for the enrichment, cultivation and isolation of Lactobacillus species from all types of materials.

Mode of Action

The MRS culture media contain polysorbate, acetate, magnesium and manganese, which are known to act as special growth factors for lactobacilli, as well as a rich nutrient base. As these media exhibit a very low degree of selectivity, Pediococcus and Leuconostoc species and other secondary bacteria may grow on them.

Typical Composition (g/litre)

Peptone from casein 10.0; meat extract 10.0; yeast extract 4.0; D(+)-glucose 20.0; dipotassium hydrogen phosphate 2.0; Tween® 80 1.0; di-ammonium hydrogen citrate 2.0; sodium acetate 5.0; magnesium sulfate 0.2; manganese sulfate 0.04; agar-agar 14.0.

Preparation

Suspend 68.2 g MRS Agar/litre, autoclave 15 min at 121°C (or 15 min at **118 °C**). Autoclavation at 118 °C result in better growth of Bifido bacterium spp.

pH: 5.7 ± 0.2 at 25 °C.

The plates filled into tubes are clear and brown.

Experimental Procedure and Evaluation

If necessary, homogenize the sample material. Inoculate the MRS Agar with this material or with the original sample; it is best to use the pour-plate method.

Incubation: up to 3 days at 35 °C or up to 5 days at 30 °C, if possible incubate the plates in a CO_2 enriched atmosphere in an anaerobic jar (e.g. with Merck Anaerocult® C or C mini).

Do not allow the surface of the plates to dry as this causes the acetate concentration to increase at the surface, which inhibits the growth of lactobacilli.

Determine the bacterial count. Identify the lactobacilli by the methods proposed by SHARPE (1962) and SHARPE et al. (1966). For further methods of differentiation and identification see ROGOSA et al. (1953), ROGOSA and SHARPE (1959) and DAVIS (1960).

Literature

DAVIS, J.G.: The lactobacilli. – **I. Prog. in Industr. Microbiol., 2** ; 3 (1960).

DE MAN, J.D., ROGOSA, M., a. SHARPE, M.E.: A Medium for the Cultivation of Lactobacilli. – **J. Appl. Bact., 23**; 130-135 (1960).

ROGOSA, M., WISEMAN, R.F., MITCHELL, J.A., DISRAELY, M.N., a. BEAMAN, A.J.: Species differentiation of oral lactobacilli from man including descriptions of Lactobacillus salivarius nov. spec. and Lactobacillus cellobiosus nov. spec. – **J. Bact., 65**; 681-699 (1953).

ROGOSA, M., a. SHARPE, M.E.: An approach to the classification of the lactobacilli. – **J. Appl. Bact., 22**; 329-340 (1959).

SHARPE, M.E.: Taxonomy of the Lactobacilli. – **Dairy Sci. Abstr., 24**; 109 (1962).

SHARPE, M.E., FRYER, T.F., a. SMITH, D.C.: Identification of the Lactic Acid Bacteria. – in GIBBS, B.M., a. SKINNER, P.A.: Identification Methods for Microbiologists, Part A; 65-79 (1966).

Ordering Information

Product	Merck Cat. No.	Pack size
MRS Agar (Lactobacillus Agar acc. to DE MAN, ROGOSA and SHARPE)	1.10660.0500	500 g
Anaerobic jar	1.16387.0001	1 jar
Anaeroclip®	1.14226.0001	1 x 25
Anaerocult® C	1.16275.0001	1 x 10
Anaerocult® C mini	1.13682.0001	1 x 25
Plate basket	1.07040.0001	1 ea

Bifidob acterium bifidum
ATCC 11863

Lactobacillus casei
ATCC 393

MRS Agar
(Lactobacillus Agar acc. to DE MAN, ROGOSA and SHARPE)

Quality control (spiral plating method)

Test strains	Inoculum (dfu/ml)	Recovery rate %
Lactobacillus acidophilus ATCC 4356	10^3-10^5	≥ 50
Lactobacillus sake ATCC 15521	10^3-10^5	≥ 50
Lactobacillus lactis ATCC 19435	10^3-10^5	≥ 50
Pediococcus damnosus ATCC 29358	10^3-10^5	≥ 50
Bifidobacterium bifidum ATCC 11863	10^3-10^5	≥ 50 (anaerobic incubation)
Escherichia coli ATCC 25922	> 10^5	no growth
Bacillus cereus ATCC 11778	> 10^5	no growth

MRS Broth
(Lactosebacillus Broth acc. to DE MAN, ROGOSA and SHARPE)

Media introduced by DE MAN, ROGOSA and SHARPE (1960) for the enrichment, cultivation and isolation of Lactobacillus species from all types of materials.

The medium complies with the German DIN-Norm 10109 and for the inspection of meat and to the regulations acc. to § 35 LMBG (06.00/35) for the inspection of food.

Mode of Action

The MRS culture media contain polysorbate, acetate, magnesium and manganese, which are known to act as special growth factors for lactobacilli, as well as a rich nutrient base. As these media exhibit a very low degree of selectivity, Pediococcus and Leuconostoc species and other secondary bacteria may grow on them.

Typical Composition (g/litre)

Peptone from casein 10.0; meat extract 8.0; yeast extract 4.0; D(+)-glucose 20.0; dipotassium hydrogen phosphate 2.0; Tween® 80 1.0; di-ammonium hydrogen citrate 2.0; sodium acetate 5.0; magnesium sulfate 0.2; manganese sulfate 0.04.

Preparation

Suspend 52.2 g MRS Broth/litre, autoclave 15min at 121°C (or 15 min at 118 °C). Autoclavation at 118 °C result in better growth of Bifido bacterium spp.

pH: 5.7 ± 0.2 at 25 °C.

The broth filled into tubes are clear and brown.

Experimental Procedure and Evaluation

If necessary, homogenize the sample material and then transfer to MRS Broth for enrichment or for determining the bacterial count by the MPN method.

Incubation: up to 3 days at 35 °C or up to 5 days at 30 °C.

Determine the bacterial count. Identify the lactobacilli by the methods proposed by SHARPE (1962) and SHARPE et al. (1966). For further methods of differentiation and identification see ROGOSA et al. (1953), ROGOSA and SHARPE (1959) and DAVIS (1960).

Literature

DAVIS, J.G.: The lactobacilli. – **I. Prog. in Industr. Microbiol., 2**; 3 (1960).

DE MAN, J.D., ROGOSA, M., a. SHARPE, M.E.: A Medium for the Cultivation of Lactobacilli. – **J. Appl. Bact., 23**; 130-135 (1960).

ROGOSA, M., WISEMAN, R.F., MITCHELL, J.A., DISRAELY, M.N., a. BEAMAN, A.J.: Species differentiation of oral lactobacilli from man including descriptions of Lactobacillus salivarius nov. spec. and Lactobacillus cellobiosus nov. spec. – **J. Bact., 65**; 681-699 (1953).

Bundesgesundheitsamt: Amtliche Sammlung von Untersuchungsverfahren nach § 35 LMBG. – Beuth Verlag Berlin, Köln.

DIN Deutsches Institut für Normung e.V.: **DIN 10109**.

ROGOSA, M., a. SHARPE, M.E.: An approach to the classification of the lactobacilli. – **J. Appl. Bact., 22**; 329-340 (1959).

SHARPE, M.E.: Taxonomy of the Lactobacilli. – **Dairy Sci. Abstr., 24**; 109 (1962).

SHARPE, M.E., FRYER, T.F., a. SMITH, D.C.: Identification of the Lactic Acid Bacteria. – in GIBBS, B.M., a. SKINNER, P.A.: Identification Methods for Microbiologists, Part A; 65-79 (1966).

Ordering Information

Product	Merck Cat. No.	Pack size
MRS Broth (Lactobacillus Broth acc. to DE MAN, ROGOSA and SHARPE)	1.10661.0500	500 g
Anaerobic jar	1.16387.0001	1 ea
Anaeroclip®	1.14226.0001	1 x 25
Anaerocult® C	1.16275.0001	1 x 10
Anaerocult® C mini	1.13682.0001	1 x 25
Plate basket	1.07040.0001	1 ea

Quality control of MRS Broth

Test strains	Growth
Lactobacillus acidophilus ATCC 4356	good / very good
Lactobacillus plantarum ATCC 8014	good / very good
Lactobacillus casei ATCC 393	good / very good
Lactobacillus fermentum ATCC 9338	good / very good
Escherichia coli ATCC 25922	fair / good
Pseudomonas aeruginosa ATCC 27853	none

MR-VP Broth (Methyl-red VOGES-PROSKAUER Broth)

Test culture medium for the methyl red test (CLARK and LUBS 1915) and the VOGES-PROSKAUER Test (VOGES and PROSKAUER 1898), which are used for biochemical differentiation, particularly within the Coli-Aerogenes group.

This culture medium complies with the recommendations of the ISO (1975), the DIN Norm 10160 for the examination of meat and the DIN Norm 10181 for the examination of milk.

Mode of Action

a. Some bacteria utilize glucose to form large amounts of acid with the result that the pH value of the medium falls to below 4.4. Other species produce less acid so that the fall in pH is not as great. This difference can be visualized by using methyl red which is yellow above pH 5.1 and red at pH 4.4.

b. Many microorganisms metabolize glucose to produce acetoin (acetylmethyl carbinol), 2,3-butanediol or diacetyl. The presence of these metabolites is established by means of O'MEARA's reagent (1931) improved by LEVINE et al. (1934), copper sulfate solution according to LEIFSON (1932), BARRIT's reagent (BARRITT 1936) or other reagents (see references). According to HOLLÄNDER et al. (1982), addition of fumarate to the broth enhances this reaction.
Details and comparative studies on the various modifications of the MR-VP test are to found in EDDY (1961), SUASSUNA et al. (1961), IJUTOV (1963) and SKERMAN (1969).

Typical Composition (g/litre)

Peptone from meat 7.0; D(+)glucose 5.0; phosphate buffer 5.0.

Preparation

Suspend 17 g/litre, dispense 5 ml aliquots into tubes and autoclave (15 min at 121 °C).

pH: 6.9 ± 0.2 at 25 °C.

The broth is clear and yellowish-brown.

Preparation of the methyl red indicator solution: Suspend 0.04 g methyl red in 60 ml absolute ethanol, adjust the pH to a value of approx. 5.0. The solution then becomes orange.

Preparation of O'MEARA's reagent: Suspend 40 g potassium hydroxide in 100 ml distilled water. Allow to cool, add 0.3g creatine (monohydrate) and dissolve. The prepared reagent solution can be stored for about 4 weeks in the refrigerator (+4 °C).

Preparation of copper sulfate solution acc. to LEIFSON: Suspend 1 g copper sulfate in 40 ml concentrated ammonia and add 690ml of an approx. 10 % potassium hydroxide solution (prepared from potassium hydroxide).

Preparation of BARRITT's reagent: Suspend 5 g naphthol(1) in 100 ml absolute ethanol.

Experimental Procedure and Evaluation

Inoculate two tubes containing MR-VP Broth with a pure culture of the microorganisms under investigation.

Incubation: up to 4 days at 35 °C.

Carry out the following tests:

Methyl red test: Add about 5 drops of the methyl red indicator solution to the first tube.

VOGES-PROSKAUER test: Add 5 ml of copper sulfate solution acc. to LEIFSON or 3 ml BARRIT's solution and 1 ml 40 % potassium hydroxide solution (prepared from extra pure potassium hydroxide) or 5 ml O'MEARA's reagent to the second tube. With the first two reagents a positive reaction is indicated, if the colour of the medium changes to red within a few minutes.

In the case of O'MEARA's reagent, the reaction is positive if, after frequent shaking, a pink coloration appears after approx. 20minutes beginning at the surface and becoming more intense within 2hours.

Colour Reaction	Microorganisms
From orange to red	Escherichia coli, Citrobacter and others
From orange to yellow	Enterobacter aerogenes, Enterobacter cloacae and others
Red (positive)	Enterobacter aerogenes, Enterobacter cloacae and others
No colour change (negative)	Escherichia coli, Citrobacter and others

Literature

ISO International Organization for Standardization: Meat and meat products. Detection of Salmonellae. Reference method. – **International Standard ISO 3565**; (1975).

DIN Deutsches Institut für Normung e.V.: Untersuchung von Fleisch und Fleischerzeugnissen. Nachweis von Salmonellen. Referenzverfahren. - **DIN10160**.

DIN Deutsches Institut für Normung e.V.: Mikrobiologische Milchuntersuchung. Nachweis von Salmonellen. Referenzverfahren. – **DIN 10181**.

BARRIT, M.: The intensification of the Voges-Proskauer reaction by the addition of α-naphthol. – **J. Path. Bact. 42**; 441-454 (1936).

CLARK, W., a. LUBS, H.: The differentiation of bacteria of the Colon-Arogenes family by the use of indicators. – **J. Inf. Dis., 17**; 160-173 (1915).

EDDY, B.P.: The Voges-Proskauer reaction and its significance: A review. - **J. Appl. Bact., 24**; 27-41 (1961).

HOLLÄNDER, R., BÖHMANN, J., a. GREWING, B.: Die Verstärkung der Voges-Proskauer-Reaktion durch Fumarat. –**Zbl. Bakt. Hyg., I Abt. Orig. A, 252**; 316-323 (1982).

LEIFSON, E.: An improved reagent for the acetyl-methyl-carbinol test. - **J.Bact. 23**; 353-354 (1932).

LEVINE, M., EPSTEIN, S.A., a. VAUGHN, R.H.: Differential reaction in the colon group of bacteria. – **Publ. Hlth., 24**; 505-510 (1934).

IJUTOV V.: Technique of Voges-Proskauer test. – **Acta path. microbiol. scand., 58**; 325-335 (1963).

O'MEARA, R.: A simple delicate and rapid method of detecting the formation of acetylmethylcarbinol by bacteria fermenting carbohydrates. – **J. Path. Bact., 34**; 401-406 (1931).

SKERMAN, V.B.D.: Abstracts of microbiological methods (Wiley-Interscience, New York, 1969).

SUASSUNA, J., SUASSUNA, J.R., a. EWING, W.H.: The methyl red and Voges-Proskauer reactions of enterobacteriaceae. – **Publ. Hlth. Lab., 19**; 67-75 (1961).

VOGES, O., a. PROSKAUER, B.: Beitrag zur Ernährungsphysiologie und zur Differentialdiagnose der hämorrhagischen Septicämie. – **Z. Hyg. Infekt., 28**; 20-32 (1898).

MR-VP Broth (Methyl-red VOGES-PROSKAUER Broth)

Ordering Information

Product	Merck Cat. No.	Pack size
MR-VP Broth (Methyl-red VOGES-PROSKAUER Broth)	1.05712.0500	500 g
Ammonia solution 25 %	1.05432.1000	1 l
Copper sulfate	1.02790.0250	250 g
Creatine (monohydrate)	8.41470.0050	50 g
Ethanol absolute	1.00983.1000	1 l
Methyl red	1.06076.0025	25 g
Naphthol-(1)	1.06223.0050	50 g
Potassium hydroxide pellets	1.05033.0500	500 g

Quality control

Test strains	Growth	Methyl red	VOGES-PROSKAUER
Escherichia coli ATCC 25922	good / very good	+	-
Klebsiella pneumoniae ATCC 13883	good / very good	+	-
Klebsiella pneumoniae ATCC 10031	good / very good	+	-
Enterobacter cloacae ATCC 13047	good / very good	-	+
Serratia marcescens ATCC 14756	good / very good	±	+

AC

MSRV Medium Base, modified

Modified Semi-solid Rappaport-Vassiliadis (MSRV) Medium

MSRV Medium is a semi-solid medium used for the isolation of Salmonella from food-stuffs and other materials.

Mode of Action

De SMEDT et al. (1986) made a semi-solid RV-medium by adding agar (MSRV). In comparison to traditional methods this formulation gave more Salmonella-positive results.

The detection principle is based on the motility of Salmonellae to migrate into the semi-solid medium thus forming opaque halos of growth.

The motility of other microorganisms is largely inhibited by selective agents (Magnesium chloride, Malachite green and Novobiocin) and the enhanced incubation temperature of 42 °C.

Typical Composition (g/litre)

Tryptose 4.59; casein hydrolysate 4.59; sodium chloride 7.34; potassium dihydrogen phosphate 1.47; magnesium chloride anhydrous 10.93; malachite green 0.037; agar-agar 2.7.

Preparation

Suspend 15.8 g in 500 ml demin. water by heating in a boiling water bath or in a flowing steam until the medium is completely dissolved.

■ **Do not autoclave / do not overheat!**

Dissolve the lyophilisate of 1 vial MSRV Selective Supplement by adding 1 ml sterile distilled water and add the solution to the medium cooled to 45-50 °C. Mix gently and pour plates.

pH: 5.6 ± 0.2 at 25 °C.

The prepared medium is clear and bright-blue and can be stored in the refrigerator at +2 °C to +8 °C for up to 2 weeks.

The plates must be well dried before use.

Drying of plates:

1. in a clean bench with air flow. Remove lids and let dry for 15-20 minutes (do not overdry!)
2. without air flow 1hour (lids removed) at room temperature.

Experimental Procedure

1. Enrich the sample material in Buffered Peptone Water (Incubation: 16-20 h at 42 °C).
2. Incubate 3 drops (0.1 ml) of the pre-enrichment culture in three different spots on the surface of the MSRV medium plates.
3. Inoculate the plates aerobically in an upright position for no longer than 24 h at 42 °C.

Evaluation

Motile microorganisms show a halo of growth originating from the inoculation spot. For the confirmation of Salmonella further biochemical and serological tests are recommended.

Literature

De SMEDT et al.: Rapid Salmonella Detection in Foods by Motility Enrichment on a Modified Semi-Solid Rappaport-Vassiliadis Medium. – **J. Food Protect. Vol. 49**, 7; 510-514 (1986).

De SMEDT, a. BOLDERDIJK, R.F.: Dynamics of Salmonella Isolation with Modified Semi-Solid Rappaport-Vassiliadis Medium. – **J. Food Protect. Vol. 50, 8**; 658-661 (1987).

Ordering Information

Product	Merck Cat. No.	Pack size
MSRV Medium Base, modified	1.09878.0500	500 g
MSRV Selective Supplement	1.09874.0001	1 x 16 vials
Peptone Water (buffered)	1.07228.0500	500 g
Peptone Water (buffered)	1.07228.5000	5 kg

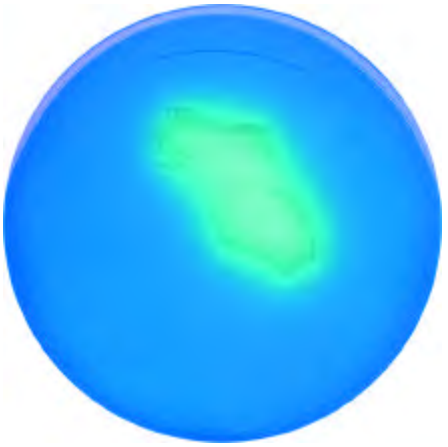
MSRV Medium Base, modified

Modified Semi-solid Rappaport-Vassiliadis (MSRV) Medium

Quality control

Test strains	Growth	Motility zone
Salmonella typhimurium ATCC 14028	good	≥ 20 mm
Salmonella enteritidis ATCC 13076	good	≥ 20 mm
Citrobacter freundii ATCC 8090	none	-
Pseudomonas aeruginosa ATCC 27853	none	-

Citrobacter freundii ATCC 8090



Salmonella enteritidis ATCC 13076

MSRV Selective Supplement

Additive for the preparation of MSRV Medium modified, Merck Cat. No. 1.09878.0500.

Mode of Action

MSRV Selective Supplement contains Novobiocin in lyophilized form.

It suppresses the growth of the accompanying flora during culturing Salmonellae.

Composition (per vial)

Novobiocin 10 mg.

Experimental Procedure

The lyophilisate is dissolved in the original vial by adding 1ml of sterile distilled water.

In the preparation of MSRV Medium, the dissolved content of one vial is evenly mixid into 500 ml of sterile, still liquid medium cooled to 45-50 °C.

Ordering Information

Product	Merck Cat. No.	Pack size
MSRV Selective Supplement	1.09874.0001	1 x 16 vials

mTSB Broth with Novobiocin

mTryptic-Soy-Broth with Novobiocin

For the selective enrichment of enterohemorrhagic E. coli (EHEC) in foods.

The culture medium complies with the requirements of the ISO Standard 16654 for the detection of Escherichia coli (E. coli) serotype 0157:H7 in foods as well as with the methods of FDA-BAM for the isolation of enterohemorrhagic E. coli (EHEC).

Mode of Action

The nutrient substrates contained in mTSB broth provide favourable growth conditions. The mixture of bile salts No. 3 and Novobiocin suppresses the growth of the Gram-positive microbial flora.

Typical Composition (g/litre)

Peptone from caseine 17.0; peptone from soymeal 3.0; sodium chloride 5.0; bile salts No. 3 1.5; D(+)-glucose 2.5; di-potassium hydrogen phosphate 4.0; novobiocin 0.02.

Preparation

Suspend 33 g in 1 litre of demin. water; autoclave (15 min at 121°C).

pH: 7.3 ± 0.2 at 25 °C.

The prepared broth is clear and yellowish. The broth is stable for up to 6 month when stored at +2 - +8 °C.

Experimental Procedure and Evaluation

Inoculate mTSB Broth with the sample material (usually add 25g test portion of the sample to 225ml of broth).

Incubation: 18 to 24 h at 37 °C or 41.5 °C. Incubation temperature depends on standard used.

Afterwards about 0.1 ml of the broth is streaked on the dry surface of a E. coli 0157:H7 Selective Agar, e.g. Fluorocult® E. coli 0157:H7 Agar, SMAC Agar or CT-SMAC Agar in a way, that single colonies can be well isolated.

Literature

DIN Deutsches Institut für Normung e.V.: Nachweis von Escherichia coli 0157 in Lebensmitteln. – **DIN 10167**.

FDA Bacteriological Analytical Manual, 8th Edition/1995, Chapter 4. Escherichia coli and the Coliform Bacteria, page 4.20: Isolation Methods for Enterohemorrhagic E. coli (EHEC).

WEAGANT, S.D., BRYANT, J.L., a. JINNEMAN, K.G.: An improved rapid technique for isolation of Escherichia coli 0157:H7 from foods. – **J. Food Prot., 58**; 7-12 (1995).

Ordering Information

Product	Merck Cat. No.	Pack size
mTSB Broth with Novobiocin	1.09205.0500	500 g
CT-Supplement	1.09202.0001	1 x 16 vials
Fluorocult® E. coli 0157:H7 Agar	1.04036.0100	100 g
Fluorocult® E. coli 0157:H7 Agar	1.04036.0500	500 g
Sorbitol-MacConkey Agar	1.09207.0500	500 g
Singlepath® E.coli 0157	1.04141.0001	25 tests

Quality control

Test strains	Growth	Singlepath® E. coli 0157
E. coli 0157:H7 ATCC 35150	good	+
E. coli ATCC 11775	fair	-
Pseudomonas aeruginosa ATCC 27853	fair	-

MUELLER-HINTON Agar

Media proposed by MUELLER and HINTON (1941) for testing the sensitivity of clinically important pathogens towards antibiotics or sulfonamides.

IVD *in vitro diagnosticum –*
For professional use only

See also General Instruction of Use
Warnings and precautions see ChemDAT®
(www.chemdat.info)

These culture media comply with the requirements of the WHO (1961, 1977) and DIN Norm 58930.

MUELLER-HINTON agar is used for agar diffusion tests while MUELLER-HINTON broth is employed for the determination of the MIC in serial dilution tests.

Principle

Microbiological method

Mode of Action

The composition of the culture media provide favourable growth conditions, the media are almost totally devoid of sulfonamide antagonists.

In order to improve the growth of fastidious microorganisms, blood can be added to MUELLER-HINTON agar. According to JENKINS et al. (1985), this may lead to false results when testing the susceptibility of enterococci to aminoglycosides.

Typical Composition (g/litre)

Meat infusion 2.0; casein hydrolysate 17.5; starch 1.5; agar-agar 13.0.

Preparation and Storage

Usable up to the expiry date when stored dry and tightly closed at +15 to +25° C. Protect from light.

After first opening of the bottle the content can be used up to the expiry date when stored dry and tightly closed at +15 to +25° C.

Suspend 34.0 g/litre, autoclave under mild conditions (10 min at 115 °C), if required cool to 45-50 °C and add 5-10 % definibrated blood, pour plates.

pH: 7.4 ± 0.2 at 25 °C.

Without blood, the plates are clear to opalescent and yellowish-brown.

Specimen

e.g. Isolated bacteria from urine,

Clinical specimen collection, handling and processing, see general instructions of use.

Experimental Procedure and Evaluation

Carry out the sensitivity or resistance test as directed.

Incubation for 24 h at 35 °C aerobically.

Literature

BAUER, A.W., KIRBY, W.M.M., SHERRIS, J.C., a. TURCK, M.: Antibiotic susceptibility testing by a standardized single disk method. - **Amer. J. Clin. Pathol., 45**; 493-496 (1966).

DIN Deutsches Institut für Normung: Methoden zur Empfindlichkeitsprüfung von bakteriellen Krankheitserregern (außer Mycobakterien) gegen Chemotherapeutika. Agar-Diffusionstest. - **DIN 58940**.

ERICSSON, H.M., a. SHERRIS, J.C.: Antibiotic Sensitivity Testing. Report of an International Collaborative Study. - **Acta path. microbiol. scand., B. Suppl., 217**; 90 pp (1971).

JENKINS, R.D., STEVENS, S.L., CRAYTHORN, J.M., THOMAS, T.W., GUINAN, M:E., a. MATSEN, J.M.: False susceptibility of enterococci to aminoglycosides with blood-enriched Mueller-Hinton agar for disk susceptibility testing. - **J. Clin. Microbiol., 22**; 369-374 (1985).

MUELLER, H.J., a. HINTON, J.: A protein-free medium for primary isolation of the Gonococcus and Meningococcus. - **Proc. Soc. Expt. Biol. Med., 48**; 330-333 (1941).

World Health Organization: Standardization of methods for conducting microbic sensitivity tests (Technical Report Series No. 210, Geneva 1961).

World Health Organization: Requirements for antibiotic susceptibility tests. I. Agar diffusion tests using antibiotic susceptibility discs. (Technical Report Series No. 610, Geneva 1977).

Ordering Information

Product	Merck Cat. No.	Pack size
MUELLER-HINTON Agar	1.05437.0500	500 g
MUELLER-HINTON Agar	1.05437.5000	5 kg
Merckoplate® MUELLER-HINTON Agar	1.10414.0001	20 plates

MUELLER-HINTON Agar

Quality control

	Inhibition zone diameter in mm acc. to WHO (revised) TEST STRAINS			
Test discs	**Esch. coli ATCC 25922**	**Staph. aureus ATCC 25923**	**Pseud. aeruginosa ATCC 27853**	**Enteroc. faecalis ATCC 33186**
Ampicillin 10 µg	16-22	27-35	-	-
Tetracyclin 30 µg	18-25	19-28	-	-
Gentamicin 10 µg	19-26	19-27	16-21	-
Polymyxin B 300 IU	12-17	7-13	-	-
Sulfamethoxazole 1.25 µg +Trimethoprim 23.75 µg	24-32	24-32	-	> 20

Staphylococcus aureus ATCC 25923

Escherichia coli ATCC 25922

MUELLER-HINTON-Broth

Media proposed by MUELLER and HINTON (1941) for testing the sensitivity of clinically important pathogens towards antibiotics or sulfonamides.

IVD *in vitro diagnosticum –*
For professional use only

See also General Instruction of Use
Warnings and precautions see ChemDAT®
(www.chemdat.info)

These culture media comply with the requirements of the WHO (1961, 1977) and DIN Norm 58930.

MUELLER-HINTON broth is employed for the determination of the MIC in serial dilution tests.

Principle

Microbiological method

Mode of Action

The composition of the culture media provide favourable growth conditions, the media are almost totally devoid of sulfonamide antagonists.

Typical Composition (g/litre)

Meat infusion 2.0; casein hydrolysate 17.5; starch 1.5.

Preparation and Storage

Usable up to the expiry date when stored dry and tightly closed at +15 to +25° C. Protect from light.

After first opening of the bottle the content can be used up to the expiry date when stored dry and tightly closed at +15 to +25°C.

Suspend 21 g/litre, dispense into test tubes, autoclave (15 min at 121 °C).

pH: 7.4 ± 0.2 at 25 °C.

The prepared broth is clear and yellowish and stable for 2 weeks at 2-8 °C.

Specimen

e.g. Isolated bacteria from urine.

Clinical specimen collection, handling and processing, see general instructions of use.MUELLER-HINTON Agar:

Experimental Procedure and Evaluation

Carry out the sensitivity or resistance test as directed.

Incubation for 24 h at 35 °C aerobically.

Literature

BAUER, A.W., KIRBY, W.M.M., SHERRIS, J.C., a. TURCK, M.: Antibiotic susceptibility testing by a standardized single disk method. - **Amer. J. Clin. Pathol., 45**; 493-496 (1966).

DIN Deutsches Institut für Normung: Methoden zur Empfindlichkeitsprüfung von bakteriellen Krankheitserregern (außer Mycobakterien) gegen Chemotherapeutika. Agar-Diffusionstest. - **DIN 58940**.

ERICSSON, H.M., a. SHERRIS, J.C.: Antibiotic Sensitivity Testing. Report of an International Collaborative Study. - **Acta path. microbiol. scand., B. Suppl., 217**; 90 pp (1971).

JENKINS, R.D., STEVENS, S.L., CRAYTHORN, J.M., THOMAS, T.W., GUINAN, M:E., a. MATSEN, J.M.: False susceptibility of enterococci to aminoglycosides with blood-enriched Mueller-Hinton agar for disk susceptibility testing. - **J. Clin. Microbiol., 22**; 369-374 (1985).

MUELLER, H.J., a. HINTON, J.: A protein-free medium for primary isolation of the Gonococcus and Meningococcus. - **Proc. Soc. Expt. Biol. Med., 48**; 330-333 (1941).

World Health Organization: Standardization of methods for conducting microbic sensitivity tests (Technical Report Series No. 210, Geneva 1961).

World Health Organization: Requirements for antibiotic susceptibility tests. I. Agar diffusion tests using antibiotic susceptibility discs. (Technical Report Series No. 610, Geneva 1977).

Ordering Information

Product	Merck Cat. No.	Pack size
MUELLER-HINTON-Broth	1.10293.0500	500 g

Quality control

Test strains	Growth
Escherichia coli ATCC 25922	good / very good
Staphylococcus aureus ATCC 25923	good / very good
Pseudomonas aeruginosa ATCC 27853	good / very good
Enterococcus faecalis ATCC 33186	good / very good
Bacillus subtilis ATCC 6633	good / very good (Antagonist test!)
Streptococcus pyogenes ATCC 12344	good / very good
Streptococcus pneumoniae ATCC 6301	fair / good
Listeria monocytogenes ATCC 19118	fair / good

MUELLER-HINTON Agar acc. to NCCLS

For the determination of antibiotic susceptibility including sulfonamides by the disc-agar diffusion method.

IVD *in vitro diagnosticum –*
For professional use only

See also General Instruction of Use
Warnings and precautions see ChemDAT®
(www.chemdat.info)

CE

Principle

Microbiological method

Mode of Action

The medium complies with the requirement of the National Committee for Clinical Laboratory Standards (NCCLS) and is manufactured to contain low concentrations of thymine and thymidine as well as appropriate levels of calcium and magnesium ions.

Thymine and thymidine concentrations are determined by the disc diffusion procedure with trimethoprim and sulfamethoxazole and Enterococcus faecalis ATCC 29212.

Calcium and/or magnesium concentrations are controlled by obtaining the correct zone diameters with aminoglycoside antibiotics and Pseudomonas aeruginosa ATCC 27853.

Typical Composition (g/litre)

Meat infusion 2.0; casein hydrolysate 17.5; starch 1.5; agar-agar 17.0..

Preparation and Storage

Usable up to the expiry date when stored dry and tightly closed at +15 to +25° C. Protect from light.

After first opening of the bottle the content can be used up to the expiry date when stored dry and tightly closed at +15 to +25° C.

Suspend 38 g in 1 litre of demin. water and autoclave (15 min at 121 °C). If required, cool to 45-50 °C and add 5-10 % defibrinated blood, pour plates.

pH: 7.3 ± 0.2 at 25 °C.

Without blood, the plates are clear to opalescent and brownish-yellow.

Specimen

e.g. Isolated bacteria from urine.

Clinical specimen collection, handling and processing, see general instructions of use.

Experimental Procedure

Carry out the sensitivity test acc. to NCCLS.

Incubation for 24 h at 35 °C aerobically.

Literature

National Committee for Clinical Laboratory Standards. Approved Standard. Performance standards for antimicrobial disc susceptibility tests, 5th ed. Natrional Committee for Clinical Laboratory Standards, Villanova, Pa. (1993).

Ordering Information

Product	Merck Cat. No.	Pack size
MUELLER-HINTON agar acc. to NCCLS	1.05435.0500	500 g

Quality control

Test strains	Growth	Zone diameters within specifications
Escherichia coli ATCC 25922	good / very good	+
Staphylococcus aureus ATCC 25923	good / very good	+
Pseudomonas aeruginosa ATCC 27853	good / very good	+
Enterococcus faecalis ATCC 29212	good / very good	+

Muller-Kauffmann Tetrathionate-Novobiocin Broth (MKTTn)

For the selective enrichment of salmonellae from food and animal feed stuffs acc. to ISO 6579

Mode of Action

Tetrathionate is produced from thiosulfate by adding iodine to the culture medium. Tetrathionate suppresses the growth of coliform and other enteric bacteria. Salmonella, Proteus and some other speices of bacteria can reduce tetrathionate and are not inhibited. Calcium carbonate buffers the sulphuric acid, which is liberated when tetrathionate is reduced. Bile promotes the growth of Salmonella, but largely inhibits the accompanying bacteria. Brillant green and novobiocin suppresses primarily Gram-positive bacteria.

Typical Composition (g/litre)

Meat extract 4.3; peptone from casein 8.6; sodium chloride 2.6; calcium carbonate 38.7; sodium thiosulfate water free 30.5 (equivalent to 47,8 g sodium thiosulphate pentahydrate); ox bile 4.78, brillant green 0.0096; novobiocin 0.040.

Also to be added:

Potassium iodide 5.0; iodine 4.0; dissolved in 20 ml water.

Preparation

Suspend 89.5 g in 1000 ml demin. water, heat briefly (5 min.) by boiling and cool rapidly. A sediment of calcium carbonate appears in the turbid broth at the bottom of the tubes. Adjust the pH, if necessary, so that it is 8.0 ± 0.2 at 25 °C.

- **Do not autoclave.**

Prior to use add 20 ml iodine/potassium iodide solution to 1000ml of basal medium. Dispense the medium aseptically into sterile flasks of suitable capacity to obtain the portions necessary for the test. Avoid further heating.

- **The basal medium without iodine/potassium iodide solution has at refrigeration a shelf life of up to 4 weeks at 2-8°C**

Preparation ot the iodine/potassium iodide solution:

Completely dissolve 5 g potassium iodide in 2 ml of water, then add 4 g iodine and dilute to 20 ml distilled water.

- **The ready-to-use broth prepared and used the same day.**

The medium is turbid and green with a white sediment (calcium carbonate).

Experimental Procedure and Evaluation

Directly suspend approximately 1 ml of culture in 10 ml Muller-Kauffmann Tetrathionate-Novobiocin broth acc. to ISO 6579

Incubation: 21-27 hours at 36-38 °C.

Streak material from the resulting cultures onto selective media acc. to ISO 6579.

Literature

BÄNFFER, J.R.: Comparison of the isolation of Salmonellae from human faeces by enrichment at 37 °C and at 43 °C. - **Zbl. Bakt. I. Orig., 217**; 35-40 (1971).

ISO 6579 2002 International Standardisation Organisation. Microbiology of Food and animal feeding stuffs – Horizontal method for the detection of Salmonella spp.

EDEL, W., a. KAMPELMACHER, E.H.: Salmonella isolation in nine European laboratories using a standardized technique. - **Bull. Wld. Hlth. Org., 41**; 297-306 (1969).

KAUFFMANN, F.: Ein kombiniertes Anreicherungsverfahren für Typhus- und Paratyphusbazillen. - **Zbl. Bakt. I. Orig., 119**; 148-152 (1930).

KAUFFMANN, F.: Weitere Erfahrungen mit dem kombinierten Anreicherungsverfahren für Salmonellenbacillen. - **Z. Hyg. Infekt.-Krkh., 117** ; 26-32 (1935).

MULLER, L.: Un nouveau milieu d'enrichissement pour la recherche du bacille typhique et des paratyphiques. - **Comp. rend. Soc. biol., 89**; 434-437 (1923).

Ordering Information

Product	Merck Cat. No.	Pack size
Muller-Kauffmann Tetrathionate-Novobiocin Broth (MKTTn)	1.05878.0500	500 g
Iodine resublimed	1.04761.0100	100 g
Potassium iodide	1.05043.0250	250 g

Quality control

Test strains	Inoculum	Growth after 24 hours
Salmonella typhimurium ATCC 14028	approx. 1 %	≥ 95 %
Escherichia coli ATCC 25922	approx. 99 %	≤ 5 %
Proteus mirabillis ATCC 29906	approx. 99 %	≤ 5 %

MYP Agar

Mannitol-Egg-yolk-Polymyxine-Agar

Medium proposed by MOSSEL et al. (1967) for the enumeration, detection and isolation of Bacillus cereus in foodstuffs.

The German Regulations for Dietetic Foodstuffs (Verordnung über diätetische Lebensmittel (Diätverordnung)) stipulate that foodstuffs should be tested for Bacillus cereus. The medium furthermore complies with the German DIN-Norm 10198 for the investigation of milk and food and to the requirements acc. to 35 LMBG (00.00/25) and 01.00/53) for the investigation of food.

Mode of Action

This culture medium is highly adapted to the properties of Bac. cereus.

a. Bac. cereus is mannitol-negative. The mannitol content of the medium thus allows differentiation of the accompanying mannitol-positive microbial flora which are identified by a change in colour of the indicator phenol red to yellow.

b. Bac. cereus is not affected by concentrations of polymyxin which inhibit the common accompanying microbial flora (DONOVAN 1958). Addition of polymyxin is necessary, however, if the sample material is suspected to contain high-numbers of accompanying microorganisms.

c. Bac. cereus produces lecithinase. The insoluble degradation products of egg-yolk lecithin accumulate around the Cereus colonies to form a white precipitate. A lecithinase reaction occurs very early in many strains, Cereus colonies can, therefore, often be rapidly identified before accompanying polymyxin-resistant microorganisms have had a chance to fully develop.

Typical Composition (g/litre)

Peptone from casein 10.0; meat extract 1.0; D(-)mannitol 10.0; sodium chloride 10.0; phenol red 0.025; agar-agar 12.0

Also to be added (per litre of medium):

egg-yolk emulsion 100 ml; polymyxin B sulfate 100,000 IU = Bacillus cereus Selective Supplement.

Preparation

Suspend 21.5 g in 450 ml demin. water, autoclave (15 min at 121°C). Cool to about 45 to 50 °C, add 50 ml (this volume can be varied depending on the degree of turbidity desired) of sterile egg-yolk emulsion and the contents of 1 vial Bacillus cereus Selective Supplement, mix. Pour plates.

pH: 7.2 ± 0.2 at 25 °C.

The plates (incl. egg-yolk) are evenly turbid and slightly orange (red without egg-yolk).

Experimental Procedure and Evaluation

Inoculate the plates by spreading the sample on the surface of the medium.

Incubation: 18-40 hours at 32 °C.

Bac. cereus appears as rough, dry colonies with a pink to purple base which are surrounded by a ring of dense precipitate. Colonies surrounded by a yellow or a clear zone are not Bacillus cereus. Further tests should be performed to confirm the identity of Bacillus cereus (anaerobic degradation of D(+)glucose, degradation of gelatin, positive nitrate reduction) (BROWN et al. 1958).

Literature

BROWN, E.R., MOODY, M.D., TREECE, E.L., a. SMITH, C.W.: Differenzial diagnosis of Bacillus cereus, Bacillus anthracis and Bacillus cereus var. mycoides. **– J. Bact., 75**; 499-509 (1958).

DONOVAN, K.O.: A selective medium for Bacillus cereus in milk. **– J. Appl. Bact., 21**; 100-103 (1958).

INAL, T.; Vergleichende Untersuchungen über die Selektivmedien zum qualitativen und quantitativen Nachweis von Bacillus cereus in Lebensmitteln.

I. Mitteilung. – Fleischwirtsch. 51; 1629-1632 (1971).

IV. Mitteilung. – Fleischwirtsch. 52; 1160-1162 (1972).

Bundesgesundheitsamt: Amtliche Sammlung von Untersuchungsverfahren nach § 35 LMBG. – Beuth Verlag Berlin, Köln.

DIN Deutsches Institut für Normierung e.V.: **DIN 10198 Teil 1 und 2**.

MOSSEL, D.A.A., KOOPMANN, M.J., a. JONGERIUS, E.: Enumeration of Bacillus cereus in foods. **– Appl. Microbiol., 15**; 650-653 (1967).

NYGREN: Phospholipase C-producing bacteria and food poisoning. An experimental study on Clostridium perfringens and Bacillus cereus. **– Acta path. microbiol. scand., 56**; Suppl. 1-160 (1962).

Ordering Information

Product	Merck Cat. No.	Pack size
MYP Agar	1.05267.0500	500 g
Bacillus cereus Selective Supplement (Polymyxin B; 50.000 IU)	1.09875.0001	16 vials
Egg-yolk emulsion sterile	1.03784.0001	10 x 100 ml

MYP Agar

Mannitol-Egg-yolk-Polymyxine-Agar

Quality control (spiral plating method)

Test strains	Inoculum (cfu/ml)	Recovery rate (%)	Colony colour	Precipitate
Bacillus cereus ATCC 11778	10^3-10^5	≥ 70	red	+
Bacillus subtilis ATCC 6051	10^3-10^5	Not limited!	yellow	-
Escherichia coli ATCC 8739	> 10^5	≥ 0.01	-	
Pseudomonas aeruginosa ATCC 25668	> 10^5	≥ 0.01	-	
Proteus mirabilis ATCC 29906	10^3-10^5	Not limited!	red	-
Staphylococcus aureus ATCC 6538	10^3-10^5	Not limited!	yellow	+

Bacillus cereus ATCC 11778

Staphylococcus aureus ATCC 6538

Nutrient Agar

Universal culture media for cultivating less fastidious microorganisms.

The nutrient agar corresponds with the recommendations of APHA (1985) for the examination of dairy products. The media comply with the recommendations of the APHA for the examination of foods (1992).

Typical Composition (g/litre)

Peptone from meat 5.0; meat extract 3.0; agar-agar 12.0.

Preparation

Suspend 20 g nutrient agar/litre or 8 g nutrient broth/litre, autoclave (15 min at 121 °C).

pH: 7.0 ± 0.2 at 25 °C.

The plates are clear and yellowish-brown.

Experimental Procedure and Evaluation

Depend on the purpose for which the media are used.

Incubation: 24 h at 35 °C aerobically.

Listeria 48 h at 35 °C aerobically.

Literature

American Public Health Association: Standard Methods for the Examination of Dairy Products (15th ed. 1985).

American Public Health Association: Compendium of methods for the microbiological examination of foods. 3rd ed., 1992.

GRAY, M.L., STAFSETH, HJ., a. THORP, F.: The use of potassium tellurite, sodium azide, and acetic acid in a selective medium for the isolation of Listeria monocytogenes. **- J. Bact., 59**, 443-444 (1950).

Ordering Information

Product	Merck Cat. No.	Pack size
Nutrient Agar	1.05450.0500	500 g

Quality control of Nutrient Agar (spiral plating method)

Test strains	Inoculum (cfu/ml)	Recovery rate %
Staphylococcus aureus ATCC 25923	10^3-10^5	≥ 70
Listeria monocyytogenes ATCC 19118	10^3-10^5	≥ 70 / 48 h
Escherichia coli ATCC 25922	10^3-10^5	≥ 70
Salmonella typhimurium ATCC 14028	10^3-10^5	≥ 70
Pseudomonas aeruginosa ATCC 27853	10^3-10^5	≥ 70
Bacillus cereus ATCC 11778	10^3-10^5	≥ 70

Escherichia coli ATCC 25922

Salmonella typhimurium ATCC 14028

AC
M
MPF
ID

Nutrient Broth

Universal culture media for cultivating less fastidious microorganisms.

The media comply with the recommendations of the APHA for the examination of foods (1992). According to GRAY et al. (1950) nutrient broth with added 0.05 % potassium tellurite is an excellent enrichment medium for Listeria monocytogenes.

Typical Composition (g/litre)

Peptone from meat 5.0; meat extract 3.0.

Preparation

Suspend 8 g nutrient broth/litre, autoclave (15 min at 121 °C).

pH: 7.0 ± 0.2 at 25 °C.

The plates are clear and yellowish-brown.

Experimental Procedure and Evaluation

Depend on the purpose for which the media are used.

Incubation: 24 h at 35 °C aerobically.

Literature

American Public Health Association: Standard Methods for the Examination of Dairy Products (15th ed. 1985).

American Public Health Association: Compendium of methods for the microbiological examination of foods. 3rd ed., 1992.

GRAY, M.L., STAFSETH, HJ., a. THORP, F.: The use of potassium tellurite, sodium azide, and acetic acid in a selective medium for the isolation of Listeria monocytogenes. **- J. Bact., 59**, 443-444 (1950).

Ordering Information

Product	Merck Cat. No.	Pack size
Nutrient Broth	1.05443.0500	500 g

Quality control of Nutrient Broth

Test strains	Growth
Staphylococcus aureus ATCC 25923	fair / very good
Streptococcus pyogenes ATCC 12344	fair / very good
Listeria monocytogenes ATCC 19118	fair / very good
Escherichia coli ATCC 25922	fair / very good
Salmonella typhimurium ATCC 14028	fair / very good
Pseudomonas aeruginosa ATCC 27853	fair / very good
Bacillus cereus ATCC 11778	fair / very good

OF Basal Medium acc. to HUGH and LEIFSON

Test culture medium proposed by HUGH and LEIFSON (1953) for deltecting oxidative and fermentative carbohydrate degradation. It is used primarily for the differentiation and classification of gram-negative intestinal bacteria.

IVD *in vitro diagnosticum –*
For professional use only

See also General Instruction of Use
Warnings and precautions see ChemDAT®
(www.chemdat.info)

A selective and differential agar for Pseudomonas cepacia was conceived by WELCH et al. (1987) on the basis of this medium, with the addition of agar-agar, lactose, polymyxin B and bacitracin.

Principle

Microbiological method

Mode of Action

A carbohydrate is added to the culture medium, degradation of the carbohydrate to acid is indicated by the pH indicator bromothymol blue which changes its colour to yellow. The degradation is allowed to take place while the medium is exposed to air (degradation may be oxidative or fermentative) or under exclusion of air (degradation by fermentation only).

Typical Composition (g/litre)

Peptone from casein 2.0; yeast extract 1.0; sodium chloride 5.0; di-potassium hydrogen phosphate 0.2; bromothymol blue 0.08; agar-agar 2.5.

also to be added:

carbohydrate 10.0 g/l.

Preparation and Storage

Usable up to the expiry date when stored dry and tightly closed at +15 to +25° C. Protect from light.

After first opening of the bottle the content can be used up to the expiry date when stored dry and tightly closed at +15 to +25° C.

Suspend 11 g/litre, autoclave (15 min at 121 °C). Cool to about 50 °C, add 100 ml/litre of a 10 % filter-sterilized solution of D(+)glucose, lactose, sucrose or other carbohydrates, mix. Dispense into tubes to give a depth of approx. 5 cm. Immediately after cooling overlay half of the tubes with an 1 cm layer of sterile paraffin oil (paraffin viscous). The prepared culture medium is dark-green to blue-green in colour and clear.

pH: 7.1 ± 0.2 at 25 °C.

Specimen

e.g. Isolated bacteria from, stool, urine, etc.

Clinical specimen collection, handling and processing, see general instructions of use.

Experimental Procedure and Evaluation

For each carbohydrate, inoculate one tube with and one tube without a paraffin seal with a pure culture of the microorganism to be examined down to the bottom of the tube by the stabbing technique. The organisms used for inoculation should be in the logarithmic phase of growth.

Incubation: at least 48 hours at 35 °C.

- **MOSSEL and MARTIN (1961) reported that this test can be performed in one tube if yeast extract is added to improve the growth of fastidious microorganisms, if the agar content is also increased to 1.5 % and if the depth of the culture medium is at least 8 cm.**
  A yellow colouration in both, the open and paraffin-sealed tubes, signifies fermentative degradation whereas yellow colouration of the open tubes alone indicate that the carbonhydrate in question is broken down by oxidation. Oxidative breakdown takes place at or close to the surface of the medium, whilst fermentative breakdown occurs both at the surface and throughout the butt. The tubes should finally be checked to see whether microbial growth produces turbidity solely along the puncture line (immotile strain) or throughout the whole medium (motile strain).

OF Basal Medium acc. to HUGH and LEIFSON

Carbohydrate metabolism of some important species (HUGH and LEIFSON, 1953):

Microorganisms	Glucose		Lactose		Sucrose		Group
	aerob	anaerob	aerob	anaerob	aerob	anaerob	
Alcalig. faecalis	-	-	-	-	-	-	I non-oxyd. spec. non-ferm. spec.
Ps. aeruginosa	A	-	-	-	-	-	II
Bact. anitratum	A	-	A	-	-	-	oxid. spec
Agrobacterium tumefaciens	A	-	-	-	A	-	non-ferm.
Malleomyces pseudomallei	A	-	A	-	A	-	spec.
Shig. dysenteriae	A	A	-	-	-	-	IIIa
Shig. sonnei	A	A	A	A	-	-	ferm. spec.
Vibrio comma	A	A	-	-	A	A	(anaerogenic)
S. enteritidis	AG	AG	-	-	-	-	IIIb
E. coli	AG	AG	AG	AG	-	-	ferm. spec.
Aerom. liquefaciens	AG	AG	-	-	AG	AG	(aerogenic)
Ent. aerogenes	AG	AG	AG	AG	AG	AG	
Non-classified species							IIIc
Some Paracolon-bacteria	A	A	A	-?	variable	variable	oxid. spec.
	AG	AG	A	-?	variable	variable	ferm. spec.

Signs and symbols: - = neutral or alkaline reaction, A = acid production, AG = acid and gas production

OF Basal Medium acc. to HUGH and LEIFSON

Use of the OF test for the diagnostic identification of some obligate and facultative aerobic, gram-negative rods of medical interest (modified according to COSTIN 1967)

Glucose-degradation	Oxidase	Type of reaction	Microorganisms
Fermntative	negative	I	1. Enterobacteriaceae 2. Yersinia pestis 3. Yersinia malassezii (pseudotuberculosis) 4. Yersinia enterocolitica
	positive	II	1. Aeromonas spp. 2. Vibrio cholerae 3. Vibrio spp. (NAG or NVC) 4. Vibrio parahaemolyticus 5. Pasteurella haemolytica 6. Pasteurella multocida 7. Pasteurella pneumotropica 8. Actinobacillus lignieresii 9. Chromobacterium violaceum
Oxidative	negative	III	1. Acinetobacter calcoaceticus (produces acid) 2. Pseudomonas maltophilia
	positive	IV	1. Pseudomonas aeruginosa 2. Pseudomonas stutzeri 3. Pseudomonas fluorescens (putida) 4. Pseudomonas mallei 5. Pseudomonas pseudomallei 6. Flavobacterium meningosepticum
Negative	negative	V	1. Acinetobacter calcoaceticus (does not produce acid) 2. Bordetella parapertussis
	positive	VI	1. Alcaligenes faecalis (denitrificans) 2. Pseudomonas alcaligenes 3. Bordetella bronchiseptica 4. Pseudomonas spp. 5. Campylobacter (Vibrio fetus) 6. Moraxella spp.

OF Basal Medium acc. to HUGH and LEIFSON

Literature

COSTIN, I.D.: An outline for the biochemical identification of aerobic and facultatively anaerobic gram-negative rods of medical interest. **- 5. Intern. Kongr. f. Chemotherapie Wien, B2/1**; 73-76 (1967).

HUGH, R., a. LEIFSON, E.: The taxonomic significance of fermentative versus oxidative metabolism of carbohydrates by various gram-negative bacteria. **- J. Bact., 66**; 24-26 (1953).

MOSSEL, D.A.A., et MARTIN, G.: Milieu simplifié permettant l'étude des divers modes d'action des bactéries sur les hydrates des carbone.**- Ann. Inst. Pasteur de Lille, 12**; 225-226 (1961).

WELCH, D.F., MUSZYNSKI, M.J., PAI, C.H., MARCON, M.J., HRIBAR, M.M., GILLIGAN, P.H., MATSEN, J.M., AHLIN, P.A:, HOLMAN, B.C., a. CHARTRAND, S.A.: Selective and differential medium for recovery of Pseudomonas cepacia from the respiratory tracts of patients with cystic fibrosis. **- J. Clin. Microbiol., 25**; 1730-1734 (1987).

Ordering Information

Product	Merck Cat. No.	Pack size
OF Basal Medium acc. to HUGH and LEIFSON	1.10282.0500	500 g
D(+)Glucose monohydrate	1.08342.1000	1 kg
Lactose monohydrate	1.07657.1000	1 kg
Paraffin viscous	1.07160.1000	1 l
Sucrose	1.07651.1000	1 kg

Quality control

Test strains	Growth	Colour change to yellow	
		with layer (aerobic)	with layer (anaerobic)
Escherichia coli ATCC 25922	good / very good	+	+
Staphylococcus aureus ATCC 25923	good / very good	+	+
Mirococcus luteus ATCC 9341	good / very good	+	(-)
Pseudomonas aeruginosa ATCC 27853	good / very good	+	-
Alcaligenes faecalis ATCC 19209	good / very good	-	-
Pseudomonas alcaligenes ATCC 14909	good / very good	-	-

OGYE Selective Supplement

Additive to OGYE Agar Base, Merck Cat. No. 1.05978. for the preparation of OGYE Selective Agar.

Mode of Action

OGYE Selective Supplement contains oxytetracycline in lyophilized form. It suppresses the growth of accompanying bacterial flora during culturing yeasts and moulds.

Composition (per vial)

Oxytetracycline in a buffered base 0.05 g.

Experimental Procedure

The lyophilisate is dissolved in the original vial by adding 10 ml of sterile, distilled water.

In the preparation of OGYE Agar, the dissolved content of one vial is evenly mixed into 500 ml sterile, still liquid medium cooled to 45-50 °C.

Ordering Information

Product	Merck Cat. No.	Pack size
OGYE Selective Supplement	1.09877.0001	1 x 15 vials

Oxytetracyclin-Glucose-Yeast Extract Agar (OGYE Agar) Base

Medium for the selective isolation and enumeration of yeasts and moulds in foods

Oxytetracycline-Glucose-Yeast Extract Agar (OGYE Agar) is described by MOSSEL et al. (1962, 1970) used for the isolation and enumeration of yeasts and molds in foods.

Mode of Action

The base medium allows good growth of yeasts and molds. Oxytetracycline inhibits the growth of bacteria.

Typical composition (g/liter)

Yeast Extract 5.0; glucose (dextrose) 20.0; Agar-agar 12.0.

Preparation

Suspend 18.5 g in 500 ml of purified water. Heat to boiling to dissolve completely. Autoclave at 121 °C for 15 minutes. Cool the medium to 45-50 °C and aseptically add the contents of 1 vial OGYE Selective Supplement. Mix well and pour into plates.

pH: 6.6 ± 0.2 at 25 °C.

The prepared medium is clear and slight yellowish-brown in color.

Experimental Procedure and Evaluation

The plates are inoculated using the pour-plate method or the surface speading method.

Incubation: up to 5 days at 20-25 °C.

Count the number of colonies per plate. Calculate the dilution factor into the final count for the sample tested.

Literature

MOSSEL, D.A.A., VISSER, M., and MENGERINK, W.H.J.: A comparison of media for the enumeration of moulds and yeasts in foods and beverages. **-Lab. Pract. 11**: 109 – 112 (1962).

MOSSEL, D.A.A., KLEYNEN-SEMMELING, A.M.C., VINCENTIE, H:M., BEERENS, H., and CATSARAS, M.: Oxytetracycline-Glucose-Yeast Extract Agar for selective enumeration of moulds and yeasts in foods and clinical material. **- J. Appl. Bact. 33**: 454 – 457 (1970).

Ordering Information

Product	Merck Cat. No.	Pack size
Oxytetracyclin-Glucose-Yeast Extract Agar (OGYE Agar) Base	1.05978.0500	500 g
OGYE Selective Supplement	1.09877.0001	1 x 15 vials

Candida albicans and Aspergillus niger

Quality control

Test strains	Growth
Candida albicans ATCC 10231	good / good
Microsporum canis ATCC 36299	fair / good
Penicillium commune ATCC 10428	good / very good
Aspergillus niger ATCC 16404	good / very good
E. coli ATCC 25922	none
Pseudomonas aeruginosa ATCC 27853	none
Bacillus cereus ATCC 11778	none

MPF

Orange-serum Agar

Medium proposed by HAYS (1951) and TROY and BEISEL (see MURDOCK et al. 1952) for the isolation, cultivation and enumeration of acid-tolerant, putrefactive microorganisms in fruit juices and fruit juice concentrates, especially citrus fruits.

This culture medium is in accordance with the recommendations of the Institut für Lebensmitteltechnologie und Verpackung (Institute for Food Technology and Packaging) (1974).

Mode of Action

The culture medium is optimally adapted to the special requirements of the microbial flora present in citrus juices (e.g. Bacillus, Lactobacillus, Leuconostoc species, fungi, etc.) due to the fact that it contains orange extract. It is therefore especially useful for the production control in the fruit juice industry (HAYS and TIESTER 1952).

Typical Composition (g/litre)

Peptone from casein 10.0; yeast extract 3.0; orange extract 5.0; D(+)glucose 4.0; di-potassium hydrogen phosphate 3.0; agar-agar 17.0.

Preparation

Suspend 42 g/litre, autoclave under mild conditions (15 min at 115 °C). Do not overheat. Pour plates.

pH: 5.5 ± 0.2 at 25 °C.

The plates are clear to opalescent and yellowish-brown.

Experimental Procedure and Evaluation

Incubate the inoculated culture medium for up to 4 days at 28 °C aerobically; if fungi are suspected to be present, incubate for up to 5 days. Determine the microbial count. Further tests can be performed to differentiate and identify the colonies.

Literature

Arbeitsgruppen des Instituts für Lebensmitteltechnologie und Verpackung der Technischen Universität München: Merkblätter für die Prüfung von Packmitteln, Merkblatt 19, "Bestimmung der Gesamtkeimzahl, der Anzahl an Schimmelpilzen und Hefen und der Anzahl an coliformen Keimen in Flaschen und vergleichbaren enghalsigen Behältern". **- Verpackgs.-Rdsch., 25**; Techn.-wiss. Beilage 569-575 (1974) und **Milchwiss., 29**; 602-606 (1974).

HAYS, G.L.: The isolation, cultivation and identification of organisms which have caused spilage in frozen concentrated orange juice. **- Proc. Florida State Hort. Soc**. (1951).

HAYS, G.L. a. RIESTER, D.W.: The control of "off-odor" spoilage in frozen concentrated orange juice. **- Food Technol., 6;** 386-389 (1952).

MURDOCK, D.I., FOLINAZZO, J.F., a. TROY, V.S.: Evaluation of plating media for citrus concentrates. **- Food Technol., 6**; 181-185 (1952).

Ordering Information

Product	Merck Cat. No.	Pack size
Orange-serum Agar	1.10673.0500	500 g

Quality control (spiral plating method)

Test strains	Inoculum (cfu/ml)	Recovery rate %
Bacillus cereus ATCC 11778	10^3-10^5	≥ 70
Lactobacillus plantarum ATCC 14917	10^3-10^5	≥ 70
Lactobacillus casei ATCC 393	10^3-10^5	≥ 70
Leuconostoc mesenteroides ATCC 9135	10^3-10^5	≥ 70
Cancida albicans ATCC 10231	10^3-10^5	≥ 70

Lactobacillus plantarum ATCC 14917

Leuconostoc mesenteroides ATCC 9135

Oxford Listia Selective Agar, Base

Selective agar for the isolation and detection of Listeria monocytogenes. This culture medium is in accordance with the standard 143:1990 of the IDF-FIL for milk and milk products for the detection of Listeria monocytogenes.

Mode of Action

The Oxford Agar formulation is based on Columbia Agar with the addition of lithium chloride, acriflavin, colistin sulfate, cefotetan, cycloheximide and fosfomycin. These ingredients suppress the growth of the common bacteria (e.g. Gram-negative bacteria and a greater part of Gram-positive bacteria).

Lithium chloride is one of the ingredients of Oxford Agar base, whereas the other substances derive from the Oxford Listeria Selective Supplement (Cat. No. 1.07006).

Listeria monocytogenes hydrolyses esculin to esculetin and forms a black complex with iron(III)ions. Therefore Listeria monocytogenes produces brown-green coloured colonies with a black halo.

Typical Composition (g/litre)

Peptone 23.0; starch 1.0; sodium chloride 5.0; agar-agar 13.0 (=Columbia agar); esculin 1.0; ammonium iron(III) citrate 0.5; lithium chloride 15.0.

Preparation

Suspend 29.25 g in 500 ml of demin. water, autoclave (15 min at 121 °C). Dissolve the lyophilisate of 1 vial Oxford Listeria Selective Supplement (Cat. No. 1.07006.) by adding 5 ml of a 1:1 mixture of ethanol and sterile distilled water. Mix gently and add the contents to the culture medium cooled to 50 °C. Pour the medium into plates and leave to solidify.

pH: 7.0 ± 0.2 at 25 °C.

The prepared agar (incl. supplement) is clear and bluish-brown.

Experimental Procedure and Evaluation

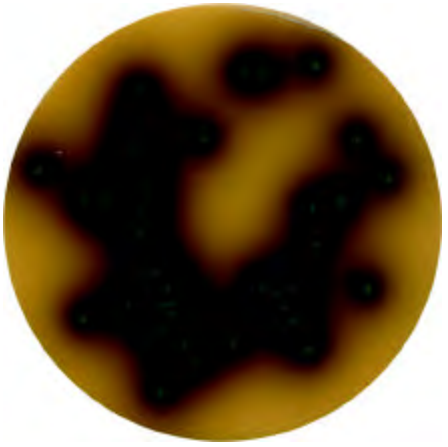
Inoculate by spreading the sample on the surface of the medium and incubate at 35 °C up to 48 h aerobically. Listeria monocytogenes grows as brown-green coloured colonies with a black halo (esculin splitting). Further biochemical tests should be carried out.

Literature

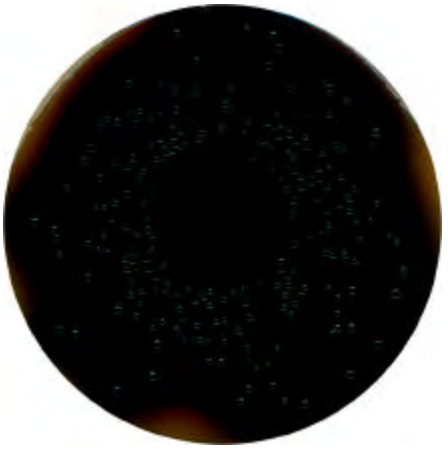
CURTIS, G.D.W., MITCHELL, R.G., KING, A.F., GRIFFIN, E.J.: A selective differential medium for the isolation of Listeria monocytogenes. **– Letters in Appl. Microbiol., 8**; 95-98 (1989).

Ordering Information

Product	Merck Cat. No.	Pack size
Oxford Listeria Selective Agar, Base	1.07004.0500	500 g
Oxford Listeria Selective Supplement	1.07006.0001	1 x 13 vials



Listeria innocua
ATCC 33090



Listeria monocytogenes
ATCC 19118

Oxford Listeria Selective Agar, Base

Quality control

Test strains	Recovery rate (%)	Black zone
Listeria monocytogenes ATCC 19118	≥ 70	+
Listeria monocytogenes ATCC 13932	≥ 70	+
Listeria monocytogenes ATCC 7973	≥ 70	+
Listeria monocytogenes ATCC 35152	≥ 70	+
Listeria innocua ATCC 33090	≥ 70	+
Staphylococcus aureus ATCC 25923	≥ 70	
Enterococcus faecalis ATCC 19433	≤ 0.01	
Erysipelothrix rhusiopathiae ATCC 19414	≤ 0.01	
Escherichia coli ATCC 25922	≤ 0.01	

Oxford Listeria Selective Supplement

Additive for the preparation of Oxford Listeria Selective Agar (Merck Cat. No. 1.07004.0500).

Mode of Action

Oxford Listeria Selective Supplement is a mixture of four antibiotics and a dye in lyophilized form. It largely inhibits the growth of accompanying bacteria in the selective cultivation of Listeria monocytogenes.

Composition (per vial))

Cycloheximide 200.0 mg, colistin sulfate 10.0 mg; acriflavin 2.5mg; cefotetan 1.0 mg; fosfomycin 5.0 mg.

Experimental Procedure

Suspend the lyophilisate by adding 5 ml of a 1:1 mixture of ethanol and sterile distilled water. Mix gently and add the contents to 500 ml of Oxford Agar Base cooled to 50 °C.

Ordering Information

Product	Merck Cat. No.	Pack size
Oxford Listeria Selective Supplement	1.07006.0001	1 x 13 vials
Ethanol absolute1	1.00983.1000	1 l

L-PALCAM Listeria Selective Enrichment Broth Base acc. to VAN NETTEN et al.

For the selective enrichment of Listeria.

Mode of Action

The nutrient substrates contained in the L-PALCAM Broth enable a very good proliferation of Listeria. Growth of the undesirable accompanying flora is inhibited by the selective substances Polymyxin-B-sulfate, acriflavine, lithium chloride and ceftazidime. Soybean lecithin has properties similar to egg-yolk emulsion, meaning that additional supplementation with egg-yolk emulsion is not required.

Esculin, ammonium iron(III) citrate, mannitol, and phenol red enable a differential-diagnostic statement regarding the possible presence of Listeria.

Listeria hydrolyse the glucoside esculin into glucose and esculetin. The latter substance reacts with iron(III) ions to form an olive-green to black complex.

When Listeria proliferate in the L-PALCAM Broth, therefore, in the majority of cases the broth turns black-brown in colour.

Typical Composition (g/litre)

Peptone 23.0; yeast extract 5.0; lithium chloride 10.0; esculin 0.8; ammonium iron(III) citrate 0.5; D(-)mannitol 5.0; phenol red 0.08; soybean lecithin 1.0; Tween® 80 2.0.

Preparation

Suspend 23.7 g in 500 ml of demin. water, autoclave (15 min at 121 °C). Suspend the contents of 1 vial of PALCAM Listeria Selective Supplement acc. to VAN NETTEN et al. in 1 ml sterile, distilled water and add to the basic broth, which has been cooled below 50 °C. Carefully swirl to mix the selective supplement into the broth homogeneously.

pH: 7.4 ± 0.2 at 25 °C.

The prepared broth (incl. supplement) is opalescent/turbid and red.

Experimental Procedure and Evaluation

Inoculate the broth with sample material (generally 25 g sample material into 225 ml broth) and incubate at 30 °C for 24-48 hours aerobically.

Approximately 0.1 ml of the broth is then smeared on the surface of a Listeria selective agar (e.g. PALCAM Agar or Oxford Agar) in a way to obtain well isolated single colonies.

Literature

VAN NETTEN, P., et al.: Liquid and solid selective differential media for the detection and enumeration of L. Int. **- J. Food Microbiol., 8 (4)**; 299-316 (1989).

LUND, A.M.: Comparison of Methods for Isolation of Listeria from Raw Milk. **– J. Food Protect., 54 (8)**; 602-606 (1991).

Ordering Information

Product	Merck Cat. No.	Pack size
L-PALCAM Listeria Selective Enrichment Broth Base acc. to VAN NETTEN et al.	1.10823.0500	500 g
PALCAM Listeria Selective Supplement acc. to VAN NETTEN et al.	1.12122.0001	1 x 16 vials

Quality control

Test strains	Growth	Color change to brown-black
Listeria monocytogenes NCTC 7973	good	+
Listeria monocytogenes ATCC 19113	good	+
Listeria monocytogenes ATCC 19114	good	+
Listeria monocytogenes ATCC 13932	good	+
Listeria ivanovii ATCC 19119	good	+
Enterococcus faecium ATCC 882	inhibited	-
Micrococcus luteus ATCC 9341	inhibited	-
Staphylococcus aureus ATCC 6538	inhibited	-

PALCAM Listeria Selective Agar Base acc. to VAN NETTEN et al.

Selective and differential medium acc. to VAN NETTEN et al. (1989) for the detection and isolation of Listeria monocytogenes from faeces, biological samples, foodstuffs and heavily contaminated material from the environment.

Mode of Action

PALCAM Agar provides a quantitative cultivation of Listeria monocytogenes, while, at the same time, inhibiting the Gram-negative and most of the Gram-positive accompanying bacteria. The selectivity of the medium results form its content of polymyxin, acriflavin, ceftazidime and lithium chloride. L.monocytogenes breaks down the esculin in the medium to glucose and esculetin. Esculetin forms an olive-green to black complex with iron(III) ions which stains the colonies of L.monocytogenes. Mannitol-positive accompanying bacteria such as staphylococci grow as yellow colonies, if they are not inhibited.

According to Hammer et al. (1990) PALCAM Agar is superior, with respect to selectivity, compared to other listeria media.

Typical Composition (g/litre)

Peptone 23.0; yeast extract 3.0; starch 1.0; sodium chloride 5.0; agar-agar 13.0 (= Columbia Agar); D(-)mannitol 10.0; ammonium iron(III) citrate 0.5; esculin 0.8; glucose 0.5; lithium chloride 15.0; phenol red 0.08.

Preparation

Suspend 35.9 g in 500 ml of demin. water, autoclave 15 min at 121 °C). Dissolve the contents of 1 vial of PALCAM Listeria Selective Supplement acc. to VAN NETTEN et al. in 1 ml sterile distilled water and add to the sterile medium cooled to 50 °C. If necessary rinse the vial with 1 ml of sterile distilled water. Mix well and pour plates.

pH: 7.2 ± 0.2 at 25 °C.

The prepared plates (incl. supplement) are clear and dark-red.

Experimental Procedure and Evaluation

Inoculate by spreading the sample on the surface of the medium and incubate at 35 °C for up to 48 hours preferably under microaerophilic conditions (using Anaerocult® C or Anaerocult® C mini).

L. monocytogenes grows as grey-green coloured colony with a black zone. If the colonies are very close together the whole medium is coloured black-brown.

PALCAM Listeria Selective Agar is highly selective. If, however, mannitol-positive enterococci or staphylococci do grow, they appear yellow with a yellow zone.

Further biochemical tests should be carried out. Suspicious colonies should be confirmed with biochemical or serological tests.

Literature

VAN NETTEN, P., PERALES, J., VAN DE MOOSDIJK, A., CURTIS, G.D.W., a. MOSSEL, D.A.A.: Liquid and solid selective differential media for the detection and enumeration of Listeria monocytogenes. **– Int. Food Microbiol., 8**; 299-316 (1989).

HAMMER, G., HAHN, G., KIRCHHOFF, H., a. HEESCHEN, W.: Vergleich der Eignung von Oxford- und PALCAM-Medium zur Isolierung von Listeria monocytogenes aus Weichkäse. **– Dtsch. Milchwirtschaft, 41**; 334-336 (1990).

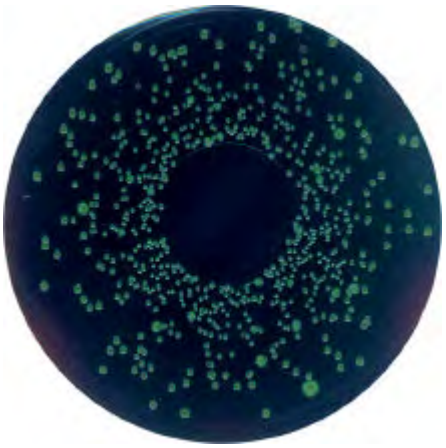
Ordering Information

Product	Merck Cat. No.	Pack size
PALCAM Listeria Selective Agar Base acc. to VAN NETTEN et al.	1.11755.0500	500 g
Anaeroclip	1.14226.0001	1 x 25
Anaerocult® C	1.16275.0001	1 x 10
Anaerocult® C mini	1.13682.0001	1 x 25
PALCAM Listeria Selective Supplement acc. to VAN NETTEN et al.	1.12122.0001	1 x 16 vials

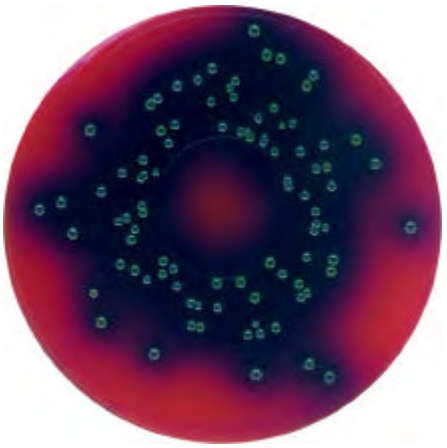
PALCAM Listeria Selective Agar Base acc. to VAN NETTEN et al.

Quality control

Test strains	Growth	Recovery rate %	Black zones
Listeria monocytogenes ATCC 19118	good / very good	≥ 30	+
Listeria monocytogenes NCTC 19113	good / very good	≥ 30	+
Listeria monocytogenes ATCC 13932	good / very good	≥ 30	+
Listeria monocytogenes NCTC 7973	good / very good	≥ 30	+
Listeria innocua ATCC 33090	good / very good	≥ 30	+
Staphylococcus aureus ATCC 25923	none	≤ 0.01	
Enterococcus faecalis ATCC 19433	none	≤ 0.01	
Erysipelothrix rhusiopathiae ATCC 19414	none	≤ 0.01	
Escherichia coli ATCC 25922	none	≤ 0.01	



Listeria innocua ATCC 33090



Listeria monocytogenes ATCC 19118

PALCAM Listeria Selective Supplement acc. to VAN NETTEN et. al.

Additive for the preparation of PALCAM Listeria Selective Agar acc. to VAN NETTEN et al. (Merck Cat. No. 1.11755.) and L-PALCAM-Listeria-Selective Enrichment Broth Base acc. to VAN NETTEN et al. (Merck Cat. No. 1.10823.).

Mode of Action

PALCAM Listeria Selective Supplement is a mixture of two antibiotics and a dye in lyophilized form. It largely inhibits the growth of accompanying bacteria in the selective cultivation of Listeria monocytogenes.

Composition (per vial)

Polymixin-B-sulfate 5.0 mg; ceftazidime 10.0 mg; acriflavine 2.5xmg.

Experimental Procedure

The lyophilisate is suspended in the original vial by adding about 1 ml of sterile distilled water. The vial contents is mixed evenly into 500 ml of sterile medium base cooled to about 45 to 50 °C.

Ordering Information

Product	Merck Cat. No.	Pack size
PALCAM Listeria Selective Supplement acc. to VAN NETTEN et al.	1.12122.0001	1 x 16 vials

Phenol-red Broth Base

Test culture medium used, together with various reactants, for the biochemical identification of microorganisms by means of fermentation tests.

Mode of Action

Fermentation of the added reactant by the inoculated culture causes phenol red to change its colour from red to yellow. When testing anaerobes, addition of a small quantity of agar stabilizes the anaerobiosis.

Typical Composition (g/litre)

Peptone from casein 5.0; peptone from meat 5.0; sodium chloride 5.0; phenol red 0.018.

Also to be added (g/litre):

reactant 5.0-10.0; if required, agar-agar 0.5-1.0.

Preparation

Suspend 15 g/litre, if requested, together with 0.5-1.0 g agar-agar/litre, dispense into test tubes, if necessary insert DURHAM tubes, autoclave (15 min at 121 °C). After cooling to about 60 °C add the desired reactants (final concentration 5.0-10.0 g/litre) as filter-sterilized solutions.

pH: 7.4 ± 0.2 at 25 °C.

The prepared broth is clear and red.

Experimental Procedure and Evaluation

Inoculate the tubes dropwise with pure cultures of the microorganisms to be identified. Tubes without reactant should also be inoculated to serve as growth controls.

In case of particularly fastidious microorganisms, it is advised to add a few drops of sterile, inactivated serum to each tube. Anaerobes should be tested under anaerobic conditions in a culture medium containing agar.

Incubation: up to 14 days at the optimal incubation temperature (usually 35 °C), in general 24 hours aerobically.

Check the tubes daily during incubation for gas formation in the DURHAM tubes and to see whether the colour has changed from red to yellow. If the phenol red is degraded, it may be freshly added dropwise as 5 % solution, when checking the tubes.

Ordering Information

Product	Merck Cat. No.	Pack size
Phenol-red Broth Base	1.10987.0500	500 g
Agar-agar purified	1.01614.1000	1 kg
Phenol red indicator	1.07241.0005	5 g

Merck reactants for fermentation tests

Product	Merck Cat. No.	Pack size
Adonitol	1.00846.0025	25 g
L(+)-Arabinose	1.01492.0100	100 g
Dulcitol	1.05990.0050	50 g
Esculin	1.00842.0005	5 g
D(-)-Fructose	1.05323.0250	250 g
D(+)-Galactose	1.04062.0050	50 g
D(+)-Glucose monohydrate	1.08342.1000	1 kg
Glycerol (about 87 %)	1.04094.0500	500 ml
Glycogen	1.04202.0001	1 g
Inulin	1.04733.0010	10 g
D(-)-Mannitol	1.07657.1000	1 kg
Maltose (monohydrate)	1.05910.0500	500 g
meso-Erythritol	1.03160.0025	25 g
myo-Inositol	1.04728.0100	100 g
Raffinose (pentahydrate)	1.07549.0100	100 g
L(+)-Rhamnose (monohydrate)	1.04736.0025	25 g
Salicin	1.07665.0025	25 g
D(-)-Sorbitol	1.07758.1000	1 kg
Starch	1.01252.0100	100 g
Sucrose	1.07651.1000	1 kg
Trehalose (dihydrate)	1.08353.0005	5 g
D(+)-Xylose	1.08689.0025	25 g

Quality control

Test strains	Growth*	Colour change to yellow	Gas formation
Staphylococcus aureus ATCC 25923	good / very good	+	-
Enterococcus faecalis ATCC 11700	good / very good	+	-
Klebsiella pneumoniae ATCC 13883	good / very good	+	+
Proteus vulgaris ATCC 13315	good / very good	+	+ (poor)
Shigella flexneri ATCC 12022	good / very good	± (orange)	-
Salmonella typhimurium ATCC 14028	good / very good	-	-

*in medium base with 1 % sucrose

Plate Count Agar (Casein-peptone Dextrose Yeast Agar)

Standard Methods Agar

This medium does not contain any inhibitors or indicators; it is mainly used to determine the total microbial content in milk, dairy products, water and other materials.

The composition of this medium complies with the Standard Methods for the Examination of Water and Wastewater (1998) and the Standard Methods for the Examination of Dairy Products (1985).

Typical Composition (g/litre)

Peptone from casein 5.0; yeast extract 2.5; D(+)glucose 1.0; agar-agar 14.0.

Preparation

Suspend 22.5 g/litre, autoclave (15 min at 121 °C). If desired, add 1.0 g skim milk powder/litre prior to sterilization.

pH: 7.0 ± 0.2 at 25 °C.

The plates are clear and yellowish.

Experimental Procedure and Evaluation

Depend on the purpose for which the medium is used.

Incubation: 48 h at 30 °C aerobically.

Literature

American Public Health Association: Standard Methods for the Examination of Dairy Products. 15th ed., 1985.

American Public Health Association, American Water Works Association and Water Pollution Control Federation: Standard Methods for the Examination of Water and Wastewater. 20th ed., Washington, 1998.

MARTLEY, F.G., JAYASHANKAR, S.R., a. LAWRENCE, R.C.: An improved agar medium for the detection of proteolytic organisms in total bacterial counts. **- J. Appl. Bact., 33**; 363-370 (1970).

Ordering Information

Product	Merck Cat. No.	Pack size
Plate Count Agar (Casein-peptone Dextrose Yeast Agar)	1.05463.0500	500 g
Plate Count Agar (Casein-peptone Dextrose Yeast Agar)	1.05463.5000	5 kg
Skim milk powder	1.15363.0500	500 g

Bacillus cereus ATCC 11778

Quality control (spiral plating method)

Test strains	Inoculum (cfu/ml)	Recovery rate
Staphylococcus aureus ATCC 6538	10^3-10^5	≥ 70 %
Streptococcus agalactiae ATCC 13813	10^3-10^5	≥ 70 %
Lactococcus lactis spp. lactis ATCC 19435	10^3-10^5	≥ 70 %
Listeria monocytogenes ATCC 19118	10^3-10^5	≥ 70 %
Lactobacillus acidophilus ATCC 4356	10^3-10^5	≥ 70 %
Bacillus cereus ATCC 11778	10^3-10^5	≥ 70 %
Escherichia coli ATCC 11775	10^3-10^5	≥ 70 %

F

Plate Count Skim Milk Agar

For determining the microbial count in milk and dairy products.

The culture medium complies with the recommendations of the International Dairy Federation (Internationaler Milchwirtschaftsverband) (1991) and the DIN Norm 10192 for the examination of milk and dairy products.

Mode of Action

Addition of skim milk to a culture medium with a superior nutrient base optimally adapts it to the neutral conditions experienced by microorganisms which grow in milk. More colonies grow and a wider range of bacteria can be cultured than in other culture media intended for the same purpose (TERPLAN et al. 1967).

Typical Composition (g/litre)

Peptone from casein 5.0; yeast extract 2.5; skim milk powder (no inhibitors) 1.0; glucose 1.0; agar-agar 10.5.

Preparation

Suspend 20.0 g/litre in cold water and allow to stand for about 15 minutes. Transfer flask to a cold water bath and heat gently, with frequent shaking, until completely dissolved, then autoclave (15 min at 121 °C).

pH: 7.0 ± 0.2 at 25 °C.

The plates are clear to opalescent and yellowish.

The reconstituted culture medium is more or less opalescent. According to DIN it can be stored for up to 3 months in the refrigerator, the temperature should not exceed 5 °C.

Experimental Procedure and Evaluation

Depend on the purpose for which the medium is used and the methods which are utilized.

Incubation: 24 h at 35 °C aerobically.

Literature

DIN Deutsches Institut für Normung e.V.: Mikrobiologische Milchuntersuchung; Bestimmung der Keimzahl (Referenzverfahren). **- DIN 10192**.

Internationaler Milchwirtschaftsverband: Milch u. Milchprodukte, Zählung von Mikroorganismen (Koloniezählung bei 30 °C) **- Internationaler Standard 100 (1991)**.

Internationaler Milchwirtschaftsverband: Flüssige Milch. Zählung von psychotrophen Mikroorganismen (Koloniezählung bei 6,5 °C). **-Internationaler Standard 101 (1991).**

TERPLAN, G., RUNDFELDT, H. u. ZAADHOF, K.-J.: Zur Eignung verschiedener Nährböden für die Bestimmung der Gesamtkeimzahl der Milch. **-Arch. Lebensmittelhyg., 18**; 9-11 (1967).

Ordering Information

Product	Merck Cat. No.	Pack size
Plate Count Skim Milk Agar	1.15338.0500	500 g
Plate Count Skim Milk Agar	1.15338.5000	5 kg

Pseudomonas aeruginosa ATCC 27853

Quality control

Test strains	Inoculum (cfu/ml)	Recovery rate %
Staphylococcus aureus ATCC 25923	10^3-10^5	≥ 70
Staphylococcus agalactiae ATCC 13813	10^3-10^5	≥ 70
Lactococcus lactis spp. lactis ATCC 19435	10^3-10^5	≥ 70
Listeria monocytogenes ATCC 19118	10^3-10^5	≥ 70
Bacillus cereus ATCC 11778	10^3-10^5	≥ 70
Escherichia coli ATCC 11775	10^3-10^5	≥ 70
Pseudomonas aeruginosa ATCC 27853	10^3-10^5	≥ 70
Candida albicans ATCC 10231	10^3-10^5	≥ 70

Potato Dextrose Agar

For the cultivation, isolation and enumeration of yeasts and moulds from foodstuffs and other materials.

This culture medium complies with the recommendations of the American Public Health Association for food (1992) and the USP (2003).

Mode of Action

Carbohydrate and potato infusion (BEEVER and BOLLARD 1970) promote the growth of yeasts and moulds while the low pH value partially inhibits the growth of the accompanying bacterial flora. If the medium is to be used for fungal counts, the pH should be adjusted to approximately 3.5. Fungi grow on this medium to develop typical morphology.

Typical Composition (g/litre)

Potato infusion 4.0 (infusion from 200 g potatoes); D(+)glucose 20.0; agar-agar 15.0.

Preparation

Suspend 39 g/litre, autoclave (15 min at 121 °C).

pH: 5.6 ± 0.2 at 25 °C.

If the pH has to be adjusted to 3.5, add approx. 14 ml of a sterile 10 % tartaric acid solution/litre at a temperature of 45-50 °C.

The plates are clear and yellowish-brown.

■ **After the tataric acid is added, do not reliquefy.**

Experimental Procedure and Evaluation

Inoculate by the pour-plate method or by spreading the sample on the surface of the culture medium.

Incubation: up to 5 days at 28 °C aerobically.

Experimental procedure depends on the purpose for which the medium is used.

Literature

BEEVER, R.E., a. BOLLARD, E.G.: The nature of the stimulation of fungal growth by potato extract. **– J. Gen. Microbiol., 60; 273-279 (1970)**.

American Public Health Association: Compendium of methods for the microbiological examination of foods. 3rd ed., 1992.

United States Pharmacopeia XXVI, Chapter "Microbial Limit Tests", 1995.

Ordering Information

Product	Merck Cat. No.	Pack size
Potato Dextrose Agar	1.10130.0500	500 g
L(+)-Tartaric acid	1.00804.0250	250 g

Aspergillus niger
ATCC 16404

Saccharomyces cerevisiae
ATCC 9080

Potato Dextrose Agar

Quality control

Test strains	Growth
Geotrichum candidum DSMZ 1240	good / very good
Aspergillus niger ATCC 16404	good / very good
Penicillium commune ATCC 10428	medium / good
Trichophyton ajelloi ATCC 28454	medium / good

Quality control (spiral plating method)

Test strains	Inoculum (cfu/ml)	Recovery rate %
Candida albicans ATCC 10231	10^3-10^5	≥ 70
Saccharomyces cerevisiae ATCC 9763	10^3-10^5	≥ 70
Saccharomyces cerevisiae ATCC 9080	10^3-10^5	≥ 70
Rhodotorula mucilaginosa DSMZ 70403	10^3-10^5	≥ 70

A
WW

Presence – Absence Broth

Selective culture medium for the detection of coliform bacteria in water.

The culture medium conforms with the recommendations of standard methods (US-EPA) for the examination of water.

Mode of Action

Peptones and meat extract give the nutrients and trace elements required for growth whereas the phosphate buffer and sodium chloride provide a good buffering capacity and the osmotic equilibrium.

Lactose-fermenting organisms form acid which is identified by the pH indicator bromocresol purple as a colour change from purple to yellow.

The selective component of the culture medium is sodium lauryl sulphate which largely inhibits the undesired accompanying flora - with the exception of the coliforms.

Typical Composition (g/litre)

(single strength)

Meat extract 3.0; peptones 5.0; lactose 7.46; tryptose 9.83; dipotassium hydrogenphosphate 1.35; potassium dihydrogenphosphate 1.35; sodium chloride 2.46; sodium lauryl sulphate 0.05; bromocresol purple 0.0085.

Preparation

For the preparation of the triple strength concentrated broth, completely dissolve 91.5 g in 1 litre of demin water. Fill 50 ml quantities in 250 ml milk dilution bottles with screw caps and autoclave for 12 min. at 121 °C. Allow broth to cool to room temperature.

The pH of the single-strength broth: 6.8 ± 0.2 at 25 °C.

The prepared medium is clear to slightly opalescent and purple-red.

Experimental Procedure

Add 100 ml water samples to the milk dilution bottles filled with 50 ml of triple strength concentrated broth, mix well. Aerobic incubation with a loose screw-cap for up to 48 hours at 35± 0.5°C. Read results after 24 h and 48 h.

Lactose-positive organisms form acid due to the fermentation of the lactose which colours the broth yellow. Gas formation can occur. To identify gas formation, the bottles are shaken gently and inspected to see if a foaming reaction occurs.

All samples with acid and/or acid and gas formation are presumptive-positive and are inoculated for confirmation in Brilliant-green Bile Lactose Broth (BRILA).

If gas formation occurs during the incubation of 48 ± 3 hours at 35 ± 0.5 °C, this can be seen as confirmation of the presence of coliforms in the 100 ml water sample.

Literature

Federal Register. 1989. National primary drinking water regulations; total coliforms (including fecal coliforms and e. coli). Fed. Regist. **54**; 27544-27568.

Weiss J.E. and **Hunter C.A.** 1939 J. Am. Water Works Ass. **31**:707 – 713.

Eaton, A. D., Clesceri L. S. and **Greenberg A. E.** (ed.). 1995. Standard methods for the examination of water and wastewater, 19th Ed. Am. Public Health Ass. Washington D.C.

Clark, J. A. 1968. A presence absence (P-A) test, providing sensitive and inexpensive detection of coliforms and faecal streptococci in municipal drinking water supplies. Can. J. Microb. 14: 13-18.

Clark, J. A. 1969 The detection of various bacteria indicative of water pollution by a presence-absence (P-A) procedure. Can. J. Microbiol. 15: 771-780.

Clark, J. A. and **Flassov L. T.** 1973 Relationships among pollution indicator bacteria isolated from raw water and distribution systems by the presence-absence (P-A) test. Health. Lab. Sci. **10**:163–172.

Clark, J. A. and **Pagel J. E.** 1977 Pollution indicator bacteria associated with municipal raw and drinking water supplies. Can. J. Micribiol. **23**: 465-470.

Clark, J. A., Burger C. A. and **Sabatinos L. E.** 1982 Characterization of indicator bacteria in municipal raw water, drinking water and new main water samples. Can. J. Microbiol. **28**: 1002-1013.

Jacobs, Leigler, Reed, Stukel and **Rice**. 1986 Appl. Environ. Microbiol. **51**: 1007

Ordering Information

Product	Merck Cat. No.	Pack size
Presence - Absence Broth	1.00414.0500	500 g

Uninoculated | Escherichia coli

Presence – Absence Broth

Quality control

Test strains	Growth	Yellow colour	Gas formation in BRILA Broth
Escherichia coli ATCC 25922	Good	Yellow	+
Escherichia coli ATCC 11755	Good	Yellow	+
Enterococcus faecalis ATCC 29212	Medium	Weakly yellow / yellow	-
Pseudomonas aeruginosa ATCC 27853	Poor / medium	None	-

Pseudomonas Agar F, Base

Elective culture media proposed by KING, WARD and RANEY (1954) for the isolation and differentiation of Pseudomonas based on the formation of pyocyanin and/or pyorubin or fluorescein.

This media comply with the recommendations of the United States Pharmacopeia XXVI (2003) and correspond to the culture media specified in the DIN Norm 38411 (examination of water).

Mode of Action

Pseudomonas Agar P favours the formation of pyocyanin and/or pyorubin and reduces that of fluorescein, whereas Pseudomonas Agar F stimulates the production of fluorescein and reduces that of pyocyanin and/or pyorubin. Simultaneous use of both culture media allows rapid, preliminary identification of most Pseudomonas species, as some strains can only synthesize pyocyanin, some form only fluorescein and others produce both pigments.

Typical Composition (g/litre)

Peptone from casein 10.0; peptone from meat 10.0; magnesium sulfate 1.5; di-potassium hydrogen phosphate 1.5; agar-agar 12.0.

Also to be added:

glycerol 10.0 ml.

Preparation

Suspend 10.0 ml glycerol/litre together with 35 g Pseudomonas Agar F Base/litre, dispense into test tubes if desired, autoclave (15 min at 121 °C). Make slant tubes or pour plates.

pH: 7.2 ± 0.2 at 25 °C.

The plates are clear to opalescent and yellowish-brown.

Experimental Procedure and Evaluation

Inoculate the surface of the culture medium with cultures suspected to contain Pseudomonas so that individual colonies develop.

Incubation: up to 7 days at 35 °C.

Check for bacterial growth after 24, 48 and 72 hours and then after 6 days.

Pseudomonas aeruginosa appears on Pseudomonas Agar F as colonies surrounded by a yellow to greenish-yellow zone resulting from fluorescein production. If pyocyanin is also synthesized, a bright green colour is produced which fluoresces under UV light.

According to BLAZEVIC et al. (1973), atypical pyocyanin-negative, fluorescein-positive Ps. aeruginosa strains can be differentiated from Ps. fluorescens and Ps. putida. BRODSKY and NIXON (1973) reported that the fluorescence of Ps. aeruginosa colonies in ultra-violet light following growth on MacCONKEY agar can be exploited to provide a rapid orientation test, Ps. fluoresce and Ps. putida do not fluorescens and show only scanty growth.

Literature

BLAZEVIC, D.J., KOEPCKE, M.H., a. MATSEN, J.M.: Incidence and identification of Pseudomonas fluorescens and Pseudomonas putida in the clinical laboratory. **– Appl. Microbiol., 25**; 107-110 (1973).

BRODSKY, M.H., a. NIXON, M.C.: Rapid method for detection of Pseudomonas aeruginosa on McCONKEY-Agar under ultraviolet light. **-Appl. Microbiol., 26**; 219-220 (1973).

DIN Deutsches Institut für Normung e.V.: Deutsche Einheitsverfahren zur Wasser-, Abwasser und Schlammuntersuchung. Mikrobiologisches Verfahren (Gruppe K). Nachweis von Pseudomonas aeruginosa (K 8). **– DIN 38411**.

GEORGIA, F.R., a. POE, C.F.: Study of bacterial fluorescence in various media. I. Inorganic substances necessary for bacterial fluorescence. **-J.Bact., 22**; 349 (1931).

GEORGIA, F.R., a. POE, C.F.: Study of bacterial fluorescence in various media. II. The production of fluorescence in media made from peptones. **-J.Bact., 23**; 135 (1932).

KING, E.O., WARD, M.K., a. RANEY, D.E.: Two simple media for the demonstration of pyocyanin and fluorescin. **– J. Lab. Clin. Med., 44**; 401-307 (1954). United States Pharmacopeia XXVI, Chapter "Microbial Limit Tests", 1995.

Ordering Information

Product	Merck Cat. No.	Pack size
Pseudomonas Agar F, Base	1.10989.0500	500 g
Glycerol	1.04091.0500	500 ml
UV Lamp (366 nm)	1.13203.0001	1 ea

Pseudomonas Agar F, Base

Quality control

Test strains	Growth	Yellow-green pigment in daylights	Fluorescence at 366 nm
Pseudomonas aeruginosa ATCC 27853	good / very good	+	+
Pseudomonas aeruginosa ATCC 9027	good / very good	+	+
Pseudomonas fluorescens ATCC 17397	good / very good (48 h)	±	±
Aeromonas hydrophila ATCC 7966	good / very good	-	-
Escherichia coli ATCC 25922	good / very good	-	-
Enterobacter cloacae ATCC 13047	good / very good	-	-

Aeromonas hydrophila ATCC 7966

Pseudomonas aeruginosa ATCC 27853

Pseudomonas Agar P, Base

Elective culture media proposed by KING, WARD and RANEY (1954) for the isolation and differentiation of Pseudomonas based on the formation of pyocyanin and/or pyorubin or fluorescein.

This media comply with the recommendations of the United States Pharmacopeia XXVI (2003) and correspond to the culture media specified in the DIN Norm 38411 (examination of water).

Mode of Action

Pseudomonas Agar P favours the formation of pyocyanin and/or pyorubin and reduces that of fluorescein, whereas Pseudomonas Agar F stimulates the production of fluorescein and reduces that of pyocyanin and/or pyorubin. Simultaneous use of both culture media allows rapid, preliminary identification of most Pseudomonas species, as some strains can only synthesize pyocyanin, some form only fluorescein and others produce both pigments.

Typical Composition (g/litre)

Peptone 20.0; magnesium chloride 1.4; potassium sulfate 10.0; agar-agar 12.6.

Also to be added:

glycerol 10.0 ml.

Preparation

Suspend 10.0 ml glycerol/litre together with 44 g Pseudomonas Agar P Base/litre, dispense into test tubes if desired, autoclave (15 min at 121 °C). Make slant tubes or pour plates.

pH: 7.2 ± 0.2 at 25 °C.

The plates are yellowish-brown (1.10989.).

Experimental Procedure and Evaluation

Inoculate the surface of the culture medium with cultures suspected to contain Pseudomonas so that individual colonies develop.

Incubation: up to 7 days at 35 °C.

Check for bacterial growth after 24, 48 and 72 hours and then after 6 days.

Pseudomonas aeruginosa can grow on Pseudomonas Agar P to form colonies surrounded by a blue to green zone due to pyocyanin formation or with a red to dark brown zone due to pyorubin production. The coloured pigments can be extracted with chloroform.

According to BLAZEVIC et al. (1973), atypical pyocyanin-negative, fluorescein-positive Ps. aeruginosa strains can be differentiated from Ps. fluorescens and Ps. putida. BRODSKY and NIXON (1973) reported that the fluorescence of Ps. aeruginosa colonies in ultra-violet light following growth on MacCONKEY agar can be exploited to provide a rapid orientation test, Ps. fluoresce and Ps. putida do not fluorescens and show only scanty growth.

Literature

BLAZEVIC, D.J., KOEPCKE, M.H., a. MATSEN, J.M.: Incidence and identification of Pseudomonas fluorescens and Pseudomonas putida in the clinical laboratory. **– Appl. Microbiol., 25**; 107-110 (1973).

BRODSKY, M.H., a. NIXON, M.C.: Rapid method for detection of Pseudomonas aeruginosa on McCONKEY-Agar under ultraviolet light. **-Appl. Microbiol., 26**; 219-220 (1973).

DIN Deutsches Institut für Normung e.V.: Deutsche Einheitsverfahren zur Wasser-, Abwasser und Schlammuntersuchung. Mikrobiologisches Verfahren (Gruppe K). Nachweis von Pseudomonas aeruginosa (K 8). **– DIN 38411**.

GEORGIA, F.R., a. POE, C.F.: Study of bacterial fluorescence in various media. I. Inorganic substances necessary for bacterial fluorescence. **-J. Bact., 22**; 349 (1931).

GEORGIA, F.R., a. POE, C.F.: Study of bacterial fluorescence in various media. II. The production of fluorescence in media made from peptones. **-J.Bact., 23**; 135 (1932).

KING, E.O., WARD, M.K., a. RANEY, D.E.: Two simple media for the demonstration of pyocyanin and fluorescin. **– J. Lab. Clin. Med., 44**; 401-307 (1954). United States Pharmacopeia XXVI, Chapter "Microbial Limit Tests", 1995.

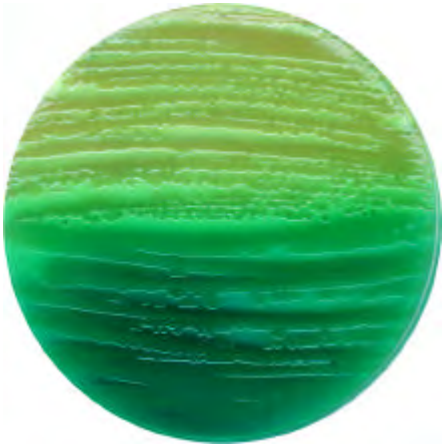
Ordering Information

Product	Merck Cat. No.	Pack size
Pseudomonas Agar P, Base	1.10988.0500	500 g
Glycerol	1.04091.0500	500 ml
UV Lamp (366 nm)	1.13203.0001	1 ea

Pseudomonas Agar P, Base

Quality control

Test strains	Growth	Blue-green pigment in day-light
Pseudomonas aeruginosa ATCC 27853	good / very good	(+)
Pseudomonas aeruginosa ATCC 9027	good / very good	+
Pseudomonas aeruginosa ATCC 25668	good / very good	+
Pseudomonas fluorescens ATCC 13535	good / very good	- (yellowish)
Aeromonas hydrophila ATCC 7966	good / very good	-
Escherichia coli ATCC 25922	good / very good	-
Enterobacter cloacae ATCC 13047	good / very good	-



Pseudomonas aeruginosa ATCC 27853 und Pseudomonas aeruginosa ATCC 8027

Pseudomonas fluorescens ATCC 13535 und Pseudomonas aeruginosa ATCC 25668

Pseudomonas Selective Agar Base

Medium for the detection and enumeration of *Pseudomonas*.

Pseudomonas CFC Selective Agar

When supplemented with Pseudomonas CFC Selective Supplement (Cat.No.1.07627.) the medium complies with the recommendations of ISO 13720 for the detection and enumeration of *Pseudomonas* spp. in foods and animal feed.

Pseudomonas CN Selective Agar

When supplemented with Pseudomonas CN Selective Supplement (Cat.No. 1.07624) the medium complies with the recommendations of DIN/EN 12780 for the detection and enumeration of *Pseudomonas aeruginosa* in water using the membrane filtration technique.

Mode of Action

The peptone mixture in Pseudomonas Selective Agar Base allows growth of a broad spectrum of Pseudomonades. The amount of potassium sulfate and magnesium chloride supports forming of pigments.

By use of the appropriate selective supplement and the incubation temperature the medium becomes selective for *Pseudomonas* spp. including *Burkholderia cepacia*, formerly known as *Pseudomonas cepacia* (CFC Agar), or *Pseudomonas aeruginosa* (CN Agar).

Typical Composition (g/Liter)

Peptone from gelatine 16.0; Casein hydrolysate 10.0; potassium sulfate 10.0; magnesium chloride 1.4; agar-agar 11.0.

Preparation

Suspend 24.2 g in 500 ml of purified water, add 5 ml glycerol and heat to boiling until dissolved completely.

Autoclave for 15 min. at 121°C.

Cool the medium to 45- 50°C and aseptically add the contents of one vial of Pseudomonas CFC Selective Supplement (Cat.No.1.07627) or Pseudomonas CN Selective Supplement (Cat.No.1.07624). Mix thoroughly and pour plates.

pH: 7.1 ± 0.2 at 25 °C.

The prepared plates are clear and colorless and can be stored for up to 4 weeks at 2 - 8°C in the refrigerator.

Protect from light and drying.

Do not keep the liquid medium (45 - 50°C) longer than 4 hours.

Do not remelt the medium several times.

Experimental Procedure and Evaluation

Pseudomonas CFC Selective Agar

Inoculate the medium using the surface spread method.

Incubation: 44 ± 4 hours at 25 ± 1°C.

All grown colonies are suspect Pseudomonas spp. and counted as such.

The suspect colonies must be confirmed. Colonies which show a positive oxidase reaction but no glucose fermentation are confirmed *Pseudomonas* spp. colonies.

Pseudomonas CN Selective Agar

Inoculate the medium using the membrane filtration technique.

The filter material impacts results. Good results were achieved using Cellulose-Mixed Ester membranes (e.g. Pall GN-6).

Incubation: 44 ± 4 hours at 36 ± 2°C.

Check the membrane filters for growth after 22 ± 2 h and 44 ± 4 h.

All grown colonies with a blue-green pigmentation are considered confirmed *Pseudomonas aeruginosa* colonies and counted as such.

Check the membrane filters under UV-light. All colonies not showing the blue-green pigmentation but fluoresce are suspect *P. aeruginosa* colonies and confirmed by use of acetamide solution.

All other reddish-brown pigmenting colonies, which do not fluoresce are considered suspect *P. aeruginosa* colonies and confirmed by the oxidase test, acetamide solution and King's B Medium.

Literature

Goto, S., and S. Enomoto. 1970. Nalidixic Acid Cetrimide Agar. A New Selective Plating Medium for the Selective Isolation of *Pseudomonas aeruginosa*. Japan. J. Microbiol. **14**: 65 - 72.

Mead, G.C., and B.W. Adams. 1977. A selective medium for the rapid isolation of Pseudomonas associated with poultry meat spoilage. Br. Poult. Sci. **18**: 661 - 670.

ISO INTERNATIONAL STANDARDISATION ORGANISATION. Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of *Pseudomonas* spp. **ISO/WD 13720:2000**.

EN EUROPEAN STANDARD. Water Quality - Detection and enumeration of Pseudomonas aeruginosa by membrane filtration. **DIN/EN 12780:2002**.

Ordering Information

Product	Merck Cat. No.	Pack size
Pseudomonas Selective Agar Base	1.07620.0500	500 g
Pseudomonas CFC Selective Supplement	1.07627.0001	1 x 16 vials
Pseudomonas CN Selective Supplement	1.07624.0001	1 x 16 vials

Pseudomonas Selective Agar Base

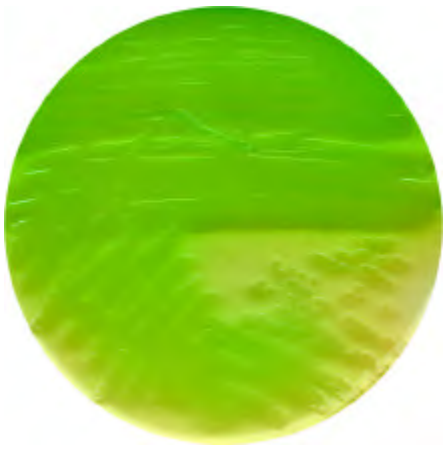
Quality Control

Pseudomonas CFC Selective Agar 44 h ± 4 h at 25 ± 1°C

Test strains	Recovery rate
Pseudomonas aeruginosa ATCC 27853	> 70%
Pseudomonas putida ATCC 12633	> 70%
Pseudomonas fluorescens ATCC 13525	> 70%
Pseudomonas fragi ATCC 27362	> 70%
Burkholderia cepacia ATCC 17759	> 70%
Proteus mirabilis ATCC 14153	< 0.01%
Staphylococcus aureus ATCC 25923	< 0.01%

Pseudomonas CN Selective Agar 44 h ± 4 h at 36 ± 2°C

Test strains	Recovery rate
Pseudomonas aeruginosa ATCC 27853	> 70%
Pseudomonas fluorescens ATCC 13525	< 0.01%
Aeromonas hydrophila ATCC 7966	< 0.01%
Klebsiella pneumoniae ATCC 13883	< 0.01%
Proteus mirabilis ATCC 14153	< 0.01%
Providencia rustigianii ATCC 13159	< 0.01%



Pseudomonas aeruginosa ATCC 27853 C-N-Supplement

Pseudomonas aeruginosa ATCC 27853 C-F-C-Supplement

Pseudomonas CN Selective Supplement

Additive for the preparation of Pseudomonas CN Selective Agar for the detection and enumeration of *Pseudomonas aeruginosa* in water by the membrane filter technique.

Mode of Action

The selective supplement is a mixture of 2 different inhibitors in lyophilized form.

Cetrimide and Nalidixic acid inhibit the Gram-positive and Gram-negative accompanying flora.

Typical Composition (per vial)

Cetrimide 100 mg ; Nalidixic acid 7.5 mg

Preparation

Aseptically add 2 ml of a 50/50 mixture of purified water and ethanol to the contents of one vial and mix gently to avoid foaming.

Aseptically add the contents of one vial to 500 ml of Pseudomonas Selective Agar Base (with 5 ml glycerol) cooled to 45- 50°C. Mix to suspend evenly.

Ordering Information

Product	Merck Cat. No.	Pack size
Pseudomonas CN Selective Supplement	1.07624.0001	1 x 16 vials
Pseudomonas Selective Agar Base	1.07620.0500	500 g

Pseudomonas aeruginosa ATCC 27853

Pseudomonas CFC Selective Supplement

Additive for the preparation of Pseudomonas CFC Selective Agar for the detection and enumeration of *Pseudomonas* spp. from food and animal feed.

Mode of Action

The selective supplement is a mixture of 3 different inhibitors in lyophilized form.

Cetrimide, Fucidin and Cephalotin inhibit the Gram-positive and Gram-negative accompanying flora.

Typical Composition (per vial)

Cetrimide 5 mg ; Fucidin 5 mg; Cephalotin 25 mg

Preparation

Aseptically add 2 ml of a 50/50 mixture of purified water and ethanol to the contents of one vial and mix gently to avoid foaming.

Aseptically add the contents of one vial to 500 ml of Pseudomonas Selective Agar Base (with 5 ml glycerol) cooled to 45- 50°C. Mix to suspend evenly.

Ordering Information

Product	Merck Cat. No.	Pack size
Pseudomonas CFC Selective Supplement	1.07627.0001	1 x 16 vials
Pseudomonas Selective Agar Base	1.07620.0500	500 g

Pseudomonas aeruginosa ATCC 27853

Pseudomonas fluorescens ATCC 13525

Pseudomonas putida ATCC 12633

Pseudomonas Selective Agar, Base (Cetrimide Agar)

CETRIMIDE Agar

A modification of the medium proposed by BROWN and LOWBURY (1965) for the isolation and differentiation of Pseudomonas aeruginosa from various materials.

This culture medium complies with the recommendations of the United States Pharmacopeia XXVI (2003) and the European Pharmacopeia II and is equivalent to the medium specified in the DIN Norm 38411.

Mode of Action

The use of cetrimide (cetyltrimethylammonium bromide) was recommended by LOWBURY (1951) and other authors; this compound largely inhibits the growth of the accompanying microbial flora. According to LOWBURRY and COLLINS (1955), a concentration of 0.3 g/litre inhibits the accompanying organisms satisfactorily and minimizes interference with the growth of Ps. aeruginosa. The pigment production of Ps. aeruginosa is not inhibited when grown on this medium.

GOTO and ENOMOTO (1970) recommend the addition of 15 µg nalidixic acid/ml to improve the inhibition of the accompanying microbial flora.

Typical Composition (g/litre)

Peptone from gelatin 20.0; magnesium chloride 1.4; potassium sulfate 10.0; N-cetyl-N,N,N-trimethylammoniumbromide (cetrimide) 0.3; agar-agar 13.0.

Also be added:

Glycerol 10 ml.

Preparation

Suspend 44.5 g/litre, add 10 ml glycerol/litre, autoclave (15 min at 121 °C). Pour plates.

pH: 7.0 ± 0.2 at 25 °C.

The plates are turbid and light brown.

Experimental Procedure and Evaluation

Inoculate by spreading the sample on the surface of the plates.

Incubation: up to 48 hours at 35 °C aerobically.

Ps. aeruginosa colonies produce a yellow-green pigment (pyocyanin) and fluoresce under UV light. Further tests should be performed for further identification (HUGH and LEIFSON 1953, KOVACS 1956, THORNLEY 1960, BÜHLMANN et al. 1961 etc).

Literature

DIN Deutsches Institut für Normung e.V.: Deutsche Einheitsverfahren zur Wasser-, Abwasser- und Schlammuntersuchung. Mikrobiologische Verfahren. Nachweis von Pseudomonas aeruginosa. **– DIN 38411**.

BROWN, V.I., a. LOWBURY, E.J.L.: Use of improved cetrimide agar medium and other culture methods for Pseudomonas aeruginosa. **– J. Clin. Pathol., 18**; 752-756 (1965).

BUHLMANN, X., FISCHER, W.A., a. BRUHN, J.: Identification of a pyocyanogenic strains of Pseudomonas aeruginosa. **– J. Bact., 82**, 787-788 (1961).

European Pharmacopeia II, Chapter VIII, 10.

GOTO, S., a. ENOMOTO, S.: Nalidixic acid cetrimide agar. A new selective plating medium for the selective isolation of Pseudomonas aeruginosa. **-Japan J. Microbiol., 14**; 65-72 (1970).

HUGH, R., a. LEIFSON, E.: The taxonomic significance of fermentative versus oxidative metabolism of carbohydrates by various gramnegative bacteria. **-J. Bact., 66**; 24-26 (1953).

KOVACS, N.: Identification of Pseudomonas pyocyanea by the oxidase reaction. **– Nature (Lond.), 178**; 703 (1956).

LOWBURY, E.J.L.: Improved culture methods for the detection of Ps. pyocyanea. **– J. Clin. Pathol., 4**; 66-72 (1951).

LOWBURY, E.J.L., a. COLLINS, A.G.: The use of a new cetrimide product in a selective medium for Pseudomonas pyocyanea. **– J. Clin. Pathol., 8** ; 47-48 (1955).

THORNLEY, M.J.: The differentiation of Pseudomonas from other gramnegative bacteria on the basis of arginine metabolism. **– J. Appl. Bact., 23**; 37-52 (1960).

United States Pharmacopeia XXVI, Chapter "Microbial Limit Tests", 1995.

Ordering Information

Product	Merck Cat. No.	Pack size
Pseudomonas Selective Agar, Base (Cetrimide Agar)	1.05284.0500	500 g
Glycerol (about 87 %)	1.04094.0500	500 ml
UV Lamp (366nm)	1.13203.0001	1 ea

Pseudomonas aeruginosa ATCC 9027

Pseudomonas Selective Agar, Base (Cetrimide Agar)

CETRIMIDE Agar

Quality control (spiral plating method)

Test strains	Inoculum (cfu/ml)	Recovery rate %	Yellow-green pigment
Pseudomonas aeruginosa ATCC 9027	10^3-10^5	≥ 30	+
Pseudomonas aeruginosa ATCC 25668	10^3-10^5	≥ 30	+
Pseudomonas aeruginosa ATCC 27853	10^3-10^5	≥ 30	+
Escherichia coli ATCC 8739	> 10^5	≤ 0.01	-
Proteus mirabilis ATCC 29906	> 10^5	≤ 0.01	-
Salmonella typhimurium ATCC 14028	> 10^5	≤ 0.01	-
Staphylococcus aureus ATCC 6538	> 10^5	≤ 0.01	-

A

RAMBACH® Agar

Differential-diagnostic culture medium for identifying non-typhi Salmonella in foodstuffs and clinical samples.

IVD *in vitro diagnosticum –*
For professional use only

CE

See also General Instruction of Use
Warnings and precautions see ChemDAT®
(www.chemdat.info)

Principle

Microbiological method

Mode of Action

The nutritive substrates in the RAMBACH® Agar enable Enterobacteriaceae to multiply readily. Sodium desoxycholate inhibits the accompanying Gram-positive flora. RAMBACH® Agar enables species of Salmonella to be differentiated unambiguously from other bacteria by means of a new procedure, for which a patent application has been submitted. This is made possible by adding propylene glycol to the culture medium. Salmonellae form acid with propylene glycol, so that, in combination with a pH indicator, the colonies have a characteristic red colour. In order to differentiate coliforms from Salmonellae, the medium contains a chromogene indicating the presence of β-galactosidase splitting, a characteristic for coliforms. Coliform microorganisms grow as blue-green or blue-violet colonies. Other Enterobacteriaceae and Gram-negative bacteria, such as Proteus, Pseudomonas, Shigella, S. typhi and S. parathyphi A grow as colourless-yellow colonies.

Typical Composition (g/litre)

Peptone 8.0; sodium chloride 5.0; sodium deoxycholate 1.0; chromogenic mix 1.5; proplylene glycol 10.5; agar-agar 15.0.

Preparation and Storage

Usable up to the expiry date when stored dry and tightly closed at +15 to +25° C. Protect from light.

After first opening of the bottle the content can be used up to the expiry date when stored dry and tightly closed at +15 to +25° C.

1. Add 1 vial of liquid-mix to 250, 1000 or 50.000 ml distilled water and mix by swirling until completely dissolved (the water quantity is dependent on the respective pack-size).
2. Add 1 vial of nutrient-powder and mix by swirling until completely suspended.
3. Heat in a boiling water-batch or in a current of steam, while carefully shaking from time to time. The medium is totally suspended, if no visual particles stick to the glass-wall.

   The medium should not be heat-treated further!

   Standard time for complete dissolution (shaking in 5-minute sequence):
   250 ml: 20-25 minutes
   1000 ml: 35-40 minutes.

   Do not autoclave, do not overheat!
4. Cool the medium as fast as possible in a water-bath (45-50 °C). During this procedure (max. 30 minutes) gently shake the medium from the time to time. Pour into plates.
5. In order to prevent any precipitate or clotting of the chromogenic-mix in the plates, we advice to place Petrid-ishes – during pouring procedure – on a cool (max. 25 °C) surface.
6. The ready-plates are opaque and pink. Before inoculation, the plates should be dry. pH: 7.3 ± 0.2 at 25 °C.
7. Shelf-life and storage conditions of fresh prepared plates: room-temperature: 12 hours.in the fridge (not below 6 °C) unsealed. 3 weeksin the fridge (not below 6 °C) sealed in plastic-pouch or with tape: 3 months.

Specimen

e.g. Stool.

Clinical specimen collection, handling and processing, see general instructions of use.

Experimental Procedure and Evaluation

Specimen enriched in appropriate Salmonella Selective Broth. Afterwards inoculate ¼ of the agar surface. In order to achieve individual colonies, the inoculation shall be continued with the same loop over the rest of the plate

Incubation: Aerobically at 35-37 °C for 24-28 hours.

Literature

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BARTOLOME, R.M., et al.: Nuevo media de cultivo para el aislamiento de salmonella sp. V Congreso de la Sociedad espanola de Immunologia clinica, Barcelona; November 1992.

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OSSMER, R.: Salmosyst and Rambach-Agar. A Rapid Alternative for the Detection of Salmonella. Congress-Poster – Salmonella and Salmonellosis – Ploufragen/Sant-Brieux – France, September 1992.

BENETT, A.R., et al.: Evaluation of Rambach Agar and Salmosyst Enrichment for the isolation of Salmonella from foods. – Congress-Poster – RAMI, London; September 1993.

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RAMBACH® Agar

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DIEHL, T., et al.: Salmonella enterica: Aktuelles aus der bakteriologischen Diagnostik. **– Tierärztl. Umschau, 48**; 703-706 (1993).

GIACCONE, V. et al.: Confronto de terreni selettivi per la ricerca di Salmonella spp. in prodotti carnei.**– Ingegneria Alimentare, 3/93**; p. 31-35.

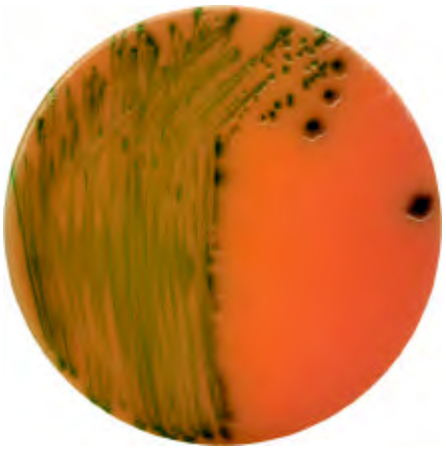
MALASPINA, P.M., et al.: Impiego di un nuovo terreno solido selettivo per la ricerca di Salmonella spp. in prodotti lattiero-caseari. **– Il latte, 7/93**; p. 770-775.

Ordering Information

Product	Merck Cat. No.	Pack size
Rambach® Agar	1.07500.0001	4 x 250 ml
Rambach® Agar	1.07500.0002	4 x 1000 ml
Rambach® Agar	1.07500.0003	1 x 50 l
Merckoplate® RAMBACH® agar	1.13999.0001	1 x 20 plates
Merckoplate® RAMBACH® agar	1.15999.0001	1 x 480 plates

Quality control

Test strains	Growth
Salmonella enteritidis ATCC 13076	red
Salmonella typhimurium ATCC 14028	red
Escherichia coli ATCC 25922	blue-green
Klebsiella pneumoniae ATCC 13883	blue-green
Shigella flexneri ATCC 29903	yellowish
Proteus mirabilis ATCC 14153	yellowish
Staphylococcus aureus ATCC 25923	inhibited
Bacillus cereus ATCC 11778	inhibited



Escherichia coli ATCC 25922

Salmonella enteritidis ATCC 13076

MPF
A
WW

R2A Agar

Nutrient medium for the determination of the heterotrophic total bacterial count in drinking water.

R2A Agar is a medium with a low nutrient content, which, in combination with a low incubation temperature and an extended incubation time, is specially suitable for the recovery of stressed and chlorine-tolerant bacteria from drinking water.

The nutrient medium conforms with recommendations of the standard methods (US-EPA) for the examination of water.

Mode of Action

The low concentration of yeast extract, casein hydrolisate, peptone and glucose allows a wide spectrum of bacteria to grow without the fast-growing bacteria suppressing the slow-growing species, such as would be the case on richly nutritious media like e.g. Plate Count Agar.

The content of starch and pyruvate allows particularly the injured bacteria to grow again more quickly.

Typical Composition (g/litre)

Yeast extract 0.5; proteose peptone 0.5; casein hydrolysate 0.5; glucose 0.5; soluble starch 0.5; sodium pyruvate 0.3; dipotassium hydrogenphosphate 0.3; magnesium sulphate 0.05; agar-agar 12.0.

Preparation

Suspend 15.2 g in 1 litre demin. water and heat in a boiling water bath or flowing steam until the medium has completely dissolved. Autoclave for 15 min. at 121°C, cool to 45–50°C and pour into sterile Petridishes.

pH : 7.2 ± 0.2 at 25°C

The prepared medium is clear to slightly opalescent and colourless.

In correct storage conditions (+2 – +8°C, protected from light and dehydration) the plates can be stored for 4 weeks.

Experimental Procedure

The determination of the total bacterial count using R2A agar can be carried out with the pour plate, spread plate and membrane filter methods.

If an incubation time of more than 3 days is used, the plates should be protected from dehydration.

Incubation temperature	Minimum Incubation time	Maximum Incubation time
35°C	72 hours	5-7 days
20 or 28°C	5 days	7 days

Evaluation

The number of colonies is counted and the bacteria count/ml is calculated noting the incubation temperature and incubation period.

Literature

Eaton, A. D., L.S. Clesceri, and A.E. Greenberg (ed.). 1995. Standard methods for the examination of water and wastewater, 19th. Ed. APHA, Washington D.C.

Fiksdal, L., E.A. Vik, A. Mills, and T. Staley. 1982. Non-standard methods for enumerating bacteria in drinking water. Journal AWWA. **74**:313-318.

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Reasoner, D.J., and E.E. Geldreich. 1979. A new medium for the enumeration and subculture of bacteria from potable water. Abstracts of the Annual Meeting of the American Society for Microbiology 79th Meeting, Paper No. N7.

Ordering Information

Product	Merck Cat. No.	Pack size
R2A Agar	1.00416.0500	500 g

Quality control

Test strains	Growth 35 °C / 24 h	Growth 20 °C / 72 h
Escherichia coli ATCC 25922	+	+
Pseudomonas aeruginosa ATCC 27853	+	+
Staphylococcus aureus ATCC 25923	+	+
Bacillus cereus ATCC 11778	+	+

Rappaport-VASSILIADIS Broth

RVS Broth

For the selective enrichment of Salmonella with the exception of S. typhi and S. paratyphi A from foodstuffs and other materials.

The medium complies with the recommendations of the APHA for the examination of foods and ISO Standard 6579 (2002 fourth edition).

This culture medium is a modification of the Salmonella Enrichment Broth acc. to RAPPAPORT (MERCK, Cat. No. 1.10236.0500) and was proposed by VASSILIADIS et al. (1976) who called it R 10 medium and later RVS Broth.. It displays a higher selectivity towards Salmonella and produces better yields than other comparable media, especially after preliminary enrichment and at an incubation temperature of 43 °C (MAIJALA et al. 1992; VAN SCHOTHORST and RENAUD 1983; FRICKER et al. 1983; TONGPIM et al. 1984; PIETZSCH 1984; KALAPOTHAKI et al. 1982; VASSILIADIS 1983; VASSILIADIS et al. 1977, 1978, 1981, 1984, JONAS et al. 1986 etc.). DE SMEDT et al. (1986) made a semi-solid RV medium by adding agar which they used for a faster Salmonella detection using mobility enrichment.

Mode of Action

The malachite green and magnesium chloride concentrations of the present culture medium are less than those of the Salmonella Enrichment Broth according to RAPPAPORT in order to improve the growth of Salmonella at 43 °C. Peptone from soymeal is also used for the same reason. Lowering pH to 5.2 increases selectivity.

ALCAIDE et al. (1982) have reported that addition of novobiocin (40 mg/litre) enhances the inhibition of accompanying flora.

Typical Composition (g/litre)

Peptone from soymeal 4.5; magnesium chloride hexahydrate 28.6; sodium chloride 7.2; di-potassium hydrogen phosphate 1.26; potassium di-hydrogen phosphate 0.18; malachite-green 0.036.

Preparation

Suspend 41.8 g/litre, heat gently, if necessary dispense into test tubes, autoclave gently **(15 min at 115 °C)**.

pH: 5.2 ± 0.2 at 25 °C.

The broth is clear and dark-blue.

The prepared culture medium can be stored in the refrigerator for at least 7 months (VASSILIADIS et al. 1985).

Experimental Procedure and Evaluation

Inoculate the culture medium with the sample or material from a pre-enriched culture (e.g. Peptone Water Buffered) and incubate for 24 hours at 41.5 °C. Streak material from the resulting cultures onto selective culture media.

Literature

ALCAIDE, E.T., MARTINEZ, J.P., MARTINEZ-GERMEX, P., a. GARAY, E.: Improved Salmonella recovery from moderate to highly polluted waters. **-J.Appl. Bact., 53**; 143-146 (1982).

FRICKER, C.R., GIRDWOOD, R.W.A., a. MONRO, D.: A comparison of enrichment media for the isolation of salmonellae from seagull cloacal swabs. **- J. Hg., 91**; 53-58 (1983).

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MAIJALA, R.: JOHANSSON, T., HIRN, J.: Growth of Salmonella and competing flora in five commercial Rappaport-Vassiliadis (RV)-media. **-Intern. J. Food Microbiology, 17**; 1-8 (1992).

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VASSILIADIS, P.: The Rappaport-Vassiliadis (RV) enrichment medium for the isolation of salmonellas: An overview. **- J. Appl. Bact., 54**; 69-76 (1983).

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VASSILIADIS, P., TRICHOPOULOS, D., PAPADAKIS, J., KALAPOTHAKI, V., ZAVITSANOS, X., a. SERIE, CH.: Salmonella Isolation with Rappaport's Enrichment Medium of Different Compositions. **- Zbl. Bakt. Hyg. I. Abt. Orig. B, 173** ; 382-389 (1981)

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Rappaport-VASSILIADIS Broth

RVS Broth

Ordering Information

Product	Merck Cat. No.	Pack size
Salmonella Enrichment Broth acc. to RAPPAPORT and VASSILIADIS (RVS Broth)	1.07700.0500	500 g
Peptone Water (buffered)	1.07228.0500	500 g
Novobiocin monosodium salt	CN Biosciences	
Singlepath® Salmonella	1.04140.0001	25 tests

Quality control

Test strains	Inoculum	Growth after 24 h	Singlepath® Salmonella
Escherichia coli ATCC 25922	approx. 99 %	≤ 10 %	-
Salmonella typhimurium ATCC14028	approx. 1 %	≥ 90 %	+
Pseudomonas aeruginosa ATCC27853	$> 10^4$	none	-
Enterococcus faecalis ATCC 29212	$> 10^4$	none	-

ReadyBag Salmonella

Peptone Water (Buffered), irradiated

Buffered Peptone Water (BPW) is used as a non-selective preenrichment to increase recovery for bacteria, particularly pathogenic Enterobacteriaceae from foodstuffs and other materials. This culture medium complies with the recommendations of the International Standard Organisation ISO 6579 2002.

The lab blender bag contains 5.75 g of granulated, Peptone water (buffered) to which 225 ml of strile water and 25g (or 25 ml) of sample material is added.

The dehydrated culture medium is pre-sterilized by gamma irradiation (25-45 kGray); this dose ensures killing of spores.

Mode of Action

The broth is rich in nutrients and produces high resuscitation rates for sublethally injured bacteria. The high buffering capacity of BPW throughout the preenrichment period allows injured cells to repair and grow.

Typical Composition (g/litre)

Peptone 10.0; sodium chloride 5.0; disodium hydrogen phosphate dodecahydrate 9.0; potassium dihydrogen phosphate 1.5.

pH: 7.0 ± 0.2 at 25 °C.

Procedure

1. Take a ReadyBag with pre-weighted sterile, granulated media
2. Open the bag
3. Add 225 ml of sterile water
4. Add 25 g or 25 ml of sample material and close the bag
5. Blend the closed ReadyBag in a blender and mix thoroughly
6. Incubate ReadyBag for 16-20 h at 37 ± 1 °C

■ **Do not autoclave.**

Experimental Procedure and Evaluation

Incubation: 16-20 hours at 37 ± 1 °C aerobically

Transfer material from the resulting culture to a selective enrichment media as recommended by appropriate standards and follow their procedures.

Ordering Information

Product	Merck Cat. No.	Pack size
Ready Bag	1.07231.0001	100 lab blender bags 5,75g/ each bag

Quality control

Test strains	Growth
Test strains	Growth
Salmonella typhimurium ATCC 14028	good / very good
Salmonella enteritidis ATCC 13076	good / very good
Enterococcus faecalis ATCC 33186	good / very good
Pseudomonas aeruginosa ATCC 27853	good / very good
Escherichia coli ATCC 25922	good / very good

Ready for incubation

ReadyBag Salmonella

Readycult® Coliforms

Rapid detection and identification of microorganisms are of high importance. The use of fluorogenic and chromogenic substrates, utilizing specific enzymatic activities of certain microorganisms, for rapid and sensitive detection of bacteria has proved to be a powerful alternative to conventional methods.

Now you have a better way to tests for enterococci and coliforms including an additional positive confirmation of E.coli. Until now, the most commonly used method to test for E.coli was based on an ONPGMUG assay, which neccessitated the use of a color Comparator to interpret initial results. The ReadyCult® Coliform test will detect total coliforms and E.coli in water samples -even in the presence of an initial background concentration of a million heterothrophic bacteria in 100ml.

Now the choice is easy:

With the ReadyCult® test the color change from yellow to blue-green is an easy-to-read, definitive positive result.

With the optional 30-second Indole reaction you have an accurate method for positive confirmation of E.coli.

With this additional Indole test you are protected two ways: Against false negatives because a lack of fluorescence doesn't always indicate absence of E.coli and against false positives because other species of bacteria can fluoresce. The ReadyCult® test method is EPA approved.

A

Readycult® Coliforms

Content: 20 snap packs

1 snap pack each for 50 ml or 100 ml of water sample.

Application

Selective enrichment broth for the simultaneous detection of total coliforms and E. coli within the bacteriological water examination.

Principle

The high nutritional quality of the peptones and the incorporated phosphate buffer guarantee rapid growth of coliforms whereas lauryl sulfate largely inhibits the accompanying flora, especially the Gram-positive. By adding the chromogenic substrate X-GAL which is cleaved by coliforms and the fluorogenic substrate MUG which is highly specific for E. coli the simultaneous detection of total coliforms and E. coli is possible. The presence of total coliforms is indicated by a blue-green colour of the broth and E.coli by a blue fluorescence under UV-light.

Composition in g / snap pack

Tryptose 0.25; sodium chloride 0.25; sorbitol 0.05; tryptophan 0.05; di-potassium hydrogen phosphate 0.135; potassium dihydrogen phosphate 0.1; lauryl sulfate sodium salt 0.005; X-GAL 0.004; MUG 0.0025; IPTG 0.005.

pH: 6.8 ± 0.2 at 25 °C.

Procedure

1. Add 50 / 100 ml of water sample into a sterile , transparent 100 / 250 ml vessel with screw cap.
   Attention: please use material e.g. glass that is not self-fluorescing!

   In case the sample is to be stored below +25 °C, the examination has to be started within 6 hours. Exceptionally the sample can be stored at +2 to +8 °C (refrigeration) for up to 24 hours.
2. Take one snap pack, shortly tap to ensure the granules are at the bottom. Bend the upper part of the snap pack until it breaks open.
   Attention: do not touch the opening to avoid contamination risk!
3. Add the content to the water sample. Seal the vessel and shake to dissolve the granules completely.

   The prepared broth is clear and yellowish.

   Incubation: 18-24h at 35°C to 37°C.

   If incubated at room temperature (+20 to +25°C) the incubation time is prolonged to 48hours.

Interpretation of results for the detection of Total Coliforms / E. coli:

Negative: No colour change.

The broth remains yellowish in colour.

Total coliforms: Any colour change of the broth to blue-green, even in the upper section of the broth only, confirms the presence of coliforms (X-GAL reaction).

No decolouration with shaking!

E. coli: check blue-green coloured vessels for fluorescence by using UV-lamp in front of the vessel. Light blue fluorescence indicates presence of E. coli (MUG reaction).

Attention: Protect your eyes from direct UV light!

To confirm E. coli in the vessel with positive fluorescence, overlay the broth with 2.5 ml of KOVAC's reagent (indole reaction).

A red ring confirms presence of E. coli.

	Colour change to blue-green	Fluores-cence	Indole-Reaction
Total coliforms	+	-	-
E. coli	+	+	+
Negative	yellow colour		

Disposal

Autoclave the broth (15 min/121 °C).

Alternatively heat the broth for 30 min. in boiling water or use a proper disinfectant
(e.g. Extran® MA 04).

Storage

Store dry at +15 °C to +25 °C.

Shelf life

If stored under recommended conditions the unopened snap pack has a shelf-life of 3 years after day of production (see expiry date on the label).

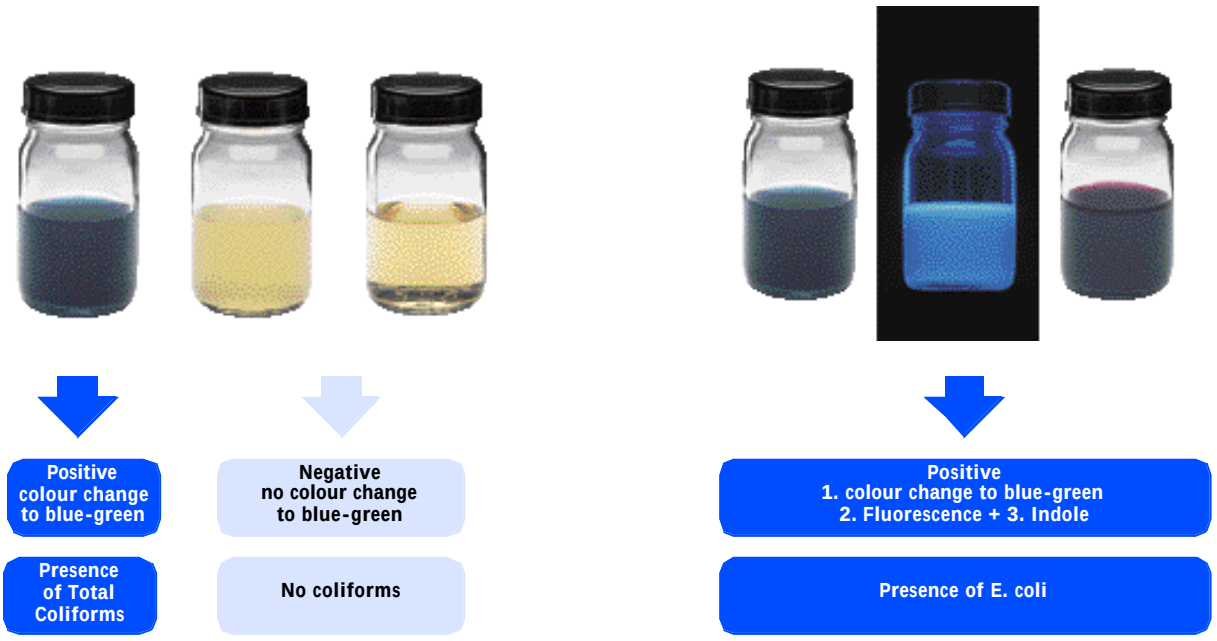
Readycult® Coliforms

Ordering Information

Product	Merck Cat. No.	Pack size
Readycult® Coliforms 50	1.01295.0001	1 x 20 tests
Readycult® Coliforms 100	1.01298.0001	1 x 20 tests
Bactident® Indole (dropper bottle)	1.11350.0001	1 x 30 ml
CULTURA® Mini-Incubator (100-110 V)	1.15533.0001	
CULTURA® Mini-Incubator (220-235 V)	1.13311.0001	1 ea
EXTRAN® MA 04 disinfectant	1.07551.2000	2 liter
KOVÁCS Indole Reagent	1.09293.0100	100 ml
UV Lamp (366 nm)	1.13203.0001	1 ea

Quality control

Test strains	Growth	Color change to blue-green	MUG	Indole
Escherichia coli ATCC 11775	+	+	+	+
Citrobacter freundii ATCC 8090	+	+	-	-
Salmonella typhimurium ATCC 14028	+	-	-	-



Readycult® Enterococci

Content: 20 snap packs

1 snap pack each for 100 ml of water sample.

Application

Selective enrichment broth for the detection of Enterococci and D-Streptococci within the bacteriological water examination.

Principle

The peptone mixture guarantees rapid growth of Enterococci. Sodium-azide largely inhibits the accompanying flora, especially the Gram-negative.

The chromogenic substrate X-GLU is cleaved, stimulated by selected peptones, by the enzyme β-D-Glucosidase present in Enterococci. This results in an intensive blue-green colour change of the broth.

Composition in g/snap pack

Peptones 0.86; sodium chloride 0.64; sodium-azide 0.06; X-GLU 0.004; Tween® 80 0.22.

pH: 7.5 ± 0.2 at 25 °C.

Procedure

1. Add 100 ml of water sample into a sterile, transparent 250 ml vessel with screw cap. In case the sample is to be stored below +25 °C, the examination has to be started within 6 hours. Exceptionally the sample can be stored at +2 to +8 °C (refrigeration) for up to 24 hours.
2. Take one snap pack, shortly tap to ensure the granules are at the bottom. Bend the upper part of the snap pack until it breaks open.
   Attention: do not touch the opening to avoid contamination risk!
3. Add the contents to the water sample. Seal the vessel and shake to dissolve the granules completely.
4. Incubation: 18-24 h at 35 to 37 °C. If incubated at room temperature (+20 to +25 °C) the incubation time is prolonged to 48 hours.

Interpretation of Results

Negative: No colour change.

The broth remains slightly yellow.

Positive: Any colour change of the broth to blue-green, even in the upper section of the broth only, confirms the presence of Enterococci (X-GLU reaction).

No decolouration with shaking!

Disposal

Autoclave the broth (15 min./121 °C). Alternatively heat the broth for 30 min. in boiling water or use a proper disinfectant (e.g. Extran® MA 04).

Storage

Store dry at +15 °C to +25 °C.

Shelf life

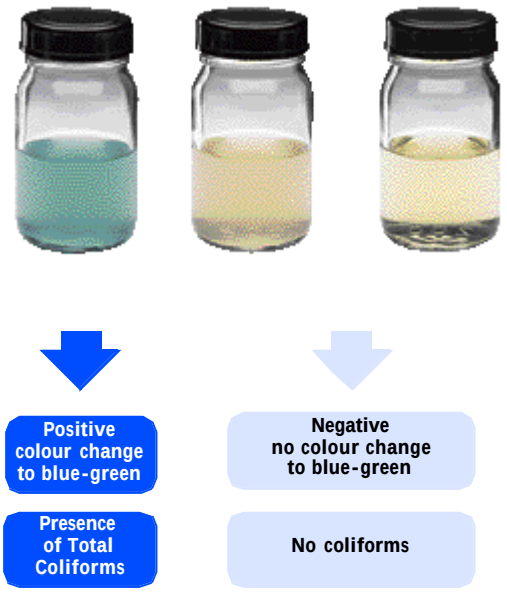
If stored under recommended conditions the unopened snap pack has a shelf-life of 3 years after day of production (see expiry date on the label).

Ordering Information

Product	Merck Cat. No.	Pack size
Readycult® Enterococci100	1.01299.0001	1 x 20 tests
CULTURA® Mini-Incubator (100-110 V)	1.15533.0001	1 ea
CULTURA® Mini-Incubator (220-235 V)	1.13311.0001	1 ea
EXTRAN® MA 04 disinfectant	1.07551.2000	2 liter

Quality control

Test strains	Growth	Colour chage to blue-green
Enterococcus faecalis ATCC 19433	fair / good	+
Staphylococcus aureus ATCC 25923	fair / good	-



Reinforced Clostridial Agar

Medium proposed by BARNES and INGRAM (1956) for the cultivation and enumeration of clostridia, other anaerobes and facultative microorganisms in foodstuffs, clinical specimens and other materials.

MUNOA and PARES (1988) developed a Bifidobacterium Iodoacetate Medium (BIM-25) on the basis of Clostridial Agar for the selective cultivation and differentiation of Bifidobacterium species.

Mode of Action

This culture medium is free from inhibitors and contains cysteine as a reducing agent. According to HIRSCH and GRINSTED (1954), Polymyxin B can be added to inhibit Gram-negative bacteria.

Typical Composition (g/litre)

Meat extract 10.0; peptone from casein 10.0; yeast extract 3.0; D(+)glucose 5.0; starch 1.0; sodium chloride 5.0; sodium acetate 3.0; L-cysteinium chloride 0.5; agar-agar 12.5.

Preparation

Suspend 50 g/litre, if desired dispense into test tubes, autoclave (15 min at 121 °C). If required, cool to 45-50 °C and add 0.02 g Polymyxin B/litre in form of a filter-sterilized aqueous solution.

pH: 6.8 ± 0.2 at 25 °C.

The medium in the tubes or Petridishes is clear and yellowish-brown.

Experimental Procedure and Evaluation

Prepare stab cultures of the sample material in test tubes or use the pour-plate technique.

Incubation: 24-48 hours at an optimal temperature (e.g. 35°C) under anaerobic conditions (e.g. Anaerocult® A, Anaerocult®A mini, or Anaerocult® P).

Count the colonies and, if necessary, perform additional tests.

Literature

BARNES, E.M., a. INGRAM, M.: The effect of redox potential on the grown Clostridium welchii strain isolated from horse muscle. – **J. Appl. Bact., 19**; 177-178 (1956).

HIRSCH, A., a. GRINSTED, E.: Methods for the growth and enumeration of anaerobic sporeformers from cheese, with observations on the effect of nisin. – **J. Dairy Res., 21**;101-110 (1954).

MUNOA, F.J., a. PARES, R.: Selective medium for isolation and enumeration of Bifidobacterium spp. – **Appl. Environm. Microbiol., 54**; 1715-1718 (1988).

Ordering Information

Product	Merck Cat. No.	Pack size
Reinforced Clostridial Agar	1.05410.0500	500 g
Anaerobic jar	1.16387.0001	1 ea
Anaeroclip®	1.14226.0001	1 x 25
Anaerocult® A	1.13829.0001	1 x 10
Anaerocult® A mini	1.01611.0001	1 x 25
Anaerocult® P	1.13807.0001	1 x 25
Anaerotest®	1.15112.0001	1 x 50
Plate basket	1.07040.0001	1 ea
Polymyxin-B-sulfate	CN Biosciences	

Quality control

Test strains	Growth
Clostridium bifermentans ATCC 19299	good / very good
Clostridium difficile 15	good / very good
Clostridium histolyticum HW-6	good / very good
Clostridium perfringens ATCC 13124	good / very good
Clostridium perfringens ATCC 10543	good / very good
Escherichia coli ATCC 25922	good / very good
Bacillus cereus ATCC 11778	good / very good

A

Reinforced Clostridial Medium (RCM)

Medium proposed by HIRSCH and GRINSTED (1954) for the cultivation and enumeration of clostridia, other anaerobes and facultative microorganisms in foodstuffs, clinical specimens and other materials.

Mode of Action

See Reinforced Clostridial Agar.

Typical Composition (g/litre)

Meat extract 10.0; peptone 5.0; yeast extract 3.0; D(+)glucose 5.0; starch 1.0; sodium chloride 5.0; sodium acetate 3.0; L-cysteinium chloride 0.5; agar-agar 0.5

Preparation

Dissolve 33 g/litre, dispense into test tubes, autoclave (15 min at 121 °C). Cool, if required add 0.02 g Polymyxin B/litre in form of an aqueous solution and mix.

pH: 6.8 ± 0.2 at 25 °C.

The prepared medium in the tubes is clear and yellowish.

Experimental Procedure and Evaluation

After inoculation it is advised to cover the medium with a layer of paraffin viscous or agar.

Incubation: 24-48 hours at 35 °C aerobically.

Count the colonies which have grown and, if necessary, perform further tests.

Literature

HIRSCH, A., a. GRINSTED, E.: Methods for the growth and enumeration of anaerobic sporeformers from cheese, with observations on the effect of nisin. – **J. Dairy Res., 21**; 101-110 (1954).

Ordering Information

Product	Merck Cat. No.	Pack size
Reinforced Clostridial Medium (RCM)	1.05411.0500	500 g
Paraffin viscous	1.07160.1000	1 l
Polymyxin-B-sulfate	CN Biosciences	

Quality control

Test strains	Growth
Clostridium bifermentans ATCC 19299	good / very good
Clostridium perfringens ATCC 10453	good / very good
Clostridium perfringens ATCC 13124	good / very good
Clostridium septicum ATCC 12464	good / very good
Clostridium novyi 17861	good / very good
Staphylococcus aureus ATCC 25923	good / very good
Escherichia coli ATCC 25922	good / very good

RINGER's Tablets

Ringer solution it utilized as a diluent for preparting suspensions in bacteriological studies, especially in the examination of milk.

The tables comply with the recommendations of the International Dairy Federation FIL-IDF (Internationaler Milchwirtschaftsverband) (1985, 1992).

Mode of Action

1/4-strength RINGER solution is isotonic with bacteria and thus prevents them from being subjected to osmotic shock or from being osmotically damaged when they are removed from their customary environment. It is also more physiologically suitable for sensitive microoganisms than physiological saline.

Preparation

1/4-strength RINGER solution is prepared by dissolving 1 tablet in 500ml neutral deionized water. Sterilize in the autoclave (15min at 121 °C).

pH: 6.9 ± 0.1 at 25°C.

Literature

Internationaler Milchwirtschaftsverband: Zählung coliformer Bakterien in Milch und Milchprodukten (Internationaler Standard FIL-IDF 73: 1985).

Internationaler Milchwirtschaftsverband: Milch und Milchprodukte - Vorbereitung der Untersuchungsproben und Herstellung der Verdünnungen für mikrobiologische Untersuchungen (Internationaler IMV-Standard 122: 1992).

Ordering Information

Product	Merck Cat. No.	Pack size
RINGER's Tablets	1.15525.0001	1 x 100 tablets

ROGOSA Agar (Lactobacillus Selective Agar)

LBS Agar

Medium proposed by ROGOSA, MITCHELL and WISEMANN (1951) for the isolation and enumeration of lactobacilli in the oral and intestinal microbial flora, meat, milk and other foodstuffs.

Mode of Action

The accompanying bacterial flora is largely suppressed by the high acetate concentration and the low pH value. Low concentrations of manganese, magnesium and iron ensure optimal growth of lactobacilli.

Typical Composition (g/litre)

Peptone from casein 10.0; yeast extract 5.0; D(+)glucose 20.0; potassium dihydrogen phosphate 6.0; ammonium citrate 2.0; Tween® 80 1.0; sodium acetate 15.0; magnesium sulfate 0.575; iron(II) sulfate 0.034; manganese sulfate 0.12; agar-agar 15.0.

Preparation

Suspend 74.5 g/litre, adjust the pH to 5.5 with acetic acid 96 % (approx. 1.3 ml/litre).

■ **Do not autoclave.**

pH: 5.5 ± 0.2 at 25 °C.

The plates are clear and yellowish-brown.

Experimental Procedure

Inoculate by the pour-plate technique or by spreading the material on the surface of the culture medium.

Incubation: up to 3 days at 35 °C or 5 days at 30 °C (SHARPE 1960) under anaerobic conditions in a 5 % carbon dioxide atmosphere.

Establish the bacterial count. For the purpose of identification, reinoculate individual colonies and subject them to the necessary tests (MITSUOKA 1969).

Literature

MITSUOKA, T.: Vergleichende Untersuchungen über Lactobazillen aus den Faeces von Menschen, Schweinen und Hühnern. – **Zbl. Bakt. I. Orig., 210**; 32-51 (1969).

ROGOSA, M.; MITCHEL, J.A., a. WISEMAN, R.F.: A selective medium for the isolation of oral und faecal lactobacilli. – **J. Bact. 62; 132-133** (1951).

SHARPE, M.E.: Selective media for the isolation and enumeration of lactobacilli. – **Lab. Practice, 9**; 223-227 (1960).

Ordering Information

Product	Merck Cat. No.	Pack size
ROGOSA Agar (Lactobacillus Selective Agar)	1.05413.0500	500 g
Acetic acid min. 96 %	1.00062.1000	1 l

Quality control (spiral plating method)

Test strains	Inoculum (cfu/ml)	Recovery rate %
Lactobacillus acidophilus ATCC 4356	10^3-10^5	≥ 70
Lactobacillus casei ATCC 393	10^3-10^5	≥ 70
Lactobacillus fermentum ATCC 9338	10^3-10^5	≥ 70
Lactobacillus plantarum ATCC 8014	10^3-10^5	≥ 70
Bifidobacterium bifidum ATCC 11863	10^3-10^5	≥ 70 (anaerobic)
Escherichia coli ATCC 11775	> 10^5	≤ 0.01
Proteus vulgaris ATCC 13315	> 10^5	≤ 0.01
Enterococcus faecalis ATCC 11700	> 10^5	≤ 0.01

Lactobacillus acidophilus ATCC 4356

Lactobacillus fermentum ATCC 9338

ww

Rose Bengal Chloramphenicol (RBC) Agar

RBC Agar

Selective agar for the enumeration of yeasts and moulds in foodstuffs, particularly proteinaceous food.

Mode of Action

The neutral pH in combination with chloramphenicol suppresses the growth of most bacteria. Rose bengal, taken up intracellular by fungi, restricts the size and the spreading of moulds, preventing overgrowth of slow growing species by luxuriant species.

Typical Composition (g/litre)

Mycological peptone 5.0; glucose 10.0; potassium dihydrogen phosphate 1.0; magnesium sulfate 0.5; Rose Bengal 0.05; chloramphenicol 0.1; agar-agar 15.5.

pH 7.2 ± 0.2 at 25 °C.

Preparation

Suspend 32.2 g in 1 liter of demin. water and heat to boiling until completely dissolved. Autoclave the medium at 121 °C for 15min. Cool to approx. 50 °C, mix well and pour plates.

The appearance of the prepared medium is pink to red .

When stored at +2 to +8 °C in the dark, the shelf life of plates is approximately 1 week and in bottles approx. 2 months.

Experimental Procedure

Directly inoculate the agar plates using surface spreading technique with serial dilutions.

Incubate at 22 °C

for 5 days in the dark.

Interpretation of Results

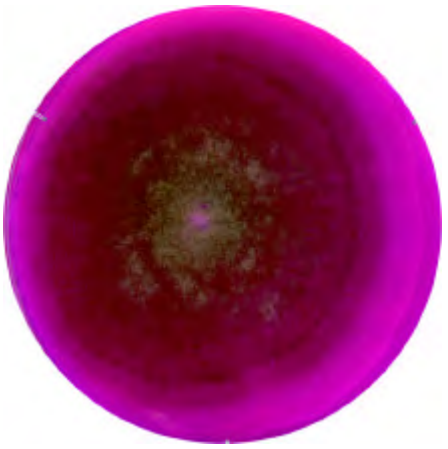
Count the number of yeast and moulds per 1 gram of food.

Literature

JARVIS, B. 1973 Comparison of an improved rose-bengal-chlortetracycline agar with other media for the selective isolation and enumeration of moulds and yeasts in food. **J. Appl. Bacteriol. 36**, 723-727.

Ordering Information

Product	Merck Cat. No.	Pack size
Rose Bengal Chloramphenicl (RBC) Agar	1.00467.0500	500 g



Mucor racemosus ATCC 42647

Quality control

Test strains	Growth
Saccharomyces cerevisiae ATCC 9763	good / very good
Rhodotorula mucilaginosa DSMZ 70403	good / very good, orange colonies
Mucor racemosus ATCC 42647	fair / good
Enterococcus faecalis ATCC 29212	none
Escherichia coli ATCC 25922	none

SABOURAUD Culture Media (introduction)

Modified media proposed by SABOURAUD (1910) for the cultivation, islolation and identficatoin of pathogenic fungi. The media containing glucose are especially suitable for dermatophytes, whilst those containing maltose are to be preferred for yeasts and moulds. The liquid SABOURAUD culture media are used primarily for sterility tests and membrane filtration. SAUBOURAUD-2 % dextrose broth corresponds to GROVE and RANDALL medium No. 13 for antibiotic assays.

Mode of Action

Optimal fungal growth is obtained on these culture media due to their relatively high carbohydrate concentration (2 or 4 %). They do not contain any agents which could selectively inhibit undesired accompanying microbial flora. The pH of 5.6 inhibits bacterial growth; this effect can be enhanced by adjusting the pH to extreme values (approx. 3.5 or 10.0).

If fungi have to be isolated from material which is heavily contaimated with bacteria, selective inhibitory agents should be added. The medium devoid of inhibitor must then also be inoculated.

Additives: 500 mg cycloheximide/litre, 20,000 I.U. penicillin/ litre and 40 mg streptomycin/litre (GEORG et al. 1954) or substitute 40 mg chloramphenicol/litre for the penicillin and streptomycin (AJELLO 1957); for the detection of yeasts add 40mg neomycin/litre and 20,000 I.U. penicillin/litre (WILLIAMS-SMITH and JONES 1963); 80 mg colistin/litre, 100 mg novobiocin/litre and 300 mg cycloheximide/litre (HANTSCHKE 1968); for the isolation of Candida albicans use SABOURAUD-4 % dextrose agar as a base and add 100 mg triphenyltetrazolium chloride/litre (PAGANO et al. 1957-1958).

Preparation

See the individual SABOURAUD culture media for details. The additives should be mixed with the media at about 50 °C after they have been sterilized.

Experimental Procedure and Evaluation

Incubate the inoculated media at approx 22 °C (room temperature) and, if necessary, at 35 °C. Dermatophytes develop after about 5-20 days, other fungi usually after 2-5 days. The procedure used depend on the purpose for which the medium is used.

Manufacturer	Product
Warner-Chillcott, USA	Colistin

Literature

AJELLO, L.: Cultural medthos for human pathogenic fungi. - **J. Chron. Dis.**; 545-551 (1957).

GEORG, L.K., AJELIO, L., a. PAPAGEORGE, C.: Use of cycloheximide in the selective isolation of fungi pathogenic to man. - **J. Lab. Clin. Med., 44**; 422e428 (1954).

HANTSCHKE, D.: Ein Colistin-Novobiocin-Actidion-Agar als Anzucht-medium für humanphathogene Pilze. - **Mykosen, 11**; 769-778 (1968).

PAGANO, J., LEVIN, J.D., a. TREJO, W.: Diagnostic medium for differentiation of species of Candida. - **Antib. Ann.; 137**-143 (1957/58).

SABOURAUD, R.: Les Teignes, (Masson, Paris 1910).

WILLIAM-SMITH, H., a. JONES, J.E.T.: Observation on the alimentary tract and its bacterial flora in healthy and disease pigs. - **J. Path., Bact., 86**; 387-412 (1963).

Ordering Information

Product	Merck Cat. No.	Pack size
2,3,5-Triphenyltetraozolium chloride	1.08380.0010	10 g
Chloramphenicol	CN Biosciences	
Straptomycin sulfate	CN Biosciences	
Novobiocin monosodium salt	CN Biosciences	
Neomycin sulfate	CN Biosciences	
Penicillin G potassium salt	CN Biosciences	

SABOURAUD-4 % Dextrose Agar

This culture medium complies with the recommendations of the United States Pharmacopeia XXVI (2003) and the European Pharmacopeia II.

Typical Composition (g/litre)

Peptone 10.0; D(+)glucose 40.0; agar-agar 15.0.

Preparation

Suspend 65 g/litre, autoclave (15 min at 121 °C).

■ **Do not overheat**

pH: 5.6 ± 0.2 at 25 °C.

The plates are clear and yellowish-brown.

Experimental Procedure and Evaluation

The plates are inoculated with sample material according to the instructions. The fungi colonies which have grown are judged macro- and microscopically.

Incubation: up to 7 days at 28 °C aerobically.

Literature

European Pharmacopeia II, Chapter VIII, 10.

United States Pharmacopeia XXIII, Chapter "Microbial Limit Tests" (1995).

Ordering Information

Product	Merck Cat. No.	Pack size
SABOURAUD-4 % Dextrose Agar	1.05438.0500	500 g
SABOURAUD-4 % Dextrose Agar	1.05438.5000	5 kg

Trichophyton rubrum

Quality control

Test strains	Growth
Trichophyton mentagrophytes ATCC 18748	fair / very good
Trichophyton rubrum ATCC 28188	fair / good
Microsporum gallinae ATCC 12108	fair / very good
Trichophyton ajelloi ATCC 28454	fair / good
Microsporum canis ATCC 36299	good / very good
Geotricum candidum DSMZ 1240	good / very good
Aspergillus niger ATCC 16404	good / very good
Penicillium commune ATCC 10428	good / very good

Quality control (spiral plating method)

Test strains	Inoculum (cfu/ml)	Recovery rate %
Candida albicans ATCC 10231	10^3-10^5	≥ 70
Candida albicans ATCC 2091	10^3-10^5	≥ 70

M
MPF
P
P

SABOURAUD-4 % Maltose Agar

This culture medium complies with the recommendations of the United States Pharmacopeia XXVI (2003) and the European Pharmacopeia II.

Typical Composition (g/litre)

Peptone 10.0; D(+)glucose 40.0; agar-agar 15.0.

Preparation

Suspend 65 g/litre, autoclave (15 min at 121 °C).

■ **Do not overheat**

pH: 5.6 ± 0.2 at 25 °C.

The plates are clear and yellowish-brown.

Experimental Procedure and Evaluation

The plates are inoculated with sample material according to the instructions. The fungi colonies which have grown are judged macro- and microscopically.

Incubation: up to 7 days at 28 °C aerobically.

Literature

European Pharmacopeia II, Chapter VIII, 10.

United States Pharmacopeia XXIII, Chapter "Microbial Limit Tests" (1995).

Ordering Information

Product	Merck Cat. No.	Pack size
SABOURAUD-4 % Maltose Agar	1.05439.0500	500 g

Quality control

Test strains	Growth
Trichophyton mentagrophytes ATCC 18748	good / very good
Trichophyton rubrum ATCC 28188	fair / good
Microsporum gallinae ATCC 12108	good / very good
Trichophyton ajelloi ATCC 28454	fair / good
Microsporum canis ATCC 36299	good / very good
Geotricum candidum DSMZ 1240	good / very good
Aspergillus niger ATCC 16404	good / very good
Penicillium commune ATCC 10428	good / very good

Quality control (spiral plating method)

Test strains	Inoculum (cfu/ml)	Recovery rate %
Candida albicans ATCC 10231	10^3-10^5	≥ 70
Candida albicans ATCC 2091	10^3-10^5	≥ 70

WW

SABOURAUD-2 % Dextrose Agar

The medium was recommended by JANKE (1961) for the cultivation of dermatophytes. GEORG et at. (1954) recommended the addition of cycloheximide, penicillin and streptomycin for the inhibition of non-pathogenic accompanying bacteria.

IVD *in vitro diagnosticum –*
For professional use only

See also General Instruction of Use
Warnings and precautions see ChemDAT®
(www.chemdat.info)

Principle

Microbiological method

Typical Composition (g/litre)

Petone 10.0; D(+)glucose 20.0; agar-agar 17.0.

Preparation and Storage

Usable up to the expiry date when stored dry and tightly closed at +15 to +25° C. Protect from light.

After first opening of the bottle the content can be used up to the expiry date when stored dry and tightly closed at +15 to +25°C.

Suspend 47g/litre, autoclave (15min at 121°C).

■ **Do not overheat.**

pH: 5.6 ± 0.2 at 25 °C.

The plates are clear and yellowish-brown.Experimental Procedure and Evaluation

The plates are inoculated with sample material according to the instructions. The fungi colonies which have grown are judged macro- and microscopically.

Incubation for up to 7 days at 28 °C aerobically.

Specimen

e.g. Skin, nails, sputum, exdates, open lessions.

Clinical specimen collection, handling and processing, see general instructions of use.

Experimental Procedure and Evaluation

The plates are inoculated with sample material according to the instructions. The fungi colonies which have grown are judged macro- and microscopically.

Incubation for up to 7 days at 28 °C aerobically.

For preparation of version acc. to EMMONS adjust pH to 6.9±0.2. Chloramphenicol (50 mg/l) may be added in addtion.

Literature

GEORG, L.K., AJELLO, L., a. PAPAGEORGE, C.: Use of cycloheximide in the selective isolation of fungi pathogenic to man. - **J. Lab. Clin. Med., 44**; 422-428 (1954).

JANKE, D.: Pilznährboden nach SABOURAUD, modifiziert MERCK, ein neuer Trockennährboden zur Züchtung von Dermatophyten. - **Zschr. Haut- u. Geschl.-Krankh., 15** ; 188-193 (1961).

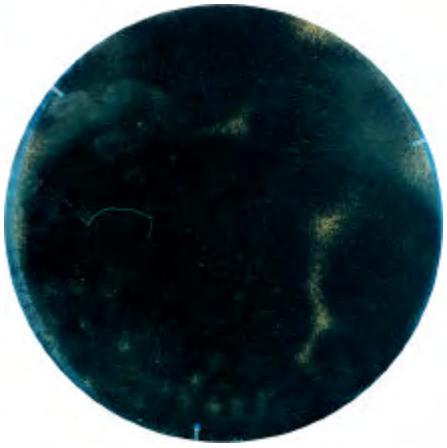
Ordering Information

Product	Merck Cat. No.	Pack size
SABOURAUD-2 % Dextrose Agar	1.07315.0500	500 g
Merckoplate® SABOURAUD-2 % glucose Agar	1.10413.0001	1 x 20 plates
Merckoplate® SABOURAUD-2 % glucose Agar	1.15404.0001	1 x 480 plates
Penicillin G potassium salt	CN Biosciences	
Streptomycin sulfate	CN Biosciences	

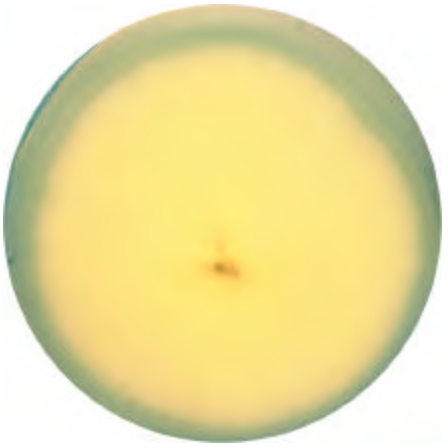
SABOURAUD-2 % Dextrose Agar

Quality control

Test strains	Growth
Trichophyton mentagrophytes ATCC 18748	good / very good
Trichophyton rubrum ATCC 28188	fair / good
Microsporum gallinae ATCC 12108	good / very good
Trichophyton ajelloi ATCC 28454	fair / very good
Microsporum canis ATCC36299	good / very good
Geotrichum candidum DSMZ 1240	good / very good
Candida albicans ATCC 10231	good / very good
Aspergillus niger ATCC 16404	good / very good
Penicillium commune ATCC 10428	good / very good



Aspergillus niger
ATCC 16404



Geotrichum candidum
DSMZ 1240

M

SABOURAUD-2 % Dextrose Broth

GROVE and RANDALL medium No. 13

Typical Composition (g/litre)

Peptone from meat 5.0; peptone from casein 5.0; D(+)glucose 20.0

Preparation

Suspend 30 g/litre, if necessary dispense into smaller vessels, autoclave (15 min at 121 °C).

pH: 5.6 ± 0.2 at 25 °C.

The plates are clear and yellowish-brown.

Experimental Procedure and Evaluation

Depend on the purpose for which the culture medium is used.

Incubation: up to 7 days at 28 °C aerobically.

Ordering Information

Product	Merck Cat. No.	Pack size
SABOURAUD-2 % Dextrose Broth	1.08339.0500	500 g

Quality control

Test strains	Growth
Trichophyton mentagrophytes ATCC 18748	good / very good
Trichophyton rubrum ATCC 28188	fair / good
Microsporum gallinae ATCC 12108	fair / good
Trichophyton ajelloiATCC 28454	fair / good
Candida albicans ATCC 10231	fair / very good
Aspergillus niger ATCC 16404	good / very good
Penicillium commune ATCC 10428	good / very good

SABOURAUD-1 % Dextrose 1 % Maltose Agar

For the cultivation of moulds and yeasts (particularly from packaging materials) and for testing antimycotic substances.

This medium complies with the recommendations of the Institute for Food Technology and Packaging of the Technical University of Munich (Institut für Lebensmitteltechnologie and Verpackung der TU München) (1974).

Typical Composition (g/litre)

Peptone from casein 5.0; peptone from meat 5.0; D(+)glucose 10.0; maltose 10.0; agar-agar 15.0.

Preparation

Suspend 45 g/litre, autoclave (15 min at 121 °C).

■ **Do not overheat!**

pH: 5.4 ± 0.2 at 25 °C.

The plates are clear and yellow.

Experimental Procedure and Evaluation

Inoculate the culture medium.

Incubation: up to 7 days at 28 °C aerobically.

Literature

Arbeitsgruppen des Instituts für Lebensmitteltechnologie und Verpackung an der Technischen Universität München, Merkblätter für die Prüfung von Packmitteln: Merkblatt 18: Prüfung auf antimikrobielle Bestandteile in Packstoffen. - **Verpackgs.-Rdsch., 25/1**; Techn.-wiss. Beilage, 5-8 (1974).

Merkblatt 19: Bestimmung der Gesamtkeimzahl, der Anzahl an Schimmelpilzen und Hefen und der Anzahl an coliformen Keimen in Flaschen und vergleichbaren enghalsigen Behältern. - **Verpackgs.-Rdsch., 25/6**; Techn.-wiss. Beilage, 569-575 (1974).

Merkblatt 21: Bestimmung der Oberflächenkeimzahl (Bakterien, Schimmelpilze, Hefen und coliforme Keime) auf nicht saugfähigen Packstoffen. - **Verpackgs.-Rdsch., 25/7**; Techn.-wiss. Beilage, 53-55 (1974).

Ordering Information

Product	Merck Cat. No.	Pack size
SABOURAUDS-1 % Dextrose 1% Maltose Agar	1.07662.0500	500 g

Quality control

Test strains	Growth
Trichophyton mentagrophytes ATCC 18748	fair / good
Trichophyton rubrum ATCC 28188	fair / good
Microsporum gallinae ATCC 12108	fair / good
Trichophyton ajelloi ATCC 28454	fair / good
Microsporum canis ATCC 36299	fair / good
Geotrichum candidum DSMZ 1240	good / very good
Candida albicans ATCC 10231	good / very good
Aspergillus niger ATCC 16404	good / very good
Penicillium commune ATCC 10428	good / very good

Microsporum canis ATCC 36299

Candida albicans ATCC 10231

Salmonella Agar acc. to ÖNÖZ

Medium introduced by ÖNÖZ (1978) for the cultivation of salmonellae.

IVD *in vitro diagnosticum –*
For professional use only

CE

See also General Instruction of Use
Warnings and precautions see ChemDAT®
(www.chemdat.info)

The most important advantage of this culture medium is that it allows more rapid bacteriological diagnosis as, according to ÖNÖZ, Salmonella and Shigella colonies can be clearly and reliably differentiated from other Enterobacteriaceae. The yields of Salmonella from stool samples obtained, when using this medium, are higher than those obtained with LEIFSON Agar or Salmonella Shigella Agar.

Principle

Microbiological method

Mode of Action

The growth of Gram-positive bacteria is almost completely inhibited while lactose- and sucrose-positive Enterobacteriaceae are partially suppressed. Furthermore their colonies can be differentiated, by means of the different shades of colour produced, in the presence of the indicators neutral red and aniline blue. Proteus colonies can be differentiated, because they deaminate phenylalanine to give phenylpyruvate, which forms a dark brown complex with iron ions. Phenylalanine also neutralizes chloramphenicol, so that during treatment with this compound detection of salmonellae is affected only slightly.

Typical Composition (g/litre)

Yeast extract 3.0; meat extract 6.0; peptone from meat 6.8; lactose 11.5; sucrose 13.0; bile salt mixture 3.825; tri-sodium citrate 5,5-hydrate 9.3; sodium thiosulfate-5-hydrate 4.25; L-phenylalanine 5.0; iron(III) citrate 0.5; magnesium sulfate 0.4; brilliant green 0.00166; neutral red 0.022; aniline blue 0.25; metachrome yellow 0.47; di-sodium hydrogen phosphate-2-hydrate 1.0; agar-agar 15.0.

Preparation and Storage

Usable up to the expiry date when stored dry and tightly closed at +15 to +25°C. Protect from light.

After first opening of the bottle the content can be used up to the expiry date when stored dry and tightly closed at +15 to +25°C.

The prepared plates can be stored in the refrigerator for at least 3 weeks. They are clear and dark brown to black.

Completely suspend 80.5 g/litre, pour plates.

pH: 7.1 ± 0.2 at 25 °C.

The prepared plates can be stored in the refrigerator for at least 3 weeks. They are clear and dark brown to black.

Specimen

e.g. Stool.

Clinical specimen collection, handling and processing, see general instructions of use.

Experimental Procedure and Evaluation

Inoculate the plates directly with the sample itself or with material taken from an enrichment culture.

Incubation: 16-24 hours at 35 °C aerobically.

Appearance of Colonies	Microorganisma
Small, blue, surrounded by a bile precipitation ring	E. coli (partly inhibited)
Large, mucoid, domed, blue-gray (domes whitish); bluish precipitation zone around the colonies	Klebsiella (partly inhibited)
Red to yellow-orange, culture medium surrounding the colonies is yellowish; if growth is dense the red colouration disappears	Citrobacter
Rust-coloured, culture medium surrounding the colonies of same colour, if growth is too dense, dark brown to black	Proteus, Providencia
Glossy, dirty yellow to greenish; culture medium is yellow	Pseudomonas
Large, mucoid, bluish or reddish, slight precipitation ring around the colonies	Enterobacter
1st day: colonies are the same colour as the medium;2nd day: slightly bluish, no change in the colour of the medium.	Shigella
1st day: colonies are the same colour as the medium;2nd day: small, yellow; with further incubation a black dot sometimes appears on the yellow colonies; colour of the culture medium changes to yellow	Salmonella typhosa
Yellow, medium size;1st day: black dots start to develop on the yellow colonies;2nd day: dear black dot visible on the yellow colonies: culture medium surrounding the colonies is yellowish	Other Salmonella species

Salmonella Agar acc. to ÖNÖZ

Literature

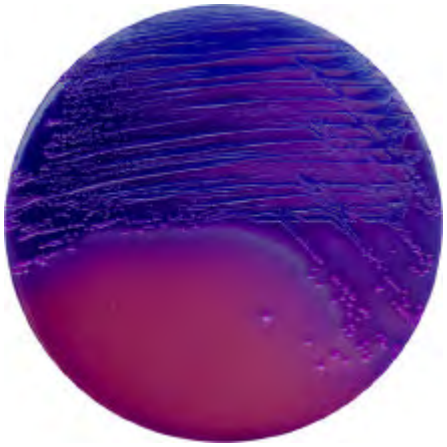
ÖNÖZ, E. u. HOFFMANN, K.: Erfahrungen mit einem neuen Nährboden für die Salmonella-Diagnostik - **Zbl. Bakt. Hyg., I. Abt. Orig., A 240**; 16-21 (1978).

Ordering Information

Product	Merck Cat. No.	Pack size
Salmonella Agar acc. to ÖNÖZ	1.15034.0500	500 g

Quality control

Test strains	Growth	Colony colour	Colour changes of medium	Black centre
Shigella flexneri ATCC 12022	fair / good		yellow to yellowish-brown	
Salmonella typhimurium ATCC 14028	good / very good		yellow to yellowish-brown	±
Salmonella enteritidis NCTC 5188	good / very good		yellow to yellowish-brown	±
Salmonella derby ATCC 6960	good / very good		yellow to yellowish-brown	±
Escherichia coli ATCC 25922	none / poor	blue-violet	blue / precipitate	
Klebsiella pneumoniae ATCC 13883	good / very good	blue	light blue	
Citrobacter freundii ATCC 8090	good / very good	yellow-violet		
Proteus mirabilis ATCC 14273	good / very good	rust coloured		
Pseudomonas aeruginosa ATCC 27853	fair / very good		yellow to yellowish-brown	
Staphylococcus aureus ATCC 25923	none			
Bacillus cereus ATCC 11778	none			



Citrobacter freundii ATCC 8090

Salmonella enteritidis NCTC 5188

Salmonella Enrichment Broth acc. to RAPPAPORT

Medium proposed by RAPPAPORT et al. (1956, 1959) for the selective enrichment of Salmonella (with the exception of S. typhosa) from fecal specimens, foodstuffs and other materials.

IVD *in vitro diagnosticum –*
For professional use only

See also General Instruction of Use
Warnings and precautions see ChemDAT®
(www.chemdat.info)

This broth is superior to other enrichments used for Salmonella - the yields obtained are several 10-fold higher (TRICHOPOULOS et al. 1972, IVESON et al. 1964). Yields are further increased by adding tetrathionate and substituting metachrome yellow with malachite green (HOFER 1969). GOOSENS et al. (1984) reported high isolation rates for salmonellae, when using a semi-soldid culture medium based on RAPPAPORT Broth.

Principle

Microbiological method

Mode of Action

Malachite green and magnesium chloride largely inhibit the growth of the microorganisms normally found in the intestine, but do not affect the proliferation of most salmonellae. Only S.typhosa and Shigellae are usually inhibited by malachite green. This culture medium is thus not suitable for the enrichment of these pathogenic organisms.

Typical Composition (g/litre)

Peptone from casein 5.0; sodium chloride 8.0; di-potassium hydrogen phosphate 0.8; magnesium chloride hexahydrate 40.0; malachite green 0.12.

Preparation and Storage Cat. No. 1.10236. Salmonella Enrichment Broth acc. to RAPPAPORT (500 g)

Usable up to the expiry date when stored dry and tightly closed at +15 to +25°C. Protect from light.

After first opening of the bottle the content can be used up to the expiry date when stored dry and tightly closed at +15 to +25°C.

Suspend 54 g/litre, dispense into test tubes, autoclave gently (20min at 115 °C).

pH: 6.0 ± 0.2 at 25 °C.

The prepared broth is clear and dark-blue.

- If the reconstituted medium is stored for a long period (about 2-3 weeks) a precipitate may occur, but this does not affect any of its properties.

Specimen

e.g. Stool.

Clinical specimen collection, handling and processing, see general instructions of use.Experimental Procedure and Evaluation

Experimental Procedure and Evaluation

Suspend the stool specimens in sterile physiological saline, ratio of up to 1:1000, add 3-4 drops of the suspension to a tube containing 5 ml enrichment broth. If only small quantities of salmonellae are suspected to be present, larger aliquots of the sample (1-2 g) should be added to 100 ml portions of the broth.

Incubation: 18-24 hours at 35 °C.

Streak samples from the resulting cultures onto selective culture media.

Ordering Information

Product	Merck Cat. No.	Pack size
Salmonella Enrichment Broth acc. to RAPPAPORT	1.10236.0500	500 g

Quality control

Test strains	Inoculum	Grwoth after 24hours
Escherichia coli ATCC 25922	approx. 99 %	≤ 10 %
Salmonella typhimurium ATCC 14028	approx. 1 %	≥ 90 %

Salmosyst® Broth Base

For the two-step enrichment of sublethally injured salmonellae, especially from foodstuffs and feeds.

Mode of Action

All the microorganisms present in the sample material are enriched during non-selective pre-enrichment in Salmosyst® Broth Base. Following the addition of the selective reagents in the form of a Salmosyst® Selective Supplement tablet, the growth of the accompanying organisms is inhibited, but the salmonellae continue to grow.

Composition of the Broth Base (g/litre)

Peptone from casein 5.0; peptone from meat 5.0; sodium chloride 5.0; calcium carbonate 10.0.

pH: 7.1 ± 0.2 at 25 °C.

Preparation of the Broth Base

Dissolve 25 g/litre, autoclave (15 min at 121 °C). A visible white precipitation of calcium carbonate which then appears does not affect the performance of the broth. During long storage of the prepared broth some of the calcium carbonate dissolves and can lead to a minimal increase in pH.

Preliminary Enrichment

Suspend 25 g sample material (if necessary homogenize) in 225ml broth base and incubate for 6-8 hours at 35 °C. Transfer 10ml of the culture to a sterile test tube.

Selective Enrichment

Add one tablet of Salmosyst® Selective Supplement to the 10ml of the preliminary enrichment culture and leave to stand for 30minutes. Shake vigorously and then incubate for further 18-22 hours at 35 °C. In order to detect the salmonellae, streak a sample of the resulting enrichment culture onto appropriate selective culture media. Identify the resulting colonies.

Literature

WEBER, A.: Über die Brauchbarkeit von Salmosyst ® zur Anreicherung von Salmonellen aus Kotproben von Tieren. - **Berl. Münch. Tierärztl. Wschr., 101**; 57-59 (1988).

OSSMER, R.: Salmosyst® and RAMBACH-Agar. A Rapid Alternative for the Detection of Salmonella. Congress-Poster - Salmonella and Solmonellosis-Ploufragan/Saint-Brieux - France, Sepetember 1992.

Ordering Information

Product	Merck Cat. No.	Pack size
Salmosyst® Broth Base	1.10153.0500	500 g
Salmosyst® Selective Supplement	1.10141.0001	250 tablets

Quality control (incl. supplement)

Test strains	Growth
Salmonella typhimurium ATCC 14028	good
Salmonella dublin ATCC 15480	good
Escherichia coli ATCC 25922	inhibited
Enterococcus faecalis ATCC 19433	inhibited

Salmosyst® Selective Supplement

For the two-step enrichment of sublethally injured salmonellae, especially from foodstuffs and feeds.

Mode of Action

All the microorganisms present in the sample material are enriched during non-selective pre-enrichment in Salmosyst® Broth Base. Following the addition of the selective reagents in the form of a Salmosyst® Selective Supplement tablet, the growth of the accompanying organisms is inhibited, but the salmonellae continue to grow.

Composition of the Selective Supplement (g/tablet)

Potassium tetrathionate 0.2; ox bile 0.08; brilliant green 0.0007; calcium carbonate 0.1.

Literature

WEBER, A.: Über die Brauchbarkeit von Salmosyst ® zur Anreicherung von Salmonellen aus Kotproben von Tieren. - **Berl. Münch. Tierärztl. Wschr., 101**; 57-59 (1988).

OSSMER, R.: Salmosyst ® and RAMBACH-Agar. A Rapid Alternative for the Detection of Salmonella. Congress-Poster - Salmonella and Solmonellosis - Ploufragan/Saint-Brieux - France, Sepetember 1992.

Ordering Information

Product	Merck Cat. No.	Pack size
Salmosyst® Selective Supplement	1.10141.0001	250 tablets
Salmosyst® Broth Base	1.10153.0500	500 g

Quality control (incl. supplement)

Test strains	Growth
Salmonella typhimurium ATCC 14028	good
Salmonella dublin ATCC 15480	good
Escherichia coli ATCC 25922	inhibited
Enterococcus faecalis ATCC 19433	inhibited

Selective Agar for Pathogenic Fungi

For the isolation of pathogenic fungi, particularly dermatophytes, from heavily contaminated sample material.

IVD *in vitro diagnosticum –*
For professional use only

CE

See also General Instruction of Use
Warnings and precautions see ChemDAT®
(www.chemdat.info)

Principle

Microbiological method

Mode of Action

Cycloheximide is used to select for dermatophytes (GEORG 1953; GEORG et al. 1954). Choramphenicol largely suppresses bacteria. Certain pathogenic fungi may also sometimes be inhibited, therefore a culture medium devoid of inhibitors should be inoculated as well. TAPLIN (1965) recommends addition of 40mg gentamicin sulfate/litre (e.g. 0.5 ml gentamicin solution/ litre), to suppress chloramphenicol-resistant bacteria, which are occasionally present.

Typical Composition (g/litre)

Peptone from soymeal 10.0; D(+)glucose 10.0; cycloheximide 0.4; chloramphenicol 0.05; agar-agar 12.5..

Preparation and Storage

Usable up to the expiry date when stored dry and tightly closed at +15 to +25° C. Protect from light.

After first opening of the bottle the content can be used up to the expiry date when stored dry and tightly closed at +15 to +25°C.

Suspend 33 g/litre completely, pour plates.

pH: 6.9 ± 0.2 at 25 °C.

The plates are clear and yellowish-brown.

■ **Do not autoclave, do not overheat. Avoid reliquefication.**

Experimental Procedure and Evaluation

Obtain the sample material by an appropriate method and inoculate on the surface of the culture medium.

Incubation: up to 3 weeks at approximately 28 °C (room temperature); if endomycoses are suspected to be present, at 35°C as well.

Any fungal colonies which develop can be identified as such (MCDONOUGH et al. 1960) or can be inoculated on media which do not contain inhibitors (e.g. SABOURAUD media) for further differentiation.

Specimen

e.g. Nails, hair, skin.

Clinical specimen collection, handling and processing, see general instructions of use.

Literature

AHEARN, D.G.: Systematics of Yeasts of Medical Interest (Pan American Health Organization: International Symposium on Mycoses). - **205**; 54-70 (1970).

GEORG, L.K.: Use of cycloheximide medium for isolation of dermatophytes from clinical materials. - **Arch. Dermat. Syphil., 67**; 355-361 (1953).

GEORG, L.K., AJELLO, D. a. PAPAGEORGE, C.: Use of cycloheximide in the selective isolation of fungi pathogenic to man. - **J. Lab. Clin. Med., 44**; 422-428 (1954).

HALEY, L.D.: Laboratory Methods in Systematic Mycoses (C.D.C. Course 8170-C, Atlanta, 1969).

MCDONOUGH, E.S., GEORG, L.K., AJELLO, L., a. BRINKMAN, S.: Growth of dimorphic human pathogenic fungi on media containing cycloheximide and chloramphenicol. - **Mycopath. Mycol. Appl., 13**; 113-120 (1960).

TAPLIN, D.: The use of gentamicin in mycology. - **J. Invest. Dermat., 45**; 549-550 (1965).

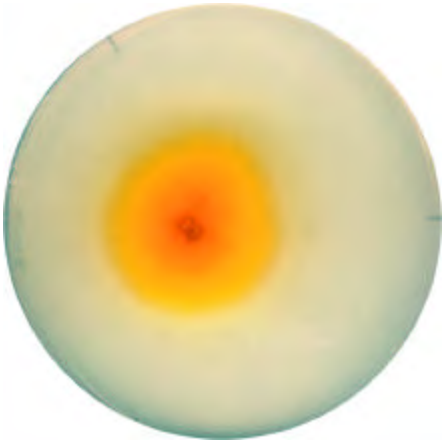
Ordering Information

Product	Merck Cat. No.	Pack size
Selective Agar for Pathogenic Fungi	1.05467.0500	500 g
Merckoplate® Selective agar for pathogenic fungi	1.10415.0001	20 plates
Gentamicin solution	1.11977.0001	10 ml

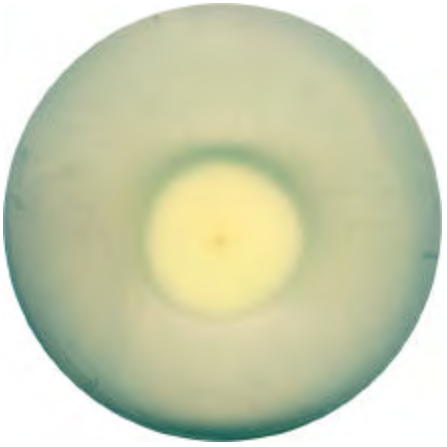
Selective Agar for Pathogenic Fungi

Quality control

Test strains	Growth
Trichophyton mentagrophytes ATCC 18748	good / very good
Trichophyton rubrum ATCC 28188	fair / good
Microsporum gallinae ATCC 12108	fair / good
Trichophyton ajelloi ATCC 28454	fair / good
Microsporum canis ATCC 36299	good / very good
Geotrichum candidum DSMZ 1240	good / very good
Candida albicans ATCC 10231	good / very good
Aspergillus niger ATCC 16404	none / poor
Penicillium commune ATCC 10428	none / poor
Bacillus cereus ATCC 11778	none



Microsporum canis
ATCC 36299



Trichophyton mentagrophytes
ATCC 18748

Selenite Cystine Broth

For the enrichment of salmonellae from faeces, foodstuffs and other materials.

in vitro diagnosticum –
For professional use only

See also General Instruction of Use
Warnings and precautions see ChemDAT®
(www.chemdat.info)

This culture medium complies with the recommendations of ISO Standard 6579 (1993), the American Public Health Association (1992), the United States Pharmacopeia XXVI (2003), the DIN Norm 10181 for the examination of milk and acc. to § 35 LMBG (German regulations) for food examination.

Principle

Microbiological method

Mode of Action

Selenite inhibits the growth of coliform bacteria and enterococci in the first 6-12 hours of incubation, its inhibitory effect then gradually declines. Salmonella, Proteus and Pseudomonas are, however, only slightly inhibited.

Typical Composition (g/litre)

Peptone from casein 5.0; L(-)cystine 0.01; lactose 4.0; phosphate buffer 10.0; sodium hydrogen selenite 4.0.

Preparation and Storage

Usable up to the expiry date when stored dry and tightly closed below +15 °C. Protect from light.

After first opening of the bottle the content can be used up to the expiry date when stored dry and tightly closed below +15 °C.

■ **Storage of the dehydrated culture medium below 15°C!**

Suspend 23 g/litre at room temperature, if the medium does not dissolve readily, heat briefly (max. 60 °C); if the medium is to be stored, filter-sterilize; dispense into suitable containers.

■ **Do not autoclave**.

pH: 7.0 ± 0.2 at 25 °C.

The prepared broth is clear and yellowish.

After a longer storage period of the dehydrated medium, the colour of the prepared broth might change to redish/red. The microbiological performance however is not affected.

Specimen

e.g. Stool .

Clinical specimen collection, handling and processing, see general instructions of use.

Experimental Procedure

Add solid material to the normal-strength broth. Mix liquid samples with double-strength broth in the ratio 1:1.

Incubation: up to 24 hours at 35-37 °C - according to BÄNFFER (1971) and other authors, 43 °C is better.

After 6-12 hours and, if necessary, after 18-24 hours inoculate material from the resulting culture onto selective culture media.

Literature

American Public Health Association: Compendium of methods for the microbiological examination of foods. - 3rd ec., 1992.

BÄNFFER, J.R.: Comparison of the isolation of Salmonellae from human faeces by enrichment at 37 °C and at 43 °C. - **Zbl. Bakt. I. Orig., 217**; 35-40 (1971).

Bundesgesundheitsamt: Amtliche Sammlung von Untersuchungsverfahren nach § 35 LMBG. - Beuth Verlag Berlin, Köln.

DIN Deutsches Institut für Normung e.V.: Mikrobiologische Milchuntersuchung. Nachweis von Salmonellen. Referenzverfahren. - **DIN 10181**.

United States Pharmacopeia XXVI, Chapter "Microbial Limit Tests" (1995).

Ordering Information

Product	Merck Cat. No.	Pack size
Selenite Cystine Broth	1.07709.0500	500 g

Quality control

Test strains	Inoculum	Growth after 24 hours
Escherichia coli ATCC 25922	approx. 99 %	≤ 10 %
Salmonella typhimurium ATCC 14028	approx. 1 %	≥ 90 %

WW

Selenite Enrichment Broth acc. to LEIFSON

Selenite-F Broth; Selenite Broth

Medium proposed by LEIFSON (1936) for the selective enrichment of Salmonella from faeces, urine, water, foodstuffs etc.

IVD *in vitro diagnosticum –*
For professional use only

See also General Instruction of Use
Warnings and precautions see ChemDAT®
(www.chemdat.info)

CE

Principle

Microbiological method

The medium complies with the recommendations of the APHA (1992) for food examination.

Mode of Action

Selenite inhibits the growth of enteric coliform bacteria and enterococci, mainly during the first 6-12 hours of incubation. Salmonella, Proteus and Pseudomonas are not suppressed.

Typical Composition (g/litre)

Peptone from meat 5.0; lactose 4.0; sodium selenite 4.0; di-potassium hydrogen phosphate 3,5; potassium dihydrogen phosphate 6.5.

Preparation and Storage

Usable up to the expiry date when stored dry and tightly closed below +15 °C. Protect from light.

After first opening of the bottle the content can be used up to the expiry date when stored dry and tightly closed below +15 °C.

■ **Storage of the dehydrated culture medium below 15°C!**

Suspend 23 g/litre at room temperature; if the medium does not dissolve readily, heat briefly (max. 60 °C); if the medium is to be stored for a longer period of time filter-sterilize, dispense into suitable containers.

■ **Do not autoclave.**

pH: 7.0 ± 0.2 at 25 °C.

The prepared broth is clear and yellowish.

After a longer storage period of the dehydrated medium, the colour of the prepared broth might change to reddish/red. The microbiological performance however is not affected.

Specimen

e.g. Stool, urine .

Clinical specimen collection, handling and processing, see general instructions of use.

Experimental Procedure and Evaluation

Add solid sample material to the normal-strength broth. Mix liquid samples with double-strength broth in the ratio 1:1.

Incubation: up to 24 hours at 37 °C - according to BÄNFFER (1971) and other authors, 43 °C is better.

After 6-12 hours and, if necessary, after 18-24 hours inoculate material from the resulting culture onto selective culture media.

Literature

BÄNFFER, J.R.: Comparison of the isolation of Salmonellae from human faeces by enrichment at 37 °C and 43 °C. - **Zbl. Bakt. I. Orig., 217**; 35-40 (1971).

LEIFSON, E.: New selenite enrichment media for the isolation of typhoid and parathyphoid (Salmonella) bacilli. - **Am. J. Hyg., 24**; 423-432 (1936).

American Public Health Association: Compendium of methods for the microbiological examination of foods. – 3rd ed., 1992.

Ordering Information

Product	Merck Cat. No.	Pack size
Selenite Enrichment Broth acc. to LEIFSON	1.07717.0500	500 g

Quality control

Test strains	Inoculum	Growth after 24 hours
Escherichia coli ATCC 25922	approx. 99 %	≤ 10 %
Salmonella typhimurium ATCC 14028	approx. 1 %	≥ 90 %

SIM Medium

Test culture medium used to detect sulfide formation, indole production and motility for the identification of Enterobacteriaceae.

The medium complies with the recommendations of APHA (1992) for food examination.

Typical Composition (g/litre)

Peptone from casein 20.0; peptone from meat 6.6; ammonium iron(II)citrate 0.2; sodium thiosulfate 0.2; agar-agar 3.0.

Preparation

Suspend 30 g/litre, dispense into tubes to give a depth of about 4 cm, autoclave (15 min at 121 °C), allow to solidify in a vertical position.

pH: 7.3 ± 0.2 at 25 °C.

The prepared medium is clear and yellowish-brown.

Experimental Procedure and Evaluation

Introduce a pure culture of the microorganism to be tested into the butt by puncture.

Incubation: 18-24 hours at 35 °C aerobically.

Motility is indicated by a diffuse turbidity of the culture medium surrounding the puncture line. In case of immotility, growth takes place solely along the puncture line. H_2S formation is shown by a blackening in those areas of the medium in which microbial growth has occured.

After checking the tubes for motility and H_2S production, the indole test is performed. The medium is covered with a layer of KOVÁCS Indole Reagent. Production of indole causes the reagent layer to become purple in colour.

Microorganisms	H_2S	Indole	Motility
Escherichia	-	+	+ / -
Enterobacter	-	-	+
Citrobacter	+	-	+
Klebsiella	-	-	-
Salmonella	+	-	+
Shigella	-	+ / -	-
Prot. vulgaris	+	+	+
Prot. mirabilis	+	-	+
Morganella	-	+	+
Rettgerella	-	+	+
Arizona	+	-	+
Hafnia	-	-	+
Serratia	-	-	+
Providencia	-	+	+
Edwardsiella	+	+	+
Yers. enterocolitica	-	- (+)	-

Literature

American Public Health Association: Compendium of methods for the microbiological examination of foods. - 3rd ed. (1992).

COSTIN, I.D.: Die biochemische Identifizierung der Enterobacteriaceae. Kritische Bemerkungen zur Prinzipien und Methoden. - **Zbl. Bakt. I. Ref., 219**; 81-151 (1961).

COSTIN, I.D.: Orientierende Identifizierung obligat- und fakultativ-aerober, anspruchsloser, gramnegativer Stäbchen von medizinischem Interesse. - **Med. Labor., 30**; 197-217 (1977).

Ordering Information

Product	Merck Cat. No.	Pack size
SIM Medium	1.05470.0500	500 g
Bactident® Indole (dropper bottle)	1.11350.0001	1 x 30 ml
KOVÁCS Indole Reagent	1.09293.0100	100 ml

Escherichia coli ATCC 25922 | **Salmonella typhimurium ATCC 14028**

SIM Medium

Quality control

Test strains	Growth	H_2S-formation	Indole formation	Motility
Escherichia coli ATCC 25922	good / very good	-	+	+
Escherichia coli ATCC 4351	good / very good	-	+	+
Klebsiella pneumoniae ATCC 13883	good / very good	-	-	-
Enterobacter cloacae ATCC 13047	good / very good	-	-	+
Salmonella typhimurium ATCC 14028	good / very good	+	-	+
Proteus vulgaris ATCC 13315	good / very good	+	+	+

SIMMONS Citrate Agar

Synthetic test agar proposed by SIMMONS (1926) for the identification of microorganisms (particularly of Enterobacteriaceae and certain fungi) on the basis of their metabolism of citrate, being the sole carbohydrate source.

IVD *in vitro diagnosticum –*
For professional use only

See also General Instruction of Use
Warnings and precautions see ChemDAT®
(www.chemdat.info)

Principle

Microbiological method

This culture medium complies with the recommendations of the APHA for the examination of water (1998) and the recommendations of the APHA for the examination of food (1992).

According to VAN KREGTEN et al. (1984) this culture medium can be used for cultivating Klebsiella by adding inositol.

Mode of Action

Metabolism of citrate leads to alkalinization of the medium, which is indicated by a change in the colour of the pH indicator bromothymol blue to deep blue.

Typical Composition (g/litre)

Ammonium dihydrogen phosphate 1.0; di-potassium hydrogen phosphate 1.0; sodium chloride 5.0; sodium citrate 2.0; magnesium sulfate 0.2; bromothymol blue 0.08; agar-agar 13.0.

Preparation and Storage

Usable up to the expiry date when stored dry and tightly closed at +15 to +25° C. Protect from light.

After first opening of the bottle the content can be used up to the expiry date when stored dry and tightly closed at +15 to +25°C.

Suspend 22.3 g/litre, autoclave (15 min at 121°C), prepare slant agar tubes or pour plates.

pH: 6.6 ± 0.2 at 25 °C.

The plates or slants are clear and green.

Preparation of Klebsiella agar: Add 10g inositol/litre before autoclaving the culture medium.

Specimen

e.g. Isolated bacteria from stool .

Clinical specimen collection, handling and processing, see general instructions of use.

Experimental Procedure and Evaluation

Streak a pure culture of the microorganism to be tested on the surface of the culture medium.

Incubation: 24 - 48 hours at 35 °C aerobically.

Growth	Microorganisms
Positive, culture medium deep blue	Citrate-positive; Citrobacter, Enterobacter, S. paratyphi B., S. enteritidis, S.typhimurium, Arizona, Klebsiella, Serratia and others
Negative or inhibited	Citrate-negative: Escherichia, Shigella, S. typh., S. parathyphi A and others

Literature

American Public Health Association: Compendium methods for the microbiological examination of foods. - 3rd ed. 1992.

American Public Health Association, American Water Works Association and Water Pollution Control Federation: Standard Methods for the Examination of Water and Wastewater, 20th ed., Wash., 1998.

SIMMONS, J.S.: A culture medium for differentiating organisms of typhoid-colon aerogenes groups and for isolating of certain fungi. - **J. Infect. Dis., 39**; 209-241 (1926).

EWING, W.H. a. EDWARDS, P.R.: The principal divisions and groups of Enterobacteriaceae and their differentiation. - **Int. Bull. Bact. Nomencl. Taxon., 10**; 1-12 (1960).

VAN KREGTEN, E., WESTERDAHL, N.A.C., a. WILLERS, J.M.N.: New, simple medium for selective recovery of Klebsiella pneumoniae and Klebsiella oxytoca from human feces. - **J. Clin. Microbiol., 20** ; 936-941 (1984).

Ordering Information

Product	Merck Cat. No.	Pack size
SIMMONS Citrate Agar	1.02501.0500	500 g
myo-Inositol	1.04728.0100	100 g

Quality control

Test strains	Growth	Colour change to blue
Enterobacter cloacae ATCC 13047	good / verygood	+
Salmonella typhimurium ATCC 14028	good / verygood	+
Klebsiella pneumoniae ATCC 13883	good / verygood	+
Escherichia coli ATCC 25922	none / poor	-
Shigella flexneri ATCC 12022	none / poor	-
Morganella morganii ATCC 25830	none / poor	-

SOB Medium

Medium used for the enrichment of recombinant strains of E. coli.

SOB Medium was developed by HANAHAN (1983) for the preparation and transformation of competent cells.

SOC Medium, which can be used at the end of the transformation process, is prepared by adding 20 ml of a sterile filtered glucose solution (20 %) to 1 liter of SOB Medium (SAMBROOK et al., 1989).

Mode of Action

Tryptone and yeast extract serve as rich nutrients to allow good growth after the transformation. Sodium chloride and potassium chloride are added for optimal osmotic conditions.

Magnesiumsulfate delivery magnesium, which is needed in a lot of enzyme reactions s.a. the DNA replication.

Typical composition (g/Liter)

Tryptone 20.0; Yeast Extract 5.0; Sodium chloride 0.5; Magnesium sulfate (anhydrous) 2.4; Potassium chloride 0.186.

Preparation

Suspend 28 g in 1 liter of purified water with frequent stirring to dissolve completely. Autoclave at 121 °C for 15minutes.

pH: 7.0 ± 0.2 at 25 °C

The prepared medium is clear and yellowish-brown.

The final medium ca be stored for 6 months at 2-8 °C.

Experimental Procedure

Refer to recommended procedures/references.

Results

Growth is indicated in form of turbidity in the medium.

Literature

Hanahan, 1983. Studies on transformation of Escherichia coli with plasmids. **J. Mol. Biol. 166** :557.

Sambrook, J., E.F. Fritsch, and T. Maniatis. 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

Ordering Information

Product	Merck Cat. No.	Pack size
SOB Medium	1.01630.0500	500 g

Quality control

Test strains	Inoculum ca. cfu/ml	Growth after 24h at 35°C aerobically cfu/ml
Escherichia coli (C600) ATCC 23724	10	> 108
Escherichia coli (HB101) ATCC 33694	10	> 108
Escherichia coli (JM103) ATCC 39403	10	> 108
Escherichia coli (JM107) ATCC 47014	10	> 108
Escherichia coli (JM110) ATCC 47013	10	> 108
Escherichia coli (DH-5) ATCC 53868	10	> 108

Sodium chloride peptone broth (buffered)

For the dilution of samples in the analysis of non-sterile products for microbial contaminants.

This broth conforms with the German Pharmacopeia DAB10 (1991) guidelines.

Mode of Action

The combination of phosphate buffer, sodium chloride and peptone increases the viability of sensitive microorganisms in particular.

Typical Composition (g/litre)

Potassium dihydrogen phosphate 3.56; di-sodium hydrogen phosphate dihydrate 7.23; sodium chloride 4.3; peptone from pepsin-digested meat 1.0.

pH: 7.0 ± 0.2 at 25 °C.

Preparation

Suspend in demineralized water to give a concentration of 16.1 g/litre, aliquot into smaller vessels if required and, if necessary, add 1-10 ml Tween® 20 or 80 per litre of culture medium. Autoclave for 15 minutes at 121 °C.

The medium is clear and yellowish.

Application and Evaluation

Samples for colony count and for the detection of Pseudomonas aeruginosa and Staphylococcus aureus should be diluted according to DAB 10.

Incubation: 24 h at 35 °C aerobically.

Literature

Deutsches Arzneibuch DAB 10, 10th ed., 1991.

Ordering Information

Product	Merck Cat. No.	Pack size
Sodium chloride peptone broth (buffered)	1.10582.0500	500 g
Sodium chloride peptone broth (buffered)	1.10582.5000	5 kg
Teween® 20	8.22184.0500	500 ml
Tween® 80	8.22187.0500	500 ml

Quality control

Test strains	No decrease in colony count within 4 hours
Escherichia coli ATCC 8739	+
Staphylococcus aureus ATCC 6538-P	+

Sorbitol-MacConkey Agar (SMAC Agar)

Selective Agar for the direct isolation and differentiation of enterohemorrhagic (EHEC) E. coli 0157:H7-strains from foodstuffs and stool.

IVD *in vitro diagnosticum –*
For professional use only

See also General Instruction of Use
Warnings and precautions see ChemDAT®
(www.chemdat.info)

Date of issue: 28.09.2004

The culture medium complies with the requirements of the DIN Norm 10167 for the detection of Escherichia coli (E. coli) serotype 0157:H7 in foods as well as with the methods of FDA-BAM for the isolation of enterohemorrhagic E. coli (EHEC).

Principle

Microbiological method.

Mode of Action

Bile salts mixture and crystal violet largely inhibit the growth of the Gram-positive microbial flora. The addition of Cefixime Potassium tellurite (CT) Supplement increases the selectivity for E. coli 0157:H7 and suppresses the remaining accompanying flora. For the detection of E. coli 0157:H7 the CT-SMAC Agar method is superior to the HC Agar (SZABO) method according to the study of WEAGANT (1995).

Sorbitol, together with the pH indicator neutral red, is used to detect sorbitol-positive colonies and turning them red in colour. Sorbitol-negative strains, on the other hand, form colourless colonies.

Typical Composition (g/litre)

Peptone 20.0; sodium chloride 5.0; bile salts No. 3 1.5; sorbitol 10.0; crystal violet 0.001; neutral red 0.03; agar-agar 15.0.

Preparation and Storage

Usable up to the expiry date when stored dry and tightly closed at +15 to +25°C. Protect from light.

After first opening of the bottle the content can be used up to the expiry date when stored dry and tightly closed at +15 to +25°C.

Suspend 51.5 g in 1 litre of demin. water, autoclave (15 min at 121 °C), pour plates.

pH: 7.1 ± 0.2 at 25 °C.

The plates are clear and red and are stable for up to 6 months when stored at +2-8 °C.

CT-SMAC Agar

Suspend 25.8 g in 500 ml of demin. water, autoclave (15 min at 121°C).

Dissolve the lyophilisate of one CT Supplement (Cat.No.1.09202.) in the original vial by adding about 1ml of sterile distilled water.

Mix gently and add the contents to the sterile, still liquid SMAC Agar cooled below 50 °C. Pour plates.

pH: 7.1 ± 0.2 at 25 °C.

The plates are clear and red and are stable for up to 6 months when stored at +2-8 °C.

Specimen

e.g. Stool, urine.

Clinical specimen collection, handling and processing, see general instructions of use.

Experimental Procedure and Evaluation

Inoculate 25 g of the food sample in 225 ml mEC broth or mTSB broth and incubate for 18-24 h at 35-37 °C aerobically.

Approximately 0.1 ml of the broth is then streaked on the surface of SMAC Agar or CT-SMAC Agar in such a way to obtain well isolated single colonies.

Incubation: 18 to 24 h at 35 °C aerobically.

Stool specimens are inoculated directly onto the plates as loop smears and incubated at 37 °C for 18-24 h.

Colourless, sorbitol-negative colonies must be subsequently isolated and tested with special antisera.

Literature

DIN Deutsches Institut für Normung e.V.: Nachweis von Escherichia coli 0157 in Lebensmitteln. - **DIN 10167**.

FDA Bacteriological Analytical Manual, 8th Edition 1995, Chapter 4. Escherichia coli and the Coliform Bacteria, page 4.20: Isolation Methods for Enterohemorrhagic E. coli (EHEC).

WEAGANT, S.D., J.L. BRYANT, and K.G. JINNEMAN, An improved rapid technique for isolation of Escherichia coli 0157:H7 from foods. - **J. Food Prot., 58**; 7-12 (1995).

ZADIK, P.M., P.A. CHAPMAN, and C.A. SIDDONS, Use of tellurite for the selection of verocytotoxigenic Escherichia coli 0157. - **J. Med. Microbiol., 39**; 155-158 (1993).

Ordering Information

Product	Merck Cat. No.	Pack size
Sorbitol-MacConkey Agar (SMAC Agar)	1.09207.0500	500 g
CT-Supplement	1.09202.0001	1 x 16 vials
mEC Broth with Novobiocin	1.14582.0500	500 g
mTSB Broth with Novobiocin	1.09205.0500	500 g

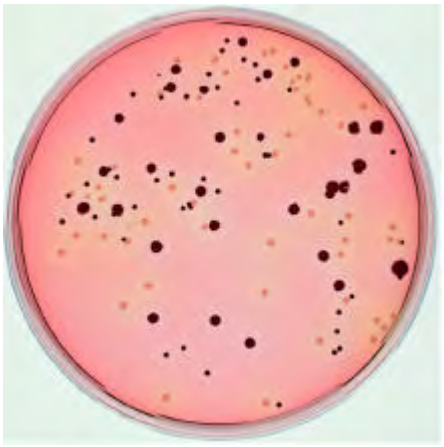
Sorbitol-MacConkey Agar (SMAC Agar)

Quality control SMAC Agar

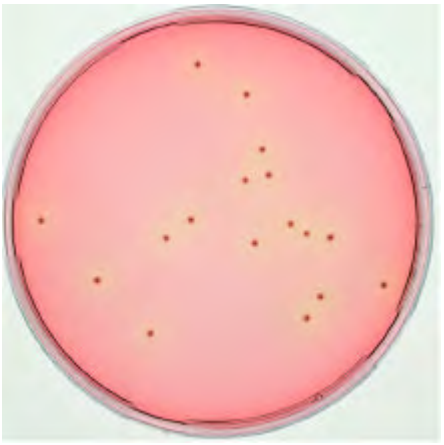
Test strains	Inoculum (cfu/ml)	Recovery rate %	Colony colour	Sorbitol
E. coli 0157:H7 ATCC 35150	10^3 - 10^5	≥ 70	colourless	-
E. coli ATCC 11775	10^3 - 10^5	≥ 70	red	+
Serratia marcescens ATCC 14756	10^3 - 10^5	≥ 70	red	+
Bacillus cereus ATCC 11778	> 10^5	≥ 0.01		

Quality control CT-SMAC Agar

Test strains	Inoculum (cfu/ml)	Recovery rate %	Colony colour	Sorbitol
E. coli 0157:H7 ATCC 35150	10^3 - 10^5	≥ 60	colourless	-
E. coli ATCC 11775	10^3 - 10^5	≤ 0.01		
E. coli ATCC 87639	> 10^5	≤ 0.01		
Serratia marcescens ATCC 14756	> 10^5	≤ 0.01		
Bacillus cereus ATCC 11778	> 10^5	≤ 0.01		



Colourless colonies: E. coli 0157:H7 (EHEC type)
Red colonies: E. coli and Servatia marcescens



Colourless colonies: E. coli 0157:H7 (EHEY type)
No growth of E. coli and Servatia marcescens

SPS Agar (Perfringens Selective Agar acc. to ANGELOTTI)

Sulfite Polymyxin Sulfadiazine Agar

Medium proposed by ANGELOTTI et al. (1962) for the isolation and enumeration of Clostridium perfringens and Clostridium botulinum in all types of foodstuffs.

Mode of Action

Sulfite Polymyxin Sulfadiazine Agar contains a broad spectrum of nutrients. Sulfite is reduced by most clostridia (including Cl. perfringens) to sulfide, which reacts with iron citrate and causes the colonies to turn black. Other sulfite-reducing microorganisms are largely suppressed by polymyxin and sulfadiazine (sulfapyrimidine). The low sulfite content allows growth of even sulfite-sensitive clostridia which also exhibit an adequate blackening of the colonies (PUT et al. 1961; BEERNS et al. 1961).

Typical Composition (g/litre)

Peptone from casein 15.0; yeast extract 10.0; iron(III) citrate 0.5; sodium sulfite 0.5; Polymyxin B sulfate 0.01; sodium sulfadiazine 0.12; agar-agar 13.9.

Preparation

Suspend 40 g/litre, autoclave (15 min at 121 °C).

pH: 7.0 ± 0.2 at 25 °C.

The prepared medium is clear and yellowish-brown.

Experimental Procdure and Evaluation

Mix the culture medium with the sample material (homogenized and diluted), pour into plates or tubes. Seal the tubes with sterile liquid paraffin. Place the plates in an anaerobic jar. Anaerocult®A, Anaerocult® A mini or Anaerocult® P can be used for this purpose.

Incubation: 24-48 hours at 35 °C.

Clostridia develop with black colonies. Further tests should be performed for purposes of identification.

Literature

ANGELOTTI, R., HALL, H.E., FOTER, M.J., a. LEWIS, K.M.: Quantitation of Clostridium perfringens in Foods. –**Appl. Microbiol., 10**; 193-199 (1962).

BEERENS, H., CASTEL, M.M., et LECLERC, H.: Contribution à l'étude des Milieux au sulphite de sodium pour l'isoelement des Clostridium. –**Ann. Inst. Pasteur Lille, 12**; 183-193 (1961).

PUT, H.M.C.: Sulphito-réduction et sulphito-sensibilité des Clostridia: considérations txonomiques et practiques. – **Ann. Inst. Pasteur Lille, 12**; 175-181 (1961).

Ordering Information

Product	Merck Cat. No.	Pack size
SPS Agar (Perfringens Selective Agar acc. to ANGELOTTI)	1.10235.0500	500 g
Anaerobic jar	1.16387.0001	1 ea
Anaeroclip®	1.14226.0001	1 x 25
Anaerocult® A	1.13829.0001	1 x 10
Anaerocult® A mini	1.01611.0001	1 x 25
Anaerocult® P	1.13807.0001	1 x 25
Anaerotest®	1.15112.0001	1 x 50
Paraffin viscous	1.07160.1000	1 l
Plate basket	1.07040.0001	1 ea

Clostridium perfringens ATCC 13124

Quality control

Test strains	Growth	Black colonies
Clostridium perfringens ATCC 10543	good / very good	+
Clostridium perfringens ATCC 13124	good / very good	+
Clostridium sporogenes ATCC 11437	good / very good	+
Escherichia coli ATCC 25922	none / fair	-
Pseudomonas aeruginosa ATCC 27853	none / poor	-

MPF

SS Agar (Salmonella Shigella Agar)

For the isolation of salmonellae and shigellae from faeces, foodstuffs and other materials.

IVD *in vitro diagnosticum –*
For professional use only

CE

See also General Instruction of Use
Warnings and precautions see ChemDAT®
(www.chemdat.info)

The medium complies with the recommendations of the APHA for the examination of food (1992).

Principle

Microbiological method

Mode of Action

Brilliant green, ox bile and high concentrations of thiosulfate and citrate largely inhibit the accompanying microbial flora. Sulfide production is detected by using thiosulfate and iron ions, the colonies turn black. The presence of coliform bacteria is established by detecting degradation of lactose to acid with the pH indicator neutral red.

Typical Composition (g/litre)

Peptones 10.0; lactose 10.0; ox bile 8.5; sodium citrate 10.0; sodium thiosulfate 8.5; ammonium iron(III) citrate 1.0; brilliant green 0.0003; neutral red 0.025; agar-agar 12.0.

Preparation and Storage

Usable up to the expiry date when stored dry and tightly closed at +15 to +25° C. Protect from light.

After first opening of the bottle the content can be used up to the expiry date when stored dry and tightly closed at +15 to +25°C.

Suspend 60 g/litre completely, pour plates.

■ **Do not autoclave.**

pH: 7.0 ± 0.2 at 25 °C.

The plates are clear and reddish-brown.

Specimen

e.g. Stool,

Clinical specimen collection, handling and processing, see general instructions of use.

Experimental Procecure and Evaluation

Spread the sample or material from an enrichment culture on the surface of the culture medium.

Incubation: 18-24 hours at 35 °C aerobically.

Lactose-negative colonies are colourless. Lactose-positive colonies are pink to red. Colonies of microorganisms producing H_2S have a black centre.

Appearance of Colonies	Microorganisms
Colourless, translucent	Shigella and some Salmonella species
Translucent with a black centre	Proteus and most Salmonella species
Pink to red	Escherichia coli
Colonies are larger than those of E. coli, pink to whitish or cream-coloured, opaque, mucoid	Enterobacter aerogenes

Literature

American Public Health Association: Compendium of methods for the microbiological examination of foods. – 3rd ed. (1992).

Ordering Information

Product	Merck Cat. No.	Pack size
SS Agar (Salmonella Shigella Agar)	1.07667.0500	500 g
Merckoplate® SS Agar	1.15178.0001	1 x 20 plates

SS Agar (Salmonella Shigella Agar)

Quality control (spiral plating method)

Test strains	Inoculum (cfu/ml)	Recovery rate %	Colony colour	Black centre	Colour change of medium
Klebsiella pneumoniae ATCC 13883	10^3-10^5	≥ 30	pink	-	pink-red (precipitate)
Shigella flexneri ATCC 29903	10^3-10^5	≥ 30	colourless	-	yellowish-brown
Salmonella typhimurium ATCC 14028	10^3-10^5	≥ 30	colourless	+	yellowish-brown
Salmonella enteritidis NCTC 5188	10^3-10^5	≥ 30	colourless	+	yellowish-brown
Proteus mirabilis ATCC 14273	10^3-10^5	≥ 30	colourless	+	yellowish-brown
Escherichia coli ATCC 25922	> 10^5	≤ 0.01	pink-red	-	pink-red (precipitate)
Staphylococcus aureus ATCC 25923	> 10^5	≤ 0.01			
Bacillus cereus ATCC 11778	> 10^5	≤ 0.01			

Salmonella enteritidis NCTC 5188

Shigella flexneri ATCC 29903

Standard Count Agar

Culture medium for determining the microbial count in milk, dairy products, water and wastewater.

This medium is highly suited as a base for preparing special culture media.

Typical Composition (g/litre)

Meat extract 3.0; peptone from casein (free from fermentable carbohydrates) 5.0; sodium chloride 5.0; agar-agar 12.0.

Preparation

Suspend 25 g/litre, autoclave (15 min at 121 °C).

pH: 7.2 ± 0.2 at 25 °C.

The prepared medium is clear and yellowish-brown.

Experimental Procedure and Evaluation

Depend on the purpose for which the medium is used.

Incubation: 24 h at 35 °C aerobically.

Ordering Information

Product	Merck Cat. No.	Pack size
Standard Count Agar	1.01621.0500	500 g

Quality control

Test strains	Growth
Staphylococcus aureus ATCC 25923	good / very good
Streptococcus agalactiae ATCC 13813	fair / good
Lactococcus lactis spp. lactis ATCC 19435	fair / good
Lactobacillus acidophilus ATCC 4356	poor / good
Escherichia coli ATCC 25922	good / very good
Pseudomonas aeruginosa ATCC 27853	good / very good
Bacillus cereus ATCC 11778	good / very good

Standard I Nutrient Agar

These culture media are suitable for the cultivation of fastidious bacteria; after addition of blood, ascites fluid or serum they can also be used to cultivate streptococci, pneumococci and erysipelas organisms etc. They are employed for the enumeration, isolation and enrichment of bacteria and also as high-grade bases for preparing special culture media.

IVD *in vitro diagnosticum – For professional use only*

See also General Instruction of Use
Warnings and precautions see ChemDAT®
(www.chemdat.info)

Principle

Microbiological method

Typical Composition (g/litre)

Peptones 15.0; yeast extract 3.0; sodium chloride 6.0; D(+)glucose 1.0; agar-agar 12.0.

Preparation and Storage

Usable up to the expiry date when stored dry and tightly closed at +15 to +25° C. Protect from light.

After first opening of the bottle the content can be used up to the expiry date when stored dry and tightly closed at +15 to +25° C.

Suspend 37 g Standard I Nutrient Agar/litre, autoclave (15 min at 121 °C).

pH: 7.5 ± 0.2 at 25 °C.

The prepared media are clear and yellowish-brown.

Specimen

e.g. Blood.

Clinical specimen collection, handling and processing, see general instructions of use.

Experimental Procedure and Evaluation

Depend on the purpose for which the media are used.

Incubation: 24 h at 35 °C aerobically.

Ordering Information

Product	Merck Cat. No.	Pack size
Standard I Nutrient Agar	1.07881.0500	500 g
Standard I Nutrient Agar	1.07881.5000	5 kg
Merckoplate® Standardl nutrient agar	1.10416.0001	1 x 20 plates

Escherichia coli ATCC 25922

Quality control (spiral plating method)

Test strains	Inoculum (cfu/ml)	Recovery rate %
Staphylococcus aureus ATCC 25923	10^3-10^5	≥ 70
Streptococcus pyogenes ATCC 12344	10^3-10^5	≥ 70
Streptococcus pneumoniae ATCC 6301	10^3-10^5	≥ 70
Listeria monocytogenes ATCC 19118	10^3-10^5	≥ 70
Erysipelothrix rhusiopathiae ATCC 19414	10^3-10^5	≥ 70
Escherichia coli ATCC 25922	10^3-10^5	≥ 70
Shigella flexneri ATCC 12022	10^3-10^5	≥ 70

Standard I Nutrient Broth

These culture media are suitable for the cultivation of fastidious bacteria; after addition of blood, ascites fluid or serum they can also be used to cultivate streptococci, pneumococci and erysipelas organisms etc. They are employed for the enumeration, isolation and enrichment of bacteria and also as high-grade bases for preparing special culture media.

in vitro diagnosticum –
For professional use only

See also General Instruction of Use
Warnings and precautions see ChemDAT®
(www.chemdat.info)

Principle

Microbiological method

Typical Composition (g/litre)

Peptones 15.0; yeast extract 3.0; sodium chloride 6.0; D(+)glucose 1.0.

Preparation and Storage

Usable up to the expiry date when stored dry and tightly closed at +15 to +25°C. Protect from light.

After first opening of the bottle the content can be used up to the expiry date when stored dry and tightly closed at +15 to +25°C.

Suspend 25 g Standard I Nutrient Broth/litre, autoclave (15 min at 121 °C).

pH: 7.5 ± 0.2 at 25 °C.

The prepared media are clear and yellowish-brown.

Specimen

e.g. Blood .

Clinical specimen collection, handling and processing, see general instructions of use.

Experimental Procedure and Evaluation

Depend on the purpose for which the media are used.

Incubation: 24 h at 35 °C aerobically.

Ordering Information

Product	Merck Cat. No.	Pack size
Standard I Nutrient Broth	1.07882.0500	500 g

Quality control

Test strains	Growth
Staphylococcus aureus ATCC 25923	good / very good
Streptococcus pyogenes ATCC 12344	good / very good
Streptococcus pneumoniae ATCC 6301	good / very good
Listeria monocytogenes ATCC 19118	good / very good
Erysipelothrix rhusiopathiae ATCC 19414	fair / good
Escherichia coli ATCC 25922	good / very good
Shigella flexneri ATCC 12022	good / very good

Standard II Nutrient Agar

This agar can be used for the cultivation and enrichment of less fastidious bacteria, for setting up stock cultures and as bases for preparing special culture media.

LEVETZOW (1971) reported that Standard II Nutrient Agar can be used in the detection of inhibitors in the bacteriological examination of meat. See also Test Agar pH 6.0, pH 8.0 and pH7.2. These media are specially developed for the examination of meat. ZAVANELLA et al. (1986) modified the medium with various additives and used it as a test comparable but simpler than the EEC four-plate-test.

Typical Composition (g/litre)

Peptone from meat 3.45; peptone from casein 3.45; sodium chloride 5.1; agar-agar 13.0

Preparation

Suspend 25 g/litre, autoclave (15 min at 121 °C).

pH: 7.5 ± 0.2 at 25 °C.

The plates are clear and yellowish-brown.

Experimental Procedure and Evaluation

Depend on the purpose for which the medium is used.

Incubation: 24 h at 35 °C aerobically.

Literature

Deutsches Fleischbeschaugesetz, Ausführungsbestimmungen A über die Untersuchung und gesundheitspolizeiliche Behandlung der Schlachttiere und des Fleisches bei Schlachtungen im Inland; Anlage 4 zu § 20 Abs. 4: Rückstandsuntersuchung.

DIN Deutsches Institut für Normung e.V.: Deutsche Einheitsverfahren zur Wasser-, Abwasser- und Schlammuntersuchung. Mikrobiologische Verfahren (Gruppe K). Nachweis von Pseudomonas aeruginosa (K 8).
- **DIN38411**.

LEVETZOW, R.: Untersuchung auf Hemmstoffe im Rahmen der bakteriologischen Fleischuntersuchung. – **Bundesgesundheitsblatt, 14**; 211-213 (1971).

ZAVANELLA, M., AURELIA, P., a. FERRINI, A.M.: Improved microbiological method for the detection of antimicrobial residues in meat.
- **Arch. Lebensmittelhyg., 37**; 118-120 (1986).

Ordering Information

Product	Merck Cat. No.	Pack size
Standard II Nutrient Agar	1.07883.0500	500 g

Quality control (spiral plating method)

Test strains	Inoculum (cfu/ml)	Recovery rate %
Escherichia coli ATCC 11775	10^3-10^5	≥ 70
Salmonella typhimurium ATCC 13311	10^3-10^5	≥ 70
Shigella flexneri ATCC 29903	10^3-10^5	≥ 70
Staphylococcus aureus ATCC 6538	10^3-10^5	≥ 70
Streptococcus pyogenes ATCC 21059	10^3-10^5	≥ 70

Staphylococcus Enrichment Broth Base acc. BAIRD

For the selective enrichment of coagulase-positive staphylococci acc. to § 35 LMBG (German food inspection legislation).

Mode of Action

Lithium chloride and potassium tellurite inhibit most of the accompanying flora, whereas the remaining components of the culture medium permit satisfying growth of staphylococci.

Typical Composition (g/litre)

Peptone from meat 8.0; peptone from casein 2.0; yeast extract 1.0; meat extract 5.0; sodium pyruvate 10.0; glycine 12.0; lithium chloride 5.0.

Preparation

Suspend 43 g/litre, fill into test tubes, autoclave (15 min at 121°C). Prior to use add 0.1 ml filter-sterilized 1 % potassium tellurite solution to 9 ml of base medium below 45 °C.

pH: 6.6 ± 0.2 at 25 °C.

The prepared culture medium base can be stored in a refrigerator for up to one month. It is clear and yellowish-brown.

Experimental Procedure and Evaluation

According to § 35 LMBG inoculate 1 ml of sample material in 9ml of culture medium. Incubate up to 48 hours at 35 °C.

Literature

Amtliche Sammlung von Untersuchungsverfahren nach § 35 LMBG; Untersuchung von Lebensmitteln. Bestimmung koagulase-positiver Staphylokokken in Trockenmilcherzeugnissen und Schmelzkäse. – L 02.07.

Ordering Information

Product	Merck Cat. No.	Pack size
Staphylococcus Enrichment Broth Base acc. BAIRD	1.07899.0500	500 g
Potassium tellurite trihydrate	1.05164.0100	100 g

Quality control

Test strains	Inoculum	Growth after 24 hours
Escherichia coli ATCC 25922	approx. 99 %	≤ 5 %
Staphylococcus aureus ATCC 6538	approx. 1 %	≥ 95 %

Sterikon® plus Bioindicator

Autoclaving control

For professional use only.

Application

Using the Merck Sterikon® plus Bioindicator System it is possible to check the efficiency of autoclaving cycle for 15 minutes at 121°C.

Furthermore it is possible to control the sterilization success of any kind of autoclave-loading after autoclaving.

For example:
pharmaceuticals, especially drugs in ampoule form, canned food, culture media, etc.

In the USP and EP the use of a bioindicator for the autoclavation control of pharmaceutical products is recommended.

Principle

The Sterikon® plus Bioindicator consists of an ampoule that contains a nutrient broth, sugar, a pH indicator and spores of a non-pathogenic organism, Geobacillus stearothermophilus ATCC 7953 (sporulation optimized). The thermal resistance is such that the spores are completly killed after 15 minutes, when heated in compressed steam at a temperature of 121 ± 0.5°C (245kPa). At lower temperatures or lower exposure times a smallnumber of spores can survive and are capable of growing.

The ampoules are placed into the autoclave along with the batch to be autoclaved. After autoclaving, the success of the sterilization process is checked by incubation of the ampoules:

No growth of Geobacillus stearothermophilus indicates adequate sterilization, whereas growth shows inadequate sterilization.

Procedure

An appropriate number of ampoules are included in the batch to be autoclaved. Autoclaves with a capacity of up to 250litres should be checked with at least 2 ampoules, whilst those with a capacity of more than 250 litres should have at least 6 ampoules. In order to avoid contamination by accidental breakage, it is advisable to place the ampoules in a glass beaker.

The ampoules are placed in the autoclave at sites where the most unfavourable conditions for sterilization are thought to exist, i.e. at the bottom and in the middle of the autoclave. If a single large volume of material is to be autoclaved (e.g. flasks containing a liquid), a test using the bioindicator is possible only when the ampoule is placed in the centre of the vessel in question (e.g. suspended in a flask or immersed in the contents of a tin of food). The Sterikon® plus Bioindikator can also be used to check the functional efficiency, i.e. to test whether the prescribed temperature of 121°C is reached within the entire autoclave and whether the temperature remains constant over the whole of the prescribed period of 15 minutes.

After sterilization, the ampoules are removed and incubated 48hours at 60 ± 2 °C. A non-sterilized ampoule should also be incubated to serve as a control. Use of the ampoules at temperatures exceeding 125°C sterilization temperature should be avoided to prevent the possibility of damaging the bioindicator.

Evaluation

If sterilization is adequate, the Geobacillus stearothermophilus spores are killed off. The contents of the ampoule remains a clear red-violet colour.

If sterilization is inadequate, some spores of Geobacillus stearothermophilus survive. The contents of the ampules then usually turn yellow-orange within 24 hours due to the formation of acid as a result of sugar fermentation and also become turbid due to microbial growth. In cases in which the spores are partially damaged, the reaction may be delayed.

The contents of the control ampoule also turn yellow-orange and become slightly turbid.

Stability

When stored at the prescribed temperature (+2 to +8 °C) in the refrigerator, the bioindicator is stable at least up to the expiry date printed on the pack.

Storage

The ampoules should be stored in the regrigerator at +2 to +8 °C. Storage at room temperature (up to approx. 25°C) is possible for a limited period of about 1-2weeks. Storage at temperatures exceeding +30C effects the product stability.

Specifications

The specifications of Sterikon® plus Bioindicator are as follows:

n	= 5×10^5 - 1×10^7 spores per unit
$\mathbf{D_{121}}$	= 1.5 to 2.0 minutes

Acc. to the USP the heat-resistance and the number of spores are optimized, when after a sterilization time of 6 minutes at 121± 0.5°C all ampoules contain living spores, whereas after 15minutes autovlaving at 121± 0.5°C all spores are dead. For the period in between there will be come ampoules which contain living spores and some ampoules where all spores are dead.

The spores are already in a nutrient broth.

Literature

I.D. costin, J. Grigo: Bioindikatoren zur Autoklavierungskontrolle. Einige theoretische Aspekte u. praktische erfahrungen bei der Entwicklung und Anwendung. - Zbl. Bakt. hyg., I. Orig. A. **227**, 483-521 (1974).

H. Seyfarth: Vorschriften der USP XXIV für die Durchführung der Sterilitätsprüfung. - Pharm. Ind. **37**/2, 87-91 (1975).

J. Grigo, I.D. Costin: Vorschriften der USP XXIV für die Anwendung von Bioindikatoren zur Sterilitätskontrolle. - Pharm. Ind. **37**/3, 179-181 (1975).

N. Holstein: Untersuchungen zur Funktionsprüfung von Autoklaven mittels Bioindikatoren. - Zbl. Bakt. Hyg., I. Orig. **160**, 443-457 (1975).

United States Pharmacopoeia 23 (1995).

European Pharmacopoeia, 3rd edition 1992.

Sterikon® plus Bioindicator

Autoclaving control

Ordering Information

Product	Merck Cat. No.	Pack size
Sterikon® plus Bioindicator	1.10274.0001	Pack containing 15ampoules, each with 2ml of spore suspension
Sterikon® plus Bioindicator	1.10274.0002	Pack containing 100 ampoules, each with 2ml of spore suspension

Non-sterile = yellow-orange (growth)

Sterile = red-violet (no growth)

AC
MPF
O

Sulfite Iron Agar, Base

For the detection and enumeration of clostridia in meat and meat products.

This culture medium complies with the recommendations of the International Organization for Standardization (ISO) (1971).

Mode of Action

Brief heating (1 min at 80 °C) kills the vegatative cells present in the sample material whilst the bacterial spores survive and germinate. The H_2S-positive ones reduce the sulfite in the culture medium to sulfide, which reacts with iron to form black iron sulfide. This stains the concerning colonies black and the H_2S-weakly-positives brown. In an anaerobic environment clostridia grow to form black colonies under these conditions.

Typical Composition (g/litre)

Peptone from casein 15.0; yeast extract 10.0; sodium sulfite 0.5; agar-agar 15.0.

Also to be added:

Iron(II) sulfate 1.4.

Preparation

Suspend 40.5 g/litre, if desired dispense into small flasks, autoclave (15 min at 121 °C); at about 50 °C add 20 ml of a 7% iron(II) sulfate solution/litre, mix and pour plates.

pH: 6.9 ± 0.2 at 25 °C.

The plates are clear and yellowish to yellowish-green.

Experimental Procedure and Evaluation

Preparation of the sample: in accordance with the ISO recommendations, homogenize the comminuted sample with 9 times its own weight of sterile diluent solution (peptone from casein 0.1 %, cysteinium chloride 0.05 %, sodium chloride 0.85%). Dispense 50 ml aliquots into 100ml flasks and heat for 1 minute at 80°C in a water bath. Cool immediately in cold water.

Spread the sample material thinly on duplicate Sulfite Iron Agar plates. Incubate one plate aerobically and the other anerobically for 2 days at 35 °C. Mesophilic clostridia are presently, if

a. only the anaerobically incubated plates show blackening and
b. a catalase thest using Bactident® Catalase performed on these plates is negative.
   Gas formation: culture is catalase-positive.
   No gas formation: culture is catalase-negative.

Note: when using vented Petridishes, the medium needs overlayer and anaerobic incubation to get black colonies.
Alternative in tubes: mixing in method, aerobic incubation

Literature

International Organization for Standardization (ISO): Meat and Meat Products. - Mesophilic Clostridial Spores - Working Draft ISO/TC/34/SC6 (1971).

Ordering Information

Product	Merck Cat. No.	Pack size
Sulfite Iron Agar, Base	1.10864.0500	500 g
Anaerobic jar	1.16387.0001	1 ea
Anaeroclip®	1.14226.0001	1 x 25
Anaerocult® A	1.13829.0001	1 x 10
Anaerocult® a mini	1.01611.0001	1 x 25
Anaerocult® P	1.13807.0001	1 x 25
Anaerotest®	1.15112.0001	1 x 50
Bactident® Catalase	1.11351.0001	1 x 30 ml
Iron(II)sulfate heptahydrate	1.03965.0100	100 g
L-Cysteinium chloride monohydrate	1.02839.0025	25 g
Peptone from casein	1.07213.1000	1 kg
Plate basket	1.07040.0001	1 ea
Sodium chloride purified	1.06400.1000	1 kg

Sulfite Iron Agar, Base

Quality control

Test strains	Growth	Black colonies
Clostridium perfringens ATCC 10543	good / very good	+
Clostridium perfringens ATCC 13124	good / very good	+
Clostridium botulinum	good / very good	+
Clostridium tetani ATCC 19406	good / very good	+
Escherichia coli ATCC 25922	fair / very good	-
Pseudomonas aeruginosa ATCC 27853	poor / good	-
Bacillus cereus ATCC 11778	poor / good	-

Bacillus cereus ATCC 11778

Clostridium botulinum

Clostridium perfringens ATCC 13124

Clostridium tetani ATCC 19406

TAT Broth (Base)

Casein-peptone Lecithin Polysorbate Broth (Base)

For diluting samples of pharmaceutical, cosmetic and other raw materials or final products when determining microbial counts.

This broth complies with the recommendations of the United States Pharmacopeia XXVI (2003).

Mode of Action

The relatively high casein peptone content of this medium provides optimal conditions for the spore germination and regeneration of even damaged microorganisms. Lecithin and polysorbate 20 inactivate many antimicrobial compounds. KOHN et al. (1963), CHIORI et al. (1965) and HUGO and FRIER (1969) reported that soya lecithin inactivates cetrimide, chlorohexidine, chlorinated phenols, desqualinium acetate and polymyxin B. According to EVANS (1964) and BROWN (1966), polysorbate 20 inactivates phenols, phenol derivatives, benzoic acid, p-hydroxybenzoic acid and their esters. The combination of the two can inactivate quaternary ammonium and phosphonium compounds. Thioglycollate has not been included to inactivate preservatives containing heavy metals as the thioamino acids of the casein peptone inactivate most of these compounds and TREADWELL et al. (1958) and GIBBS (1964) have shown that thioglycollate inhibits the spores of many Bacillus and Clostridium species, especially when they are already damaged by heat.

Typical Composition (g/litre)

Peptone from casein 20.0; soya lecithin 5.0.

Also to be added:

polysorbate (Tween® 20) 40 ml.

Preparation

Suspend 25 g in 0.96 litre in accordance with USP, heat in a water bath set at 50 °C for about 30 minutes until completely dissolved. Add 40 ml polysorbate, autoclave (15 min at 121°C).

pH: 7.1 ± 0.2 at 25 °C.

The prepared broth is clear and yellowish; slight turbidity may occur because of the contents of lecithin.

Experimental Procedure and Evaluation

As recommended by USP or in accordance with the purpose for which the medium is used.

Literature

BROWN, M.R.W.: Turbidimetric method for the rapid evaluation of antimicrobial agents. Inactivation of preservatives by nonionic agent. – **J. Soc. Cosm. Chem., 17**; 185-195 (1966).

CHIORO, C.O., MAMBLETON, R.Q., a. RIGBY, G.: The inhibition of spores of Bacillus subtilis by cetrimide retained on washed membrane spores. – **J. Appl. Bact., 28**; 322-330 (1965).

EVANS, W.P.: The solubilisation and inactivation of preservatives by nonionic detergents. – **J. Pharm. Pharmacol., 16**; 323-331 (1964).

GIBBS, P.A.: Factors affecting the germination of spores of Clostridium bifermentans. – **J. gen. Microbiol., 37**; 41-48 (1964).

HUGO, W.B., a. FRIER, M.: Mode of action on the antibacterial compound desqualinium acetate. – **Appl. Microbiol., 17**; 118-127 (1969).

KOHN, S.R., GERSHENFELD, L., a. BARR, M.: Effectiveness of antibacterial agents presently employed in ophthalmic preparations as preservatives against Pseudomonas aeruginosa. – **J. Pharm. Sci., 52**; 967-974 (1963).

TREADWELL, P.E., JANN, G.J., a. SALLE, A.J.: Studies on factors affecting the rapid germination of spores of Clostridium botulinum. – **J.Bact., 76**; 549-556 (1958).

United States Pharmacopeia XXVI. Chapter "Microbial Limit Tests", 2003.

Ordering Information

Product	Merck Cat. No.	Pack size
Casein-peptone Lecithin Polysorbate Broth (Base)	1.11723.0500	500 g
Tween® 20	8.17072.1000	1000 ml

Quality control

Test strains	Growth
Escherichia coli ATCC 25922	good
Streptococcus pyogenes ATCC 12344	good
Staphylococcus aureus ATCC 25923	good
Clostridium perfringens ATCC 10543	good
Candida albicans ATCC 10231	good

TBG-Broth (Tetrathionate-Brilliant-green Bile Enrichment Broth), modified

Selective enrichment of Salmonella for the examination of pharmaceutical products in raw materials as well as feces, foodstuffs, meat etc.

The broth complies with the recommendations of the German DAB 10 and the European Pharmacopeia II.

Note: for granting good functioning of the TBG-Broth, the preparation and the incubation temperature had to be modified versus the recommendations of DAB 10/EP.

Mode of Action

Bile supports the growth of enteric bacteria and inhibits bacteria, which do not normally live in the intestine.

Brilliant-green specifically inhibits the Gram-positive accompanying flora. For suppressing Proteus, the pH of the medium can be adjusted to approx. 6.5. JEFFRIES (1959) reported that it is advantageous to add 0.04 g/litre novobiocin for suppressing Proteus.

Typical Composition (g/litre)

Peptone 8.6; ox-bile 8.0; sodium chloride 6.4; calcium carbonate 20.0; potassium tetrathionate 20.0; brilliant green 0.07.

Preparation

Suspend 63 g in 1 litre of demin. water, if needed short heating to a maximum of 50 °C. Any undissolved calcium carbonate should be homogenously mixed before pouring.

- **Do not autoclave!**
- adjust pH to a value of 7.0 ± 0.2.

The preparared broth is turbid, green with white sediment.

Experimental Procedure and Evaluation

1. Sample to be enriched in Lactose-Broth.
2. From the pre-enrichment inoculate an appropriate amount into TBG-Broth and incubate for 18-24 h at 35-37 °C.
3. Streak onto appropriate Salmonella media.
4. Brown cultures to be examined further.

Literature

Deutsches Arzneibuch (DAB), 10. Auflage, Kapitel VIII, 10.

European Pharmacopeia II, Kapitel VIII, 10

JEFFRIES, L.: Novobiocin-tetrathionate broth: A medium of improved selectivity for the isolation of salmonellae from faeces. - **J. Clin. Path., 12**; 568-571 (1959)

Ordering Information

Product	Merck Cat. No.	Pack size
TBG-Broth (Tetrathionate-Brilliant-green Bile Enrichment Broth), modified	1.05178.0500	500 g
BPLS Agar (USP)	1.07232.0500	500 g
Lactose Broth	1.07661.0500	500 g
LEIFSON Agar	1.02896.0500	500 g
XLD Agar	1.05287.0500	500 g

Quality control

Test strains	Inoculum	Growth 6 h	after 24 h
Escherichia coli ATCC 25922	approx. 99 %	≤ 30 %	≤ 5 %
Salmonella typhimurium ATCC 14028	approx. 1 %	≥ 70 %	≥ 95 %

TB Medium Base acc. to LÖWENSTEIN-JENSEN

Medium proposed by LÖWENSTEIN (1931) and modified by JENSEN (1932) for the detection and testing the resistance of tubercle bacilli.

This culture medium complies with recommendations of the Bull. Int. (1954) and the DIN-Norm 58943.

Typical Composition (g/1.6 litre)

Potassium dihydrogen phosphate 2.5; magnesium sulfate heptahydrate 0.24; tri-magnesium dicitrate 14-hydrate 0.6; L-asparagine 3.6; potato meal 30.0; malachite green 0.4.

Also to be added:

Glycerol 12 ml; whole-egg homogenate 1 litre.

Preparation

Suspend 37.5 g in 0.6 litre demin. water, if required add 12 ml glycerol, mix, autoclave (15 min at 121 °C). Cool to about 50 °C, add 1 litre whole-egg homogenate prepared from fresh hen eggs under sterile conditions; stir to give a homogeneous mixture avoiding formation of bubbles. Dispense into sterile test tubes and allow to coagulate in a slant position by heating for 45minutes at 85 °C in an inspissator saturated with water vapour or in free-flowing steam. The culture medium should be heated once more in this way after about 24 hours to guarantee its sterility.

pH: 4.8 ± 0.2 at 25 °C (before adding the homogenate).

The prepared medium is green and non-transparent.

Experimental Procedure and Evaluation

Inoculate the culture medium massively by spreading the sample on the surface. Use the glycerol-free culture medium when culturing glycerophobic mycobacteria.

Incubation up to 2 weeks at 35 °C.

Check the tubes for colony growth after 10-14 days and then in weekly intervals. The cultures should be carefully aerated.

Appearance of Colonies	Microorganisms
On medium containing glycerol, colony growth is "eugonic", i.e. abundant, raised, crumbly, dry, usually yellowish (navel form). This growth pattern develops poorly on medium which does not contain glycerol	Mycobacterium tuberculosis type humanus (R-variant)
On medium containing glycerol, sparse growth or no growth at all. On medium without glycerol growth is "dysgonic", i.e. flat, moist, glossy, confluent colonies (often nipple form) without pigment formation	type bovinus (S-variant)
Rapid growth in the form of a moist, fairly abundant "lawn":	
optimal temperature 41-42 °C	type poikilothermorum
optimal temperature 25 °C	type gallinaceus

Literature

Bull. Intern. Un. Tuberc., 24; 102 (1954).

CRUCKSHANK, R.A., STEWART, S.M.: Detection of resistance to streptomycin PAS and isoniazid in tubercle bacilli (Assoc. of Clin. Pathologists, broadsheet No. 32, 1961).

DIN Deutsches Institut für Normung e.V.: Tuberkulosediagnostik, Modifiziertes Löwenstein-Jensen-Kulturmedium für Anzüchtung von Tuberkulosebakterien - **DIN 58943**.

JENSEN, K.A.: Reinzüchtung und Typenbestimmung von Tuberkelbazillen-stämmen. - **Zbl. Bakt. I. Orig., 125**; 222-239 (1932).

LÖWENSTEIN, E.: Die Züchtung der Tuberkelbazillen aus dem strömenden Blute. - **Zbl. Bakt. I. Orig., 120**; 127-129 (1931).

Ordering Information

Product	Merck Cat. No.	Pack size
TB Medium Base acc. to LÖWENSTEIN-JENSEN	1.05400.0500	500 g
Glycerol (about 87 %)	1.04094.0500	500 ml

Quality control

Test strains	Growth
Mycobacterium tuberculosis ATCC 25177	good / very good
Mycobacterium fortuitum ATCC6841	good / very good
Mycobacterium kansasii ATCC 12478	good / very good
Mycobacterium phlei ATCC11758	good / very good
Mycobacterium smegmatis ATCC 14468	good / very good

AC
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TCBS Agar (Vibrio Selective Agar)

Thiosulfate Citrate Bile Sucrose Agar

Thiosulfate Citrate Bile Sucrose Agar proposed by NAKANISHI (1962), modified by KOBAYASHI et al. (1963) is used for the isolation and selective cultivation of Vibrio cholerae and other enteropahtogenic vibrios (V. parahaemolyticus, NAG vibrios).

IVD *in vitro diagnosticum – For professional use only*

See also General Instruction of Use
Warnings and precautions see ChemDAT®
(www.chemdat.info)

This culture medium complies with the recommendations of the World Health Organization WHO (1965, 1967) and the APHA (1992).

Principle

Microbiological method

Mode of Action

The high concentrations of thiosulfate and citrate and the strong alkalinity of this medium largely inhibit the growth of Enterobacteriaceae. Ox bile and cholate suppress primarily enterococci. Any coliform bacteria, which may grow, cannot metabolize sucrose. Only a few sucrose-positive Proteus strains can grow to form yellow, vibrid-like colonies. The mixed indicator thymol blue-bromothymol blue changes its colour to yellow, when acid is formed, even in this strongly alkaline medium.

Typical Composition (g/litre)

Peptone from casein 5.0; peptone from meat 5.0; yeast extract 5.0; sodium citrate 10.0; sodium thiosulfate 10.0; ox bile 5.0; sodium cholate 3.0; sucrose 20.0; sodium chloride 10.0; iron(III) citrate 1.0; thymol blue 0.04; bromothymol blue 0.04; agar-agar 14.0.

Preparation and Storage

Cat. No. 1.10263. TCBS Agar (Vibrio Selective Agar) (500 g)

Usable up to the expiry date when stored dry and tightly closed at +15 to +25° C. Protect from light.

After first opening of the bottle the content can be used up to the expiry date when stored dry and tightly closed at +15 to +25° C.

Suspend 88 g/litre and pour plates.

■ **Do not autoclave.**

pH: 8.6 ± 0.2 at 25 °C.

The plates are clear and green-blue.

Specimen

e.g. Stool

Clinical specimen collection, handling and processing, see general instructions of use.

Experimental Procedure and Evaluation

Inoculate by spreading the sample or material from an enrichment culture, Alkaline Peptone water, on the surface of the plates.

Incubation: 18-24 hours at 35 °C aerobically.

According to BURKHARDT (1969), it is advised to use, in addition to a liquid enrichment medium, two different solid culture media - a highly selective (e.g. TCBS Agar) and a less selective culture medium (e.g. Nutrient Agar: Merck Cat. No.1.05450.).

Appearance of Colonies	Microorganisms
Appearance of Colonies	Microorganisms
Flat, 2-3 mm in diameter, yellow	Vibrio cholerae, Vibrio cholerae type El Tor
Small, blue-green centre	Vibrio parahaemolyticus
Large, yellow	Vibrio alginolyticus
Blue	Pseudomonas, Aeromonas and others
Very small, translucent	Enterobacteriaceae and others

Further tests are necessary for complete identification (MUCKERJEE 1961, FINKELSTEIN and MUCKERJEE 1963, ROY et al. 1965, BOCKEMÜHL 1974 etc.).

Literature

American Public Health Association: Compendium of methods for the microbiological examination of foods. – 3rd edition (1992).

BOCKEMÜHL, J.: Einfache Laboratoriumsdiagnostik der El Tor-Cholera. - **Ärztl. Lab., 20**; 32-41 (1974).

BURKHARDT, F.: Die bakteriologische Diagnose der Vibrio El Tor-Infektion. - **Zbl. Bakt. I. Orig., 212** ; 177-189 (1969).

FINKELSTEIN, R.A., a. MUCKERJEE, S.: Haemagglutination a rapid method for differentiating V. cholerae and El Tor vibrios. - **Proc. Soc. Exp. Biol. 112**; 335-359 (1963).

KAMPELMACHER, E.H., MOSSEL, D.A.A., VAN NOORLE-JANSEN, a. VINCENTIE, H.: A survey on the occurrence of Vibrio parahaemolyticus on fish and shellfish, marketed in the Netherlands. - **J. Hyg. Camp., 68**; 189-196 (1970).

KOBAYASHI, T., ENOMOTO, S., SAKAZAKI, R., a. KUWAHARA, S.: A new selective isolation medium for pathogenic vibrios: TCBS-Agar. - **Jap. J. Bact., 18** ; 391-397 (1963).

MUCKERJEE, S.: Diagnostic use of bacteriophage. - **J. Hyg., 59;** 109-115 (1961).

NAKANISHI, Y.: An isolation agar medium for cholerae and enteropathogenic halophilic vibrios. - **Modern Media, 9**; 246 (1963).

ROY, C., MRIDHA, K., a. MUCKERJEE, S.: Action of polymyxin on cholera vibrios. Techniques of determinatoin of polymyxin sensitivity. - **Proc. Soc. Exp. Biol., 119**; 893-896 (1965).

WHO Expert Committee on Cholera (2nd Rep. Techn. Rep. Series No. 352, 1967). WHO: Cholera Information (1965).

TCBS Agar (Vibrio Selective Agar)

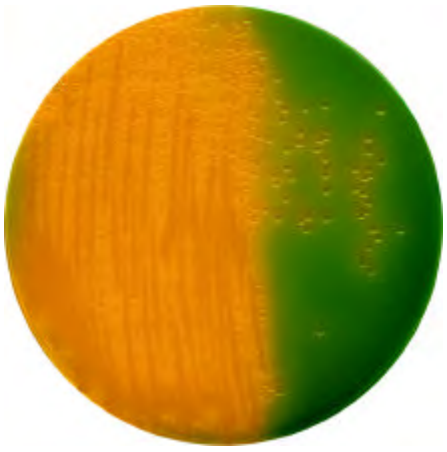
Thiosulfate Citrate Bile Sucrose Agar

Ordering Information

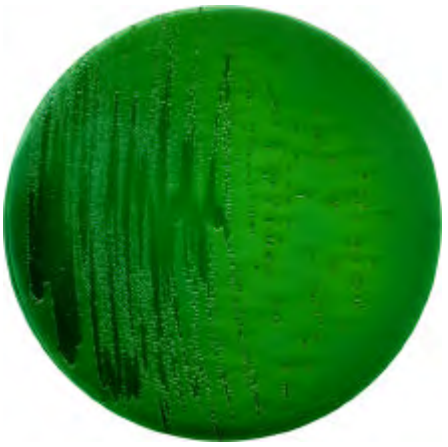
Product	Merck Cat. No.	Pack size
TCBS Agar (Vibrio Selective Agar)	1.10263.0500	500 g
Alkaline Peptone water	1.01800.0500	500 g

Quality control

Test strains	Growth	Colour change to yellow
Vibrio alginolyticus	good / very good	+
Vibrio cholerae Inaba NIH 35	good / very good	+
Vibrio cholerae El Tor Inaba CH 38	good / very good	+
Vibrio cholerae Ogawa NIH 41	good / very good	+
Vibrio cholerae El Tor Ogawa CH 60	good / very good	+
Vibrio parahaemolyticus ATCC 17802	good / very good	-
Escherichia coli ATCC 25922	none / poor	-
Enterobacter cloacae ATCC 13047	none / poor	-
Proteus mirabilis ATCC 14273	none / poor	-
Pseudomonas aeruginosa ATCC 27853	none / poor	-



Vibrio cholerae Inaba NIH 35



Vibrio parahaemolyticus ATCC 17802

Terrific Broth

For cultivating recombinant strains of Escherichia coli.

Terrific Broth was developed by TARTOFF and HOBBS (1987) to improve yield of plasmid DNA from transformed E. coli.

Mode of Action

Tryptone and yeast extract serve as nutritious base to allow higher plasmid yield. The medium is phosphate buffered to prevent cell death due to a drop inpH. Glycerol serves as carbon and energy source.

Typical Composition (g/liter)

Tryptone 12.0; Yeast Extract 24.0; Potassium Hydrogen Phosphate, dibasic 9.4; Potassium Phosphate, monobasic 2.2.

Preparation

Suspend 47,6 g in 1 liter of purified water, add 4 ml Glycerol and autoclave for 15 minutes at 121°C.

pH: 7.2 ± 0.2 at 25 °C.

the prepared medium is clear and yellowish-brown. When stored at 2-8 °C in the refrigerator the medium can be used for up to 4 weeks.

Experimental Procedure and Evaluation

Use appropriate references for recommended test procedures.

Results

Growth is indicated when the medium gets turbid.

Literature

Tartoff, K.D., and c.a. Hobbs. 1987. Improved media for growing plasmid and cosmid clones. Bethesda Research Laboratories Focus **9**:12.

Sambrook, J., E. F. Fritsch, and T. Maniatis. 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

Ordering Information

Product	Merck Cat. No.	Pack size
Terrific Broth	1.01629.0500	500 g
Glycerol	1.04093.1000	1000 ml

Qaulity control

Test strains	Inoculum ca. cfu/ml	Growth after 24 h at 35°C aerobically cfu/ml
Escherichia coli (C600) ATCC 23724	10	> 10^8
Escherichia coli (HB101) ATCC 33694	10	> 10^8
Escherichia coli (JM103) ATCC 39403	10	> 10^8
Escherichia coli (JM107) ATCC 47014	10	> 10^8
Escherichia coli (JM110) ATCC 47013	10	> 10^8
Escherichia coli (DH-5) ATCC 53868	10	> 10^8

Test Agar for the Residue Test acc. to KUNDRAT

Medium introduced by KUNDRAT (1968, 1972) for the routine qualitative detection of residues of antibiotics, sulfonamides and other chemotherapeutic agents in meat and other foodstuffs derived from animals.

The test is carried out using a spore suspension of Bacillus stearothermophilus (MERCK, Cat. No. 1.11499.) as test microorganisms. Cleaning agents, disinfectants and preservatives are not covered by this test. The medium can be used for rapid or long-term tests.

Mode of Action

The test is performed in the form of an agar diffusion test. Any inhibitors present produce inhibition zones devoid of bacterial growth surrounding the applied samples. With further incubation, the test organism ferments glucose present in the medium to form acid; this causes bromocresol purple to change its colour to yellow. Only the inhibition zones still retain the original violet colour of the indicator. When performing the rapid test, growth of the test organism is enhanced by preincubating the inoculated plates; the inhibition zones then appear more rapidly after application of the samples.

Typical Composition (g/litre)

Peptone 17.0; sodium chloride 3.0; D(+)glucose 3.0; starch 3.0; gelatin 2.5; bromocresol purple 0.016; sucrose 2.0; agar-agar 10.0.

Preparation

Suspend 40.5 g/litre and autoclave (15 min at 121 °C). Cool to 50-60 °C, to each 200 ml add the contents of 1 ampoule of Bacillus stearothermophilus spore suspension, mix, pour plates. Acc. to the German DIN 10182 part 1, a volume of 5 ml/Petridish (Ø 90 mm) is recommended.

pH: 6.8 ± 0.2 at 25 °C.

The plates are clear and purple.

Plates which are to be used for the rapid test should be preincubated for 135 minutes at 65 °C. The plates should not be stacked in order to ensure even temperature.

- **If the ready-to-use plates are sealed with air-tight adhesive tape, they may be stored for up to 3 months in the refrigerator. The preincubated plates can be stored for up to one month under the same conditions. It is also advised to put the sealed plates into plastic bags.**

Experimental Procedure and Evaluation

Filter paper discs with a diameter of 6 mm are soaked with the liquid specimen or placed on organ (kidney, liver) or muscle sections. The discs are then slightly pressed onto the surface of the culture medium (up to 6 discs per plate).

Two methods are recommended for performing the test:

1. 45 minutes incubation, rapid test: After placing the discs on the preincubated plates, incubate them for a further 45minutes at 65 °C without prediffusion.
2. 3 hour incubation: The plates are not preincubated. After the filter paper discs have been applied to the plates, they should be incubated for 3 hours at 65 °C without prediffusion.

In the case of rapid test, formation of inhibition zones can be seen after 15-25 minutes incubation in the medium, which is otherwise turbid as a result of spore growth. After the 45 minutes incubation, the inhibition zones become even more distinct due to the fact that the culture medium changes colour. Formation of inhibition zones is to be regarded as a positive result.

In the case of the 3 hours incubation, only those inhibition zones with a diameter of more than 10 mm can be considered positive.

If a distinct colour change has not occurred after 45 minutes or 3hours, incubation can be prolonged.

Literature

KUNDRAT, W.: Methoden zur Bestimmung von Antibiotika-Rückständen in tierischen Produkten. - **Z. Anal. Chem.**; 624-630 (1968).

KUNDRAT, W.: 45-Minuten-Schnellmethode zum mikrobiologischen Nachweis von Hemmstoffen in tierischen Produkten. - **Fleischwirtsch., 52**; 485-487 (1972).

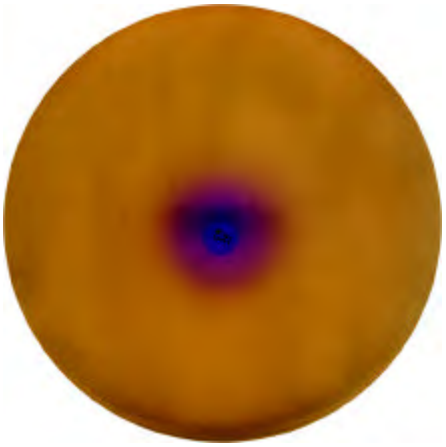
Ordering Information

Product	Merck Cat. No.	Pack size
Test Agar for the Residue Test acc. to KUNDRAT	1.10662.0500	500 g
Bacillus stearothermophilus spore suspension	1.11499.0001	5 x 2 ml

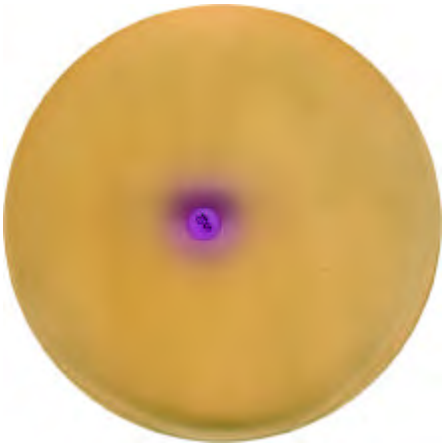
Test Agar for the Residue Test acc. to KUNDRAT

Quality control

Test strains	Growth after 3-3.5 h at 65 °C	Colour change to yellow	Inhibition zones in mm Ø			
			Gentamicin		Penicillin	Strepto-mycin
			10 µg	30 µg	10 IU	10 µg
Bacillus stearothermophilus ATCC 7953	good / very good	+	18-24	20-26	35-40	14-21



Gentamycin30yg



Streptomycin10yg

Test Agar pH 6.0 for the Inhibitor Test

For the detection of antimicrobial inhibitors in meat and organ samples together with Bacillus subtilis (BGA) spore suspension and Micrococcus luteus ATCC 9341 as test organisms.

The nutrient media are suitable both, for the inhibitor test (LEVETZOW, 1971) according to the German Meat Inspection Law as well as for the EEC Four-Plate-Test (BOGAERTS and WOLF, 1980) suggested by the Scientific Veterinary Commission of the European Economic Community. Test agar pH 7.2 with addition of trimethoprim is used particularly for determination of sulfonamide residues.

Mode of Action

The test is carried out according to the agar diffusion procedure. Small slices of the meat sample are placed on the inoculated Test Agar plates and incubated. Antimicrobial inhibitors contained in the samples diffuse into the nutrient media and cause growth-free inhibition zones to develop on the otherwise thickly covered plates. Repeated tests with pH 6.0, pH 8.0 and pH 7.2 are necessary, as penicillin and streptomycin are optimally active at pH 6.0 and 8.0 respectively (PICHNARCIK et al., 1969) and the activity optimum of sulfonamide is found at pH 7.2. Addition of trimethoprim to Test Agar pH 7.2 considerably increases the sensitivity of the test system to sulfonamides (GUDDING, 1976; EBRECHT, 1982).

Typical Composition (g/litre)

Peptone from casein, tryptic 3.45; peptone from meat, tryptic 3.45; sodium chloride 5.1; agar-agar 13.0.

Preparation

Suspend 25 g/litre Test Agar pH 6.0, autoclave (15 min at 121°C), test the pH and if necessary adjust. Cool to 50-45 °C, mix in 1ml/litre Bacillus subtilis (BGA) spore suspension.

After mixing the spore suspension, immediately pour the plates and place in the refrigerator.

The plates are clear and yellowish-brown.

Storage of the Plates

The ready-to-use plates can be sealed with air-tight adhesive tape and, when cooled (+4 to +6 °C) may be kept for up to 2 weeks. Additional packing into plastic bags is recommended. If stored for longer periods, the temperature should not be more than +3 °C; however, freezing of the culture medium must be avoided.

Experimental Procedure and Evaluation

Inhibitor Test

Test Agar pH 6.0 with Bacillus subtilis (BGA).

EEC Four-Plate-Test

Test Agar pH 6.0 with Bacillus subtilis (BGA).

For details regarding the collection of samples, transportation as well as the execution of the test, see the Fleischbeschaugesetz (Meat Inspection Law) or BOGAERTS and WOLF (1980).

According to these specifications the cylinder-shaped tissue sections (8 mm in diameter and 2 mm thick) are stamped out under conditions, which are as aseptic as possible and laid on the plates; according to BOGAERTS and WOLF two sections are required per plate. As a control, one test disc with 10 IU of penicillin G-sodium is placed on a plate with pH 6.0, one test disc with 10 µg of streptomycin on each of the two plates with pH 8.0 and one test disc with 0.5 µg sulfadimidine on a plate with pH 7.2. The test discs can also be made by the user using filter paper discs, 6 mm in diameter.

Incubation: 18-24 hours at 30 °C (Bacillus subtilis BGA).

Manufacturers	Product
Burroughs Wellcome, GB	Trimethoprim
Intern. Chemical Industries, GB	Sulfadimidine
Schleicher & Schüll, No. 2668, FRG	Filter paper discs 6 mm in diameter
American Type Culture Collection, 12301 Parklawn Drive, Rockeville, Maryland 20852, USA	Micrococcus luteus ATCC 9341
Beiersdorf AG, Hamburg, FRG	Adhesive tape Tesaflex 166

The inhibition zones between the edge of the tissue section or the test disc and the growth limit of the test organism are measured. Complete inhibition of growth with an inhibitory zone of at least 2mm is regarded as positive result, an inhibitory zone of 1-2mm should be considered as doubtful. This only applies, however, if the controls, prepared at the same time, display zones of inhibition measuring about 6 mm.

For possible methodological improvements see FORSCHNER and SEIDLER (1976).

Test Agar pH 6.0 for the Inhibitor Test

Literature

Arbeitsgruppe des Instituts für Lebensmitteltechnologie und Verpackung der Technischen Universität München: Merkblätter für die Prüfung von Packmitteln, Merkblatt 18 "Prüfung auf antimikrobielle Bestandteile in Packstoffen". - **Verpackgs.-Rdsch., 25**; Techn.-wiss. Beilagen; 5-8 (1974).

BAUR, E.: Untersuchungen von Fleisch- und Wurstwaren mit dem Hemmstofftest im Rahmen der tierärztlichen Lebensmittelüberwachung. - **Fleischwirtsch., 55**; 843-845 (1975)

BOGAERTS, R., u. WOLF, F.: Eine standardisierte Methode zum Nachweis von Rückständen antibakteriell wirksamer Substanzen in frischem Fleisch. - **Fleischwirtsch., 60**; 667-675 (1980).

Deutsches Fleischbeschaugesetz: Ausführungsbestimmungen A über die Untersuchung und gesundheitspolizeiliche Behandlung der Schlachttiere und des Fleisches bei Schlachtungen im Inland; Anlage 4 zu § 20 Abs. 4: Rückstandsuntersuchung.

EBRECHT, A.: Verbesserung des Hemmstofftestes durch Zusatz von Trimethoprim zum Nachweis von Sulfonamiden. - **Arch. Lebensmittelhyg. 33**; 109-115 (1982).

FORSCHNER, E., u. SEIDLER, M.: Alternativvorschläge zum Hemmstofftest. Rationalisierung und Absicherung. - **Fleischwirtsch., 56**; 1008-1013 (1976).

GUDDING, R.: An improved bacteriological method for the detection of sulfonamide residues in food. - **Acta Vet. Scand., 17**; 458-464 (1976).

LEVETZOW, R.: Untersuchungen auf Hemmstoffe im Rahmen der Bakteriologischen Fleischuntersuchung (BU). - Bundesgesundheitsblatt, 14; 15/16, 211-213 (1971).

PICHNARCIK, J., WENZEL, S., u. GISSKE, W.: Beitrag zur Methodik des Hemmstoffnachweises in Organen und Muskulatur von Schlachttieren. - **Arch. Lebensmittelhyg., 20**; 272-279 (1969).

Ordering Information

Product	Merck Cat. No.	Pack size
Test Agar pH 6.0 for the Inhibitor Test	1.10663.0500	500 g
Bacillus subtilis (BGA)-spore suspension	1.10649.0001	15 x 2 ml
Penicillin G potassium salt	CN Biosciences	
Streptomycin sulfate	CN Biosciences	

Quality control

Test strains	Inhibition zones in mm Ø			
	Gentamicin		Penicillin	Streptomycin
	10 µg	30 µg	10 IU	10 µ
Bacillus subtilis strain BGA (DSMZ 618)	20-28	22-30	36-48	19-27

Gentamicin 30µg

Penicillin 10IE

Test Agar pH 8.0 for the Inhibitor Test

For the detection of antimicrobial inhibitors in meat and organ samples together with Bacillus subtilis (BGA) spore suspension and Micrococcus luteus ATCC 9341 as test organisms.

The nutrient media are suitable both, for the inhibitor test (LEVETZOW, 1971) according to the German Meat Inspection Law as well as for the EEC Four-Plate-Test (BOGAERTS and WOLF, 1980) suggested by the Scientific Veterinary Commission of the European Economic Community. Test agar pH 7.2 with addition of trimethoprim is used particularly for determination of sulfonamide residues.

Mode of Action

The test is carried out according to the agar diffusion procedure. Small slices of the meat sample are placed on the inoculated Test Agar plates and incubated. Antimicrobial inhibitors contained in the samples diffuse into the nutrient media and cause growth-free inhibition zones to develop on the otherwise thickly covered plates. Repeated tests with pH 6.0, pH 8.0 and pH 7.2 are necessary, as penicillin and streptomycin are optimally active at pH 6.0 and 8.0 respectively (PICHNARCIK et al., 1969) and the activity optimum of sulfonamide is found at pH 7.2. Addition of trimethoprim to Test Agar pH 7.2 considerably increases the sensitivity of the test system to sulfonamides (GUDDING, 1976; EBRECHT, 1982).

Typical Composition (g/litre)

Peptone from casein, tryptic 3.45; peptone from meat, tryptic 3.45; sodium chloride 5.1; tri-sodium phosphate 12-hydrate 2.4; agar-agar 13.0.

Preparation

Suspend 27.5 g/litre Test Agar pH 8.0, autoclave (15 min at 121 °C), test the pH and if necessary adjust. Cool to 50-45 °C, mix in 1 ml/litre Bacillus subtilis (BGA) spore suspension. If necessary add Micrococcus luteus ATCC 9341 (microbial count in culture medium: approximately 104 cfu/ml) to Test Agar pH 8.0.

After mixing the spore suspension, immediately pour the plates and place in the refrigerator.

The plates are clear and yellowish-brown.

Storage of the Plates

The ready-to-use plates can be sealed with air-tight adhesive tape and, when cooled (+4 to +6 °C) may be kept for up to 2 weeks. Additional packing into plastic bags is recommended. If stored for longer periods, the temperature should not be more than +3 °C; however, freezing of the culture medium must be avoided.

Experimental Procedure and Evaluation

Inhibitor Test

Test Agar pH 8.0 with Bacillus subtilis (BGA)

EEC Four-Plate-Test

Test Agar pH 8.0 with Bacillus subtilis (BGA) and Test Agar pH 8.0 with Micrococcus luteus ATCC 9341.

For details regarding the collection of samples, transportation as well as the execution of the test, see the Fleischbeschaugesetz (Meat Inspection Law) or BOGAERTS and WOLF (1980).

According to these specifications the cylinder-shaped tissue sections (8 mm in diameter and 2 mm thick) are stamped out under conditions, which are as aseptic as possible and laid on the plates; according to BOGAERTS and WOLF two sections are required per plate. As a control, one test disc with 10 IU of penicillin G-sodium is placed on a plate with pH 6.0, one test disc with 10 µg of streptomycin on each of the two plates with pH 8.0 and one test disc with 0.5 µg sulfadimidine on a plate with pH 7.2. The test discs can also be made by the user using filter paper discs, 6 mm in diameter.

Incubation: 18-24 hours at 30 °C (Bacillus subtilis BGA) and 37°C (Micrococcus luteus ATCC 9341).

Manufacturers	Product
Burroughs Wellcome, GB	Trimethoprim
Intern. Chemical Industries, GB	Sulfadimidine
Schleicher & Schüll, No. 2668, FRG	Filter paper discs 6 mm in diameter
American Type Culture Collection, 12301 Parklawn Drive, Rockeville, Maryland 20852, USA	Micrococcus luteus ATCC 9341
Beiersdorf AG, Hamburg, FRG	Adhesive tape Tesaflex 166

The inhibition zones between the edge of the tissue section or the test disc and the growth limit of the test organism are measured. Complete inhibition of growth with an inhibitory zone of at least 2mm is regarded as positive result, an inhibitory zone of 1-2mm should be considered as doubtful. This only applies, however, if the controls, prepared at the same time, display zones of inhibition measuring about 6 mm.

For possible methodological improvements see FORSCHNER and SEIDLER (1976).

Test Agar pH 8.0 for the Inhibitor Test

Literature

Arbeitsgruppe des Instituts für Lebensmitteltechnologie und Verpackung der Technischen Universität München: Merkblätter für die Prüfung von Packmitteln, Merkblatt 18 "Prüfung auf antimikrobielle Bestandteile in Packstoffen". - **Verpackgs.-Rdsch., 25**; Techn.-wiss. Beilagen; 5-8 (1974).

BAUR, E.: Untersuchungen von Fleisch- und Wurstwaren mit dem Hemmstofftest im Rahmen der tierärztlichen Lebensmittelüberwachung. - **Fleischwirtsch., 55**; 843-845 (1975)

BOGAERTS, R., u. WOLF, F.: Eine standardisierte Methode zum Nachweis von Rückständen antibakteriell wirksamer Substanzen in frischem Fleisch. - **Fleischwirtsch., 60**; 667-675 (1980).

Deutsches Fleischbeschaugesetz: Ausführungsbestimmungen A über die Untersuchung und gesundheitspolizeiliche Behandlung der Schlachttiere und des Fleisches bei Schlachtungen im Inland; Anlage 4 zu § 20 Abs. 4: Rückstandsuntersuchung.

EBRECHT, A.: Verbesserung des Hemmstofftestes durch Zusatz von Trimethoprim zum Nachweis von Sulfonamiden. - **Arch. Lebensmittelhyg. 33**; 109-115 (1982).

FORSCHNER, E., u. SEIDLER, M.: Alternativvorschläge zum Hemmstofftest. Rationalisierung und Absicherung. - **Fleischwirtsch., 56**; 1008-1013 (1976).

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LEVETZOW, R.: Untersuchungen auf Hemmstoffe im Rahmen der Bakteriologischen Fleischuntersuchung (BU). - Bundesgesundheitsblatt, 14; 15/16, 211-213 (1971).

PICHNARCIK, J., WENZEL, S., u. GISSKE, W.: Beitrag zur Methodik des Hemmstoffnachweises in Organen und Muskulatur von Schlachttieren. - **Arch. Lebensmittelhyg., 20**; 272-279 (1969).

Ordering Information

Product	Merck Cat. No.	Pack size
Test Agar pH 8.0 for the Inhibitor Test	1.10664.0500	500 g
Bacillus subtilis (BGA)-spore suspension	1.10649.0001	15 x 2 ml
Penicillin G potassium salt	CN Biosciences	
Streptomycin sulfate	CN Biosciences	

Quality control

Test strains	Growth	Inhibition zones in mm Ø			
		Gentamicin		Penicillin	Streptomycin
		10 µg	30 µg	10 IU	10 µg
Bacillus subtilis strain BGA (DSMZ 618)	good / very good	36-44	38-47	34-45	30-36
Micrococcus luteus ATCC 9341	good / very good	28-36	32-40	50-60	30-36

BGA

Micrococcus luteus ATCC 9341

Tetrathionate Broth, Base

For the selective enrichment of salmonellae from various materials.

This broth complies with the specifications given in the United States Pharmacopeia XXVI (2003) and the recommendations of the APHA (1992).

Mode of Action

Tetrathionate and excess thiosulfate (PALUMBO and ALFORD 1970) suppress coliform microorganisms and other accompanying bacteria, whereas all tetrathionate-reducing bacteria (e.g. salmonellae and Proteus) can multiply more or less normally in this medium. Acidic tetrathionate decomposition products are formed, which are neutralized by calcium carbonate

Bile salts largely inhibit all microorganisms, which do not normally live in the intestine. The United States Pharmacopeia recommends the addition of brilliant green, which suppresses, above all, the Gram-positive microbial flora. The resulting culture medium has a very strong inhibitory effect; it is therefore sometimes better to omit the brilliant green in order to obtain satisfactory yields of salmonellae. According to JEFFRIES (1959), Proteus can be suppressed by adding 0.04 g novobiocin/litre.

Typical Composition (g/litre)

Peptone from casein 2.5; peptone from meat 2.5; bile salt mixture 1.0; calcium carbonate 10.0; sodium thiosulfate 30.0.

Also to be added:

Potassium iodide 5.0; iodine 6.0; if required brilliant green 0.01.

Preparation

Suspend 46 g/litre, heat briefly to the boil and cool rapidly.

- **Do not autoclave.**

Prior to use, add 20 ml iodine/potassium iodide solution/litre, if desired 10 ml of a 0.1 % brilliant green solution/litre and if required 0.04 g novobiocin/litre. Avoid any further heating. When dispensing the prepared medium, make sure that any precipitate formed is evenly suspended.

Preparation of the iodine/potassium iodide solution: Iodine 6 g; potassium iodide 5 g; distilled water 20 ml.

- **The ready-to-use broth should be prepared and used the same day.**

The medium is turbid and green with white sediment (calcium carbonate).

Experimental Procedure and Evaluation

Inoculate the culture medium massively with the sample material.

Incubation: 18-24 hours at 35-37 °C or 43 °C respectively (BÄNFFER 1971).

The resulting cultures are then subjected to further tests.

Literature

American Public Health Association: Compendium of methods for the microbiological examination of foods. – 3rd ed. (1992).

BÄNFFER, J.R.: Comparison of the isolation of Salmonellae from human faeces by enrichment at 37 °C and 43 °C. - **Zbl. Bakt. I. Orig., 217**; 35-40 (1971).

JEFFRIES, L.: Novobiocin - tetrathionate broth: A medium of improved selectivity for the isolation of salmonellae from faeces. - **J. Clin. Path., 12**; 568-571 (1959).

KNOX, R., POLLOCK, M.R., a. GELL, F.G.H.: The selective action of tetrathionate in bacteriological media. - **J. Hyg., 43**; 147-158 (1943).

PALUMBO, S., a. ALFORD, J.: Inhibitory action of tetrathionate enrichment broth. - **Appl. Microbiol., 20**; 970-976 (1970).

United States Pharmacopeia XXIII, Chapter "Microbiol Limit Tests", 1995.

Ordering Information

Product	Merck Cat. No.	Pack size
Tetrathionate Broth, Base	1.05285.0500	500 g
Brilliant green (C.I. 42040)	1.01310.0050	50 g
Iodine resublimed	1.04761.0100	100 g
Potassium iodide	1.05043.0250	250 g
Novobiocin monosodium salt	CN Biosciences	

Quality control

Test strains	Inoculum	Growth after 24 hours
Escherichia coli ATCC 25922	approx. 99 %	≤ 5 %
Salmonella typhimurium ATCC 14028	approx. 1 %	≥ 95 %

Tetrathionate Broth Base acc. to MULLER-KAUFFMANN

For the selective enrichtment of salmonellae from various materials, particularly meat, meat products and other foodstuffs.

This culture medium complies with the recommendations of the DIN Norm 10160 for the examination of meat and the DIN Norm 10181 for the examination of milk.

Mode of Action

Tetrathionate is produced from thiosulfate by adding iodine to the culture medium. Tetrathionate suppresses the growth of coliform and other enteric bacteria. Salmonella, Proteus and some other species of bacteria can reduce tetrathionate and are not inhibited. Calcium carbonate buffers the sulphuric acid, which is liberated when tetrathionate is reduced. Bile promotes the growth of Salmonella, but largely inhibits the accompanying bacteria. Brilliant green suppresses primarily Gram-positive bacteria.

Typical Composition (g/litre)

Meat extract 0.9; peptone from meat 4.5; yeast extract 1.8; sodium chloride 4.5; calcium carbonate 25.0; sodium thiosulfate 40.7; oc bile 4.75.

Also to be added:

Potassium iodide 5.0; iodine 4.0; brilliant green 0.01.

Preparation

Suspend 82g/litre, heat briefly to the boil and cool rapidly. A sediment of calcium carbonate appears in the turbid broth at the bottom of the tubes.

- **Do not autoclave.**

Prior to use add iodine/potassium iodide solution (20mg/litre) and a 0.1 % solution of brilliant green (10ml/litre), dispense into test tubes takin care to suspend any precipitate evenly. Avoid further heating.
pH: 7.6 ± 0.2 at 25 °C.

Preparation of the iodine/potassium iodide solution: Potassium iodide 5g; iodine 4g; distilled water 20ml.

- **The ready-to-use broth should be prepared and used the same day.**

The medium is turbid and green with a white sediment (calcium carbonate).

Experimental Procedure and Evaluation

Directly suspend approximately 10g of sample material in 100 ml Tetrathionate Broth acc. to MULLER-KAUFFMANN.

Incubation: 18-24 hours at 35-37 °C or 43 °C respectively (BÄNFFER 1971, EDEL and KAMPELMACHER 1969).

The resulting cultures should be subjected to further tests.

Literature

BÄNFFER, J.R.: Comparison of the isolation of Salmonellae from human faeces by enrichment at 37 °C and 43 °C. - **Zbl. Bakt. I. Orig., 217**; 35-40 (1971).

DIN Deutsches Institut für Normung e.V.: Untersuchung von Fleisch und Fleischerzeugnissen. Nachweis von Salmonellen. Referenzverfahren. - **DIN 10160**

DIN Deutsches Institut für Normung e.V.: Mikrobiologische Milchuntersuchung. Nachweis von Salmonellen. Referenzverfahren. - **DIN 10181**.

EDEL, W., a. KAMPELMACHER, E.H.: Salmonella isolation in nine European laboratories using a standardized technique. - **Bull. Wld. Hlth. Org., 41**; 297-306 (1969).

KAUFFMANN, F.: Ein kombiniertes Anreicherungsverfahren für Typhus- und Paratyphusbazillen. - **Zbl. Bakt. I. Orig., 119**; 148-152 (1930).

KAUFFMANN, F.: Weitee Erfahrungen mit dem kombinierten Anreicherungsverfahren für Salmonellenbacillen. - **Z. Hyg. Infekt.-Krkh., 117** ; 26-32 (1935).

MULLER, L.: Un nouveau milieu d'enrichissement pour la recherce du bacille typhique et des paratyphiques. - **Comp. rend. Soc. biol., 89**; 434-437 (1923).

Ordering Information

Product	Merck Cat. No.	Pack size
Tetrathionate Broth Base acc. to MULLER-KAUFFMANN	1.10863.0500	500 g
Brilliant green (C.I. 42040)	1.01310.0050	50 g
Iodine resublimed	1.04761.0100	100 g
Potassium iodide	1.05043.0250	250 g

Quality control

Test strains	Inoculum	Growth after 24 hours
Escherichia coli ATCC 25922	approx. 99 %	≤ 5 %
Salmonella typhimurium ATCC 14028	approx. 1 %	≥ 95 %

Tetrathionate Crystal-violet Enrichment Broth acc. to PREUSS

Medium proposed by PREUSS (1949) for the selective enrichment of Salmonella from meat, foodstuffs etc.

This culture medium complies with the specifications prescribed in the Fleischbeschaugesetz (German Meat Inspection Law) and the Einfuhruntersuchungsverordnung (German Regulations for the Examination of Imported Goods).

Mode of Action

Tetrathionate and crystal violet largely inhibit the entire accompanying bacterial flora including Shigella.

Typical Composition (g/litre)

Peptone from meat 4.3; peptone from casein 4.3; sodium chloride 6.4; potassium tetrathionate 20.0; crystal violet 0.005.

Preparation

Suspend 35 g/litre, heat gently, if necessary (max. 50 °C), dispense into suitable containers.

- **Do not autoclave.**

pH: 6.5 ± 0.2 at 25 °C.

The prepared broth is clear and violet.

- **The culture medium is not stable and must therefore be prepared always fresh.**

Experimental Procedure

Inoculate the broth with the sample material

Incubation: 18-24 hours at 35-37 °C.

Spread material from the resulting cultures on selective culture media.

Literature

Deutsches Fleischbeschaugesetz: Aufführungsbestimmungen A über die Untersuchung und gesundheitspolizeiliche Behandlung der Schlachttiere und des Fleisches bei Schlachtungen im Inland, Anlage 1 zu § 20 Abs. 4: Vorschriften über die bakteriologische Fleischuntersuchung.

Verordnung über die Untersuchung des in das Zollgebiet eingehenden Fleisches (Einfuhruntersuchungsverordnung). Anlage 1 zu § 20 Abs. 1: Untersuchungsverfahren.

PREUSS, H.: Über eine neue Tetrathionat-Anreicherung. - **Z. Hyg., 129**; 187-214 (1949).

Ordering Information

Product	Merck Cat. No.	Pack size
Tetrathionate Crystal-violet Enrichment Broth acc. to PREUSS	1.05173.0500	500 g

Quality control

Test strains	Inoculum	Growth 6 hours	after 20 hours
Escherichia coli ATCC 25922	approx. 99 %	≤ 50 %	≤ 10 %
Salmonella typhimurium ATCC 14028	approx. 1 %	≥ 50 %	≥ 90 %

AC
MPF

TGE Agar (Tryptone Glucose Extract Agar)

For determining the total aerobic microbial count in water and other materials.

This culture medium complies with the specifications given by the APHA for the examination of water (1998) and for food (1992) and the recommendations of the "American Petroleum Institute" (1959). For details concerning the examination of foodstuffs also see BAUMGARTEN and LEVETZOW (1969).

Typical Composition (g/litre)

Peptone from casein 5.0; meat extract 3.0; D(+)glucose 1.0; agar-agar 15.0.

Preparation

Suspend 24 g/litre, autoclave (15 min at 121 °C).

pH: 7.0 ± 0.2 at 25 °C.

The plates are clear and yellowish-brown.

Experimental Procedure and Evaluation

The culture medium is usually inoculated by the pour-plate method. Other details depend on the purpose for which the medium is used.

Incubation: 24 hours at 35 °C aerobically.

Literature

American Public Health Association, American Water Works Association and Water Pollution Control Federation: Standard Methods for the Examination of Water and Wastewater, 20th ed., Washington, 1998.

American Public Health Association: Compendium of methods for the microbiological examination of foods. - 3rd ed. (1992).

BAUMGARTEN, H.J., u. LEVETZOW, R.: Untersuchungen zu hygienischen Beschaffenheit von im Handel befindlicher Speisegelatine. - **Arch. f. Lebensmittelhyg., 20**; 38-42 (1969).

Recommended Practice for Biological Analyses of Subsurface Injection Waters. Vol. 38, 1st ed., **American Petroleum Institute** (1959).

Ordering Information

Product	Merck Cat. No.	Pack size
TGE Agar (Tryptone Glucose Extract Agar)	1.10128.0500	500 g

Escherichia coli ATCC 25922

Quality control (spiral plating methods)

Test strains	Inoculum (cfu/ml)	Recovery %
Staphylococcus aureus ATCC 25923	10^3-10^5	≥ 70
Streptococcus agalactiae ATCC 13813	10^3-10^5	≥ 70
Enterococcus faecalis ATCC 11700	10^3-10^5	≥ 70
Escherichia coli ATCC 25922	10^3-10^5	≥ 70
Salmonella typhimurium ATCC 14028	10^3-10^5	≥ 70
Pseudomonas aeruginosa ATCC 27853	10^3-10^5	≥ 70
Bacillus cereus ATCC 11778	10^3-10^5	≥ 70

Thioglycollate Broth

For cultivation and isolation of obligate and facultative anaerobic and microaerophilic bacteria and for sterility tests.

Both culture media comply with the recommendations of United States Pharmacopeia XXVI (2003), the European Pharmacopeia and APHA (1992).

Mode of Action

The reducing agents thioglycollate and cystine ensure an anaerobiosis which is adequate even for fastidious anaerobes. The sulfhydryl groups of these substances also inactivate arsenic, mercury and other heavy metal compounds.

The thioglycollate media are thus suitable for the examination of materials which contain heavy metals or heavy metal preservatives. The higher viscosity of the Fluid Thioglycollate Medium prevents rapid uptake of oxygen. Any increase in the oxygen content is indicated by the redox indicator sodium resazurin which changes its colour to red.

Typical Composition (g/litre)

Peptone from casein 15.0; yeast extract 5.0; D(+)glucose 5.5; L-cystine 0.5; sodium chloride 2.5; sodium thioglycollate 0.5.

Preparation

Suspend 29 g Thioglycollate Broth/litre,dispense into tubes, autoclave 15min at 121 °C).

pH: 7.1 ± 0.2 at 25 °C.

The prepared media are clear and yellowish.

■ **The culture media should always be freshly prepared.**

Experimental Procedure and Evaluation

Inoculate the culture medium with the sample material taking care that the sample reaches the bottom of the tubes. In order to ensure anaerobiosis, the medium can then be overlayed with 1cm of sterile liquid paraffin or agar solution.

Incubation: several days at the optimal incubation temperature (35-37 °C).

Anaerobes grow in the lower part of the culture.

Literature

American Public Health Association: Compendium of methods for the microbiological examination of foods. - 3rd ed. (1992).

European Pharmacopeia II, Chapter VIII. 3.

United States Pharmacopeia XXVI, Chapter "Microbial Limit Tests", 2003.

Ordering Information

Product	Merck Cat. No.	Pack size
Thioglycollate Broth	1.08190.0500	500 g
Thioglycollate Broth	1.08190.5000	5 kg
Agar-agar purified	1.01614.1000	1 kg
Paraffin viscous	1.07160.1000	1 l

Quality control

Test strains	Growth
Clostridium sporogenes ATCC 11437	good
Clostridium sporogenes ATCC 19404	good (anaerobic)
Bacillus subtilis ATCC 6633	good
Micrococcus luteus ATCC 9341	good
Pseudomonas aeruginosa ATCC 9027	good
Bacteroides vulgatus ATCC 8482	good (anaerobic)
Staphylococcus aureus ATCC 6538	good
Escherichia coli ATCC 25922	good

Tributyrin Agar, Base

Medium proposed by ANDERSON (1939) for the detection and enumeration of lipolytic microorganisms in foodstuffs and other materials. The medium can also be used for the detection of lipase in various bacterial species such as staphylococci (INNES 1956), clostridia (WILLIS 1960), Pseudomonas, marine flavobacteria (HAYES 1963) etc.

Mode of Action

The culture medium contains tributyrin as a reactant; degradation of this compound gives rise to clear zones surrounding the lipolytic colonies in the otherwise turbid culture medium.

Typical Composition (g/litre)

Peptone from meat 2.5; peptone from casein 2.5; yeast extract 3.0; agar-agar 12.0.

Also to be added:

Tributyrin 10.0 ml.

Preparation

Suspend 20 g/litre, add 10 ml neutral tributyrin/litre, mix uniformly and autoclave (15 min at 121 °C). While shaking frequently (emulsification of the tributyrin) cool to at least 50°C (stabilization of the emulsion) and pour plates. Allow the plates to solidify rapidly.

pH: 7.5 ± 0.2 at 25 °C.

The plates are turbid and yellowish.

- **The culture medium must contain a uniformly turbid emulsion. If the emulsion separates, the effectiveness of the culture medium is affected.**

EL SADEK and RICHARDS (1957) reported that other glycerides such as triolein and trilinolein can be used instead of tributyrin. According to RAPP (1978), better emulsification of tributyrin can be achieved if 4 ml polyoxyethylene-(20)-hydrated ricinus oil is added to 1 litre of the culture medium.

Experimental Procedure and Evaluation

Inoculate the culture medium by the pour-plate method or by spreading the sample material on the surface of the plates.

Incubation: up to 72 hours under optimal conditions (e.g. 28 °C).

Lipolytic microorganisms produce colonies which are surrounded by clear zones in the otherwise turbid culture medium.

Literature

ANDERSON, J.A.: The use of tributyrin agar in dairy bacteriology. - **Ber. 3. Int. Mikrobiol. Kongress, 3**; 726-728 (1939)

EL SADEK, G.M., a. RICHARDS, T.: Nile blue, aniline blue and neutral red as indicators of lipolysis. - **J. Appl. Bact., 20**; 137 (1959).

INNES, A.G.: Coagulase positive Staphylococci from bulk milk supplies low in solids-notfat. - **J. Appl. Bact., 19**; 39-45 (1956).

HAYES, P.R.: Studies on marine flavobacteria. - **J. Gen. Microbiol., 30**; 1-19 (1963).

RAPP, M.: Elektive Nährmedien zum Nachweis von Lipolyten. - **Milchwirtsch., 33**; 493-496 (1978).

WILLIS, A.T.: The lipolytic activity of some clostridia. - **J. Path. Bact., 80**; 379-390 (1960).

Ordering Information

Product	Merck Cat. No.	Pack size
Tributyrin Agar, Base	1.01957.0500	500 g
Glycerol tributyrate (Tributyrin)	1.01958.0100	100 ml

Manufacturer	Product
ICI Chemicals, Essen, BRD	Polyoxyethylene-(20)-hydrated Ricinus oil

Quality control

Test strains	Growth	Clear zones
Escherichia coli ATCC 25922	good / very good	-
Salmonella typhimurium ATCC 14028	good / very good	-
Pseudomonas aeruginosa ATCC 27853	good / very good	+
Staphylococcus aureus ATCC 25923	good / very good	+
Bacillus subtilis ATCC 6633	good / very good	+
Penicillium commune ATCC 10428	poor / fair	+

Triple Sugar Iron Agar

TSI-Agar

Culture medium proposed by SULKIN and WILLETT (1940) and modified by HAJNA (1945) for identifying Enterobacteriaceae.

This medium complies with the recommendations of the International Organization for Standardization (ISO) (1975), DIN Norm 10160 for the examination of meat and DIN Norm 10181 for the examination of milk. Its composition is equivalent to that recommended by the United States Pharmacopeia XXVI (2003), the European Pharmacopeia II and the German examination procedure for food acc. to § 35 LMBG.

Mode of Action

Degradation of sugar and accompanying acid production are detected by the pH indicator phenol red, which changes its colour from red-orange to yellow, on alkalinization it turns deep red. Thiosulfate is reduced to hydrogen sulfide by several species of bacteria, the hydrogen sulfide reacts with an iron salt to give black iron sulfide.

Typical Composition (g/litre)

Peptone from casein 15.0; peptone from meat 5.0; meat extract 3.0; yeast extract 3.0; sodium chloride 5.0; lactose 10.0; sucrose 10.0; D(+)glucose 1.0; ammonium iron(III) citrate 0.5; sodium thiosulfate 0.5; phenol red 0.024; agar-agar 12.0.

Preparation

Suspend 65 g/litre, dispense into test tubes, autoclave (15 min at 121 °C). Allow the medium to solidify to give slant-agar tubes.

pH: 7.4 ± 0.2 at 25 °C.

The prepared medium is clear and red.

Experimental Procedure and Evaluation

Streak the pure culture under investigation on the sloped surface and inoculate the butt of the same tube by a central stab.

Incubation: up to 48 hours at 35 °C aerobically.

Microorganisms	Butt	Slant surface	H_2S-production	
S.-typhosa	S	OA	+	Only in the upper part of the butt, often accompanied by ring formation, may take 48 hours
S. paratyphi A	SG			
S. choleraesuis	SG	OA	-	
S. pullorum	SG	OA	+	
S. paratyphi B	SG	OA	+	Butt black
S. typhimurium	SG	OA	+	
S. enteritidis	SG	OA	+	
S. gallinarum	S	OA	+	
Sh. dysenteriae type 1	S	OA	-	
Sh. schmitzii	S	OA	-	
Sh. boydii	S	OA	-	
Sh. flexneri	S	OA	-	
Sh. flexneri type 6 var. Newcastle	S/SG	OA	-	
Alkalescens	S	A/S***	-	
Sh. sonnei	S	S	-	
Dispar	S	S	-	
Ent. aerogenes	SG	S	-	
Ent. cloacae	SG	S	-	
E. coli	SG	S	-	
Citrobacter	SG	S	+	
Klebsiella	SG	S	-	
Pr. vulgaris	SG**	S***	+	
Pr. mirabilis	SG**	A	+	Dirty black-green
Pr. morganii	SG**	OA	-	
Pr. rettgeri	S(A)	OA	-	
K. pneumoniae	S/SG	OA	-	
Ps. aeruginosa	OA	OA*	-	
Al. faecalis	OA	OA		

Triple Sugar Iron Agar

TSI-Agar

Abbreviations:

A = Colour changes to red due to alkalinization

OA = No change in the original colour of the culture medium or colour changes to red due to alkalinization

S = Colour changes to yellow due to acid production

SG = Colour changes to yellow and gas is produced

\+ = Blackening due to H_2S production

\- = No blackening

* May be due to pigment production

** Some strains: A, possibly without gas production

*** On KLIGLER (double sugar iron agar): OA

Literature

Bundesgesundheitsamt: Amtliche Sammlung von Untersuchungsverfahren nach § 35 LMBG. Beuth Verlag Berlin, Köln.

Deutsches Arzneibuch, 10. Auflage, Chapter VIII, 10.

DIN Deutsches Institut für Normung: Untersuchung von Fleisch und Fleischerzeugnissen. - Nachweis von Salmonellen (Referenzverfahren). - **DIN10160**.

DIN Deutsches Institut für Normung e.V.: Mikrobiologische Milchuntersuchung. Nachweis von Salmonellen. Referenzverfahren. - **DIN 10181**.

European Pharmacopeia II, Chapter VIII, 10.

HAJNA, A.A.: Triple-Sugar Iron Medium for the identification of the intestinal group of bacteria. - **J. Bact., 49**; 516-517 (1945).

International Organization for Standardization: Meat and meat products. -Detection of Salmonella (Reference method). - **International Standard ISO 3565** (1975).

SULKIN, E.S., a. WILLETT, J.C.: A Triple Sugar-Ferrous Sulphate Medium for use in identification of enteric organisms. - **J. Lab Clin. Med., 25**; 649-653 (1940).

United States Pharmacopeia XXVI, Chapter "Microbial Limit Tests", 2003.

Ordering Information

Product	Merck Cat. No.	Pack size
Triple Sugar Iron Agar	1.03915.0500	500 g

Quality control

Test strains	Growth	Butt	Slant surface
Escherichia coli ATCC 25922	good / very good	yellow	yellow
Citrobacter freundii ATCC 8090	good / very good	yellow and black	yellow
Enterobacter cloacae ATCC 13047	good / very good	yellow	yellow
Shigella flexneri ATCC 12022	good / very good	yellow	red
Salmonella typhimurium ATCC 14028	good / very good	yellow and black	red
Salmonella enteritidis ATCC 13076	good / very good	yellow and black	red
Proteus mirabilis ATCC 14153	good / very good	yellow and black	red and black
Proteus vulgaris ATCC 13315	good / very good	yellow and black	yellow

Tryptic Soy Agar (CASO) with Polysorbate 80 and Lecithin

Medium for environmental monitoring and for determining efficiency of containers, equipment and surfaces.
The medium conforms to the United States Pharmacopeia XXVI (2003).

Mode of Action

Casein and soy peptones provide the replication of even fastidious microorganisms. Sodium chloride maintains osmotic equilibrium. QUISNO et al. (1946), BRUMMER (1976) and ERLANDSON et al. (1953) reported that Lecithin and Polysorbate80 inactivate many residual desinfectants. Polysorbate 80 neutralizes phenols, hexachlorophene and formalin. Lecithin inactivates quaternary ammonium compounds.

Typical Composition (g/litre)

Peptone from casein 15.0; peptone from soymeal 5.0; sodium chloride 5.0; polysorbate 80 5.0; lecithin 0.7; agar-agar 15.0.

Preparation

Suspend 45.7 g in 1 litre of distilled or dem. water and heat to boiling, if necessary, with frequent agitation until completely dissolved. Autoclave at 121 °C for 15 minutes.

Cool the medium to about 45 °C, mix well and pour in Petridishes or **RODAC** (**R**eplicate **O**rganism **D**etection and **C**ounting) plates (about 17 ml).

pH 7.3 ± 0.2 at 25 °C.

The prepared medium is clear and yellowish-brown.

Experimental Procedure

Inoculate the medium by spreading method (in Petridish). Using RODAC plates for checking the cleanliness and disinfection efficiency of surfaces, press the plate with even pressure onto the surface. Avoid rubbing to prevent damage of the agar bed. **Clean the surface afterwards to remove any remainings of the agar.**

Incubation: 24-48 hours at 35 °C aerobically.

Literature

QUISNO, R., I. W. GIBBY, AND M. J. FOTER: A neutralizing medium for evaluating the germicidal potency of the quaternary ammonium salts. - **Am.J.Pharm., 118**; 320-323 (1946).

BRUMMER, B.: Influence of possible disinfectant transfer on Staphylococcus aureus plate counts after contact sampling. – **App. Environ. Microbiol., 32**; 80-84 (1976).

ERLANDSON, A. L., Jr., and C. A. LAWRENCE: Inactivating medium for hexachlorophene (G-11) types of compounds and some substituted phenolic disinfectants. – **Science, 118**; 274-276 (1953).

Ordering Information

Product	Merck Cat. No.	Pack size
Tryptic Soy Agar (CASO) with Polysorbate80 and Lecithin	1.07324.0500	500 g

Quality control

Test strains	Growth after 24 hours	Pigment	Colony colour
Staphylococcus aureus ATCC 25923	good / very good	-	yellow to white
Pseudomonas aeroginosa ATCC 10145	good / very good	+	green-blue

Pseudomonas aeruginosa ATCC 10145

Staphylococcus aureus ATCC 25923

Tryptic Soy Agar (TSA)

CASO Agar (Casein-peptone Soymeal-peptone Agar)

Universal culture media free from inhibitors and indicators for a wide spectrum of applications.

They comply with the recommendations of the United States Pharmacopeia XXVI (2003) and the European PharmacopeiaII.

Typical Composition (g/litre)

Peptone from casein 15.0; peptone from soymeal 5.0; sodium chloride 5.0; agar-agar 15.0.

Preparation

Suspend 40 g/litre, autoclave (15 min at 121 °C).

pH: 7.3 ± 0.2 at 25 °C.

After preparation both media are clear and yellowish-brown.

Experimental Procedure and Evaluation

Depend on the purpose for which the media are used.

Incubation: 24 hours at 35 °C aerobically, for up to 7 days for the sterility-test at room-temperature.

Literature

European Pharmacopeia II. Chapter VIII. 3. und VIII. 10.

HAWKEY, P.H., MCCORMICK, A., a. SIMPSON, R.A.: Selective and differential medium for the primary isolation of members of the proteae. – **J. Clin. Microbiol. 23**; 600-603 (1986).

United States Pharmacopeia XXVI, Chapter "Microbial Limit Tests", 1995.

Ordering Information

Product	Merck Cat. No.	Pack size
Tryptic Soy Agar (TSA)	1.05458.0500	500 g
Tryptic Soy Agar (TSA)	1.05458.5000	5 kg

Quality control (spiral plating method)

Test strains	Inoculum (cfu/ml)	Recovery rate (%)
Escherichia coli ATCC 8739	10^3-10^5	≥ 70
Staphylococcus aureus ATCC 6538	10^3-10^5	≥ 70
Streptococcus pyogenes ATCC 21059	10^3-10^5	≥ 70
Bacillus subtilis ATCC 6633	10^3-10^5	≥ 70
Candida albicans ATCC 10231	10^3-10^5	≥ 70
Candida albicans ATCC 2091	10^3-10^5	≥ 70

Tryptic Soy Broth (TSB)

CASO Broth (Casein-peptone Soymeal-peptone Broth)

Universal culture media free from inhibitors and indicators for a wide spectrum of applications.

They comply with the recommendations of the United States Pharmacopeia XXVI (2003) and the European Pharmacopeia II.

Typical Composition (g/litre)

Peptone from casein 17.0; peptone from soymeal 3.0; D(+)glucose 2.5; sodium chloride 5.0; di-potassium hydrogen phosphate 2.5.

Preparation

Suspend 30 g/litre, autoclave (15 min at 121 °C).

pH: 7.3 ± 0.2 at 25 °C.

After preparation both media are clear and yellowish-brown.

Experimental Procedure and Evaluation

Depend on the purpose for which the media are used.

Incubation: 24 hours at 35 °C aerobically, for up to 7 days for the sterility-test at room-temperature.

Literature

European Pharmacopeia II. Chapter VIII. 3. und VIII. 10.

HAWKEY, P.H., MCCORMICK, A., a. SIMPSON, R.A.: Selective and differential medium for the primary isolation of members of the proteae. – **J. Clin. Microbiol. 23**; 600-603 (1986).

United States Pharmacopeia XXVI, Chapter "Microbial Limit Tests", 1995.

Ordering Information

Product	Merck Cat. No	Pack size
Tryptic Soy Broth (TSB)	1.05459.0500	500 g
Tryptic Soy Broth (TSB)	1.05459.5000	5 kg
Tryptic Soy Broth (TSB)	1.05459.9025	25 kg

Quality control (inoculum: about 100 microorganisms)

Test strains	Growth
Incubation 24 h at 35 °C	
Escherichia coli ATCC 8739	+
Staphylococcus aureus ATCC 6538	+
Streptococcus pneumoniae ATCC 6301	+
Bacillus subtilis ATCC 6633	+
Pseudomonas aerugionas ATCC 9027	+
Salmonella typhimurium ATCC 14028	+
Icubation 3 days at 20 - 25 °C	
Staphylococcus aureus ATCC 6538	+
Staphylococcus aureus ATCC 25923	+
Staphylococcus aureus ATCC 12228	+
Bacillus subtilis ATCC 6633	+
Incubation 5 day at 20 - 25 °C	
Candida albicans ATCC 2091	+
Candida albicans ATCC 10231	+
Aspergillus niger ATCC 16404	+

Tryptic Soy (CASO) Broth, irradiated

Dehydrated culture medium sterilized by irradiation for the microbiological validation of aseptic filling (Media Fill Test).

The dehydrated culture medium is irradiated with 48-62 kGy gamma. The intensity of irradiation guarantees that even spores are destroyed. The test for sterility is carried out by incubation of part of the prepared medium.

Mode of Action

The medium complies with the recommendations of USP XXVI (2003), EP (2004) and German edition.

Due to the rich nutrient base, this medium is also suitable for the cultivation of even fastidious microorganisms.

Typical Composition (g/litre)

Peptone from casein 17.0; peptone from soymeal 3.0; D(+)glucose 2.5; sodium chloride 5.0; di-potassium hydrogen phosphate 2.5.

Preparation

Dissolve 30 g in 1 litre of sterile, demin. water and use according to the purpose required.

pH: 7.3 ± 0.2 at 25 °C.

The prepared medium is clear and yellowish-brown.

Experimental Procedure and Evaluation

The medium is very suitable for the simulation of aseptic filling of sterile powder. The filled powder is readily soluble in sterile purified water and can be dissolved directly together with the filled units. The previously aseptically prepared medium can be used for the simulation of the aseptic filling of liquids. For the validation of the aseptic filling at least 3,000 units per run are expected to be filled. The contamination rate should be <1/1000 at a probability of 95 %. (EU-GMP Guidelines, Annex 1, ISO13408-1). A growth control should be carried out for each run. The strains listed in the table below serve as growth controls, whereby bacteria should demonstrate good growth after 3 days of incubation at the latest and yeasts and moulds after 5 days.

Literature

Deutsches Arzneibuch, 10. Auflage; European Pharmacopeia II, United States Pharmacopeia XXVI; ISO 13408-1, 1998-08-01, Aseptic processing of health care products – Part 1: General requirements.

Ordering Information

Product	Merck Cat. No.	Pack size
Tryptic Soy (CASO) Broth, irradiated	1.00800.5000	5 kg

Quality control (inoculum about 10-100 microorganisms)

Test strains	Growth
Incubation 24 h at 35 °C	
Escherichia coli ATCC 8739	+
Staphylococcus aureus ATCC 6538	+
Streptococcus pneumoniae ATCC 6301	+
Bacillus subtilis ATCC 6633	+
Pseudomonas aerugionas ATCC 9027	+
Salmonella typhimurium ATCC 14028	+
Icubation 3 days at 20 - 25 °C	
Staphylococcus aureus ATCC 6538	+
Staphylococcus aureus ATCC 25923	+
Staphylococcus aureus ATCC 12228	+
Bacillus subtilis ATCC 6633	+
Incubation 5 day at 20 - 25 °C	
Candida albicans ATCC 2091	+
Candida albicans ATCC 10231	+
Aspergillus niger ATCC 16404	+

Sterility test (2 weeks at 20 - 25 °C and 30 - 35 °C): no growth

Tryptic Soy Broth (TSB) non animal origin

Universal culture medium free from inhibitors and indicators for a wide spectrum of applicaitons. The Peptones used in the broth are from non animal origin. It conforms with the requirements of the pharmaceutical industry and biotechnology.

Mode of Action

The medium comply with the recommendations of USP XXVI (2003), EP (2003) and the German Edition of Ph.Eur. (1999). The microbiological performance of the Tryptic Soy Broth (TSB) non animal origin complies with the classical CASO Broth (TSB).

Typical Composition (g/litre)

Peptone non animal origin 20.0; D(+)-Glucose 2.5; Sodium chloride 5.0; di-potassium hydrogen phosphate 2.5

Preparation

Suspend 30 g in 1 litre of demin. water, autoclave (15 min. at 121°C).

pH: 7.3 ± 0.2 at 25 °C.

The prepared medium is clear and yellowish-brown.

Experimental Procedure and Evaluation

Depend on the purpose for which the medium is used.

Incubation: 24 hours at 35 °C aerobically, for up to 7 days for the sterility-test at room-temperature.

Literature

Deutsches Arzneibuch, 10. Auflage; European Pharmacopeia II, United States Pharmacopeia XXVI

Ordering Information

Product	Merck Cat. No.	Pack size
Tryptic Soy Broth (TSB) non animal origin	1.00525.5000	5 kg

Quality control (Inoculum approx. 10 – 100 microorganisms)

Test strains	Growth
Incubation 24 h at 35 °C	
Escherichia coli ATCC 8739	+
Staphylococcus aureus ATCC 6538	+
Streptococcus pneumoniae ATCC 6301	+
Bacillus subtilis ATCC 6633	+
Pseudomonas aerugionas ATCC 9027	+
Salmonella typhimurium ATCC 14028	+
Icubation 3 days at 20 - 25 °C	
Staphylococcus aureus ATCC 6538	+
Staphylococcus aureus ATCC 25923	+
Staphylococcus aureus ATCC 12228	+
Bacillus subtilis ATCC 6633	+
Incubation 5 day at 20 - 25 °C	
Candida albicans ATCC 2091	+
Candida albicans ATCC 10231	+
Aspergillus niger ATCC 16404	+

Tryptic Soy Broth (TSB) non-animal origin, irradiated

Caso Broth non-animal origin, irradiated

Dehydrated culture media sterilised by irradiation for the microbiological validation of aseptic filling (Media Fill Test)

The dehydrated culture medium is irradiated with 48-62 kGy gamma. The intensity of irradiation guarantees that Bacteria, spores, viruses and mycoplasma are destroyed. The test for sterility is carried out in compliance with European Pharmacopoeia and United States Pharmacopoeia (Ph. Eur. 2.6.1. Sterility).

Mode of Action

The medium complies with the recommendations of USP XXVI (2003), EP (1999) and the pH. Eur. (1999) German edition.

The microbiological performance of CASO Broth non-animal origin is equivalent to the common CASO Broth.

Typical Composition (g/litre)

Peptone, non-animal origin 20.0; D(+)-glucose 2.5; sodium chloride 5.0; di-potasium hydrogen phosphate 2.5.

Preparation

Suspend 30.0 g in 1 litre of sterile, demin. Water and use according to the purpose required.

pH: 7.3 0.2 at 25 °C

The prepared medium is clear and yellowish-bown.

Experimental Procedure and Evaluation

The medium is very suitable for the simulation of aseptic filling of sterile powder. The filled powder is readily soluble in sterile purified water and can be dissolved directly together with the filled units. The previously aseptically prepared nutrient medium can be used for the simulation of the aseptic filling of liquids. for the validation of the aseptic filling at least 3.000 units per run are expected to be filled. The contamination rate should be <1/1000 at a probability of 95 %. (EU-GMP guidelines, Annex 1, ISO 13408-1). A growth control should be carried out for each run. The strains listed in the table below serve as growth controls, whereby bacteria should demonstrate good growth after 3days of incubation at the (latest and yeasts and moulds after 5days.

Literature

Deutsches Arzneibuch, 10. Auflage; Eruopean Pharmacopeia II; United States Pharmacopoeia XXVI; ISO 13408-1, 1998-08-00, Aseptische Herstellung von Produkten für die Gesundheitsfürsorge-Teil1, Allgemeine Anforderungen.

Ordering Information

Product	Merck Cat. No.	Pack size
Tryptic Soy Broth (TSB) non-animal origin, irradiated	1.00550.5000	5 kg

Quality control (inoculum about 10 – 100 microorganisms)

Test strains	Growth
Incubation 24 h at 35 °C	
Escherichia coli ATCC 8739	+
Staphylococcus aureus ATCC 6538	+
Streptococcus pneumoniae ATCC 6301	+
Bacillus subtilis ATCC 6633	+
Pseudomonas aerugionas ATCC 9027	+
Salmonella typhimurium ATCC 14028	+
Icubation 3 days at 20 - 25 °C	
Staphylococcus aureus ATCC 6538	+
Staphylococcus aureus ATCC 25923	+
Staphylococcus aureus ATCC 12228	+
Bacillus subtilis ATCC 6633	+
Incubation 5 day at 20 - 25 °C	
Candida albicans ATCC 2091	+
Candida albicans ATCC 10231	+
Aspergillus niger ATCC 16404	+

Sterility test (2 weeks at 20-25 °C and 30-35 °C): no growth

Tryptone Water

For the detection of microbial indole formation when identifying microorganisms by biochemical methods.

This culture medium is recommended by the International Organization for Standardization (ISO) (1975) for the detection of E. coli in the examination of meat and meat products. It can be used instead of Tryptophane Broth recommended in the Standard Methods for the Examination of Water and Wastewater (1992). The medium also complies with the APHA (1998) recommendations for food examination.

Mode of Action

Peptone from casein (= tryptone) contains a high proportion of tryptophane which is degraded by indole-positive organisms to form indole. Indole can be detected with KOVACS Indole Reagent.

Typical Composition (g/litre)

Peptone from casein 10.0; sodium chloride 5.0.

Preparation

Suspend 15 g/litre, dispense into tubes, autoclave (15 min at 121°C).

pH: 7.3 ± 0.2 at 25 °C.

The prepared broth is clear and yellowish.

Experimental Procedure and Evaluation

Inoculate the tubes with pure cultures of the microorganisms to be tested.

Incubation: 24 hours at 35 °C aerobically.

Cover the medium with a 0.5 cm layer of KOVÁCS Indole Reagent. If the culture is indole-positive, the reagent turns cherry red in colour after a few minutes.

Literature

American Public Health Association: Compendium of methods for the microbiological examination of foods. – 3rd ed. (1992).

American Public Health Association, American Water Works Association, Water pollution Control Federation: Standard Methods for the Examination of Water and Wastewater, 20th ed., Washington, 1998.

International Organization for Standardization: Meat and meat products. – Detection and enumeration of presumptive coliform bacteria and presumptive Escherichia coli (Reference method). – **Draft International Standard ISO/DIS 3811** (1975).

Ordering Information

Product	Merck Cat. No.	Pack size
Tryptone Water	1.10859.0500	500 g
Bactident® Indole (dropper bottle)	1.11350.0001	1 x 30 ml
KOVÁCS Indole Reagent	1.09293.0100	100 ml

Quality control

Test strains	Growth	Indole formation
Escherichia coli ATCC 25922	good / very good	+
Proteus vulgaris ATCC 13315	fair / good	+
Morganella morganii ATCC 25830	fair / good	+
Enterobacter cloacae ATCC 13047	good / very good	-
Salmonella typhimurium ATCC 14028	fair / very good	-
Staphylococcus aureus ATCC 25923	fair / good	-

M
MPF

Tryptose Broth

For the enrichment and cultivation of streptococci, pneumococci, meningococci, Listeria, pasteurellae and other pathogenic microorganisms.

IVD *in vitro diagnosticum –*
For professional use only

See also General Instruction of Use
Warnings and precautions see ChemDAT®
(www.chemdat.info)

Tryptose culture media are recommended by HAUSLER and KOONTZ (1970) in diagnostic procedures.

Principle

Microbiological method

Mode of Action

Addition of crystal violet inhibits the Gram-positive bacterial flora (HAUSLER and KOONTZ 1970). I Isolation of Listeria monocytogenes from brain (GRAY et al. 1948), preparation of a Listeria Selective Agar by adding potassium tellurite (GRAY et al. 1950). Tryptose Agar also serves as a satisfactory base for preparing blood agar.

Typical Composition (g/litre)

Tryptose 20.0; D(+)glucose 1.0; sodium chloride 5.0; thiaminium dichloride 0.005;

Preparation and Storage

Cat. No. 1.10676. Tryptose Broth (500 g)

Usable up to the expiry date when stored dry and tightly closed at +15 to +25° C. Protect from light.

After first opening of the bottle the content can be used up to the expiry date when stored dry and tightly closed at +15 to +25° C.

Suspend 26 g Tryptose Broth/litre, autoclave (15 min at 121 °C).

pH: 7.3 ± 0.2 at 25 °C.

The prepared media are clear and yellowish-brown.

Preparation of tryptose crystal violet agar: before autoclaving, add 1.4 ml of an aqueous 1 % crystal violet solution/litre and 13 g/litre agar agar, mix homogeneously.

Preparation of tryptose blood agar: sterile Tryptose Broth plus 13,0 g/l Agar,cooled to 45-50 °C, add 5 % sterile defibrinated blood and mix taking care not to form any bubbles.

Specimen

e.g. Stool, blood.

Clinical specimen collection, handling and processing,see general instructions of use.

Experimental Procedure and Evaluation

A pre-enrichment with Tryptose Broth should be carried out if only small numbers of fastidious bacteria are expected. Incubation of anaerobic microorganisms should be carried out, in each case, for up to 5 days at 35 °C in a 10 % carbon dioxide atmosphere. This can be achieved using Anaerocult® C or Anaerocult® C mini.

For the cultivation of other microorganisms, Tryptose Agar and Tryptose Broth are used. The incubation should be carried out, in each case, under optimum conditions.

Tryptose citrate broth can be used to prepare blood cultures. 2 to 5ml of fresh blood taken from the patient are mixed with 20ml of the broth.

Appearance of Colonies	Microorganisms
Pale pink, opaque, rough surface, large	streptococci

Further differentiation is possible, if Brucella Differential Agar is inoculated with pure Brucella colonies. Instead of employing culture media containing dyes, differentiation can also be performed with strips of paper (CRUICKSHANK 1948) or filter paper discs (PICKETT et al. 1953, SCHINDLER 1955) soaked in the dye solutions and placed on the surface of Tryptose Agar.

Literature

GRAY, M.L., STAFSEHT, H.J., THORP, F., a. RILEY, W.F.: A new technique for isolation of Listerella from bovine brain. - **J. Bact., 55**; 471-476 (1948).

GRAY, M.L., STAFSEHT, H.J., a. THORP, F. jr.: The use of potassium tellurite, sodium azide and acetic acid in a selective medium for the isolation of Listeria monocytogenes. - **J. Bact., 59**; 443-444 (1950).

HAUSLER, W.J., a. KOONTZ, F.P.: Brucellosis in Diagnostic procedures for Bacterial, Mycotic and Parasitic Infections; 5th ed., APHA, New York (1970).

Ordering Information

Product	Merck Cat. No.	Pack size
Tryptose Broth	1.10676.0500	500 g
Agar-agar purified	1.01614.1000	1 kg
Anaerobic jar	1.16387.0001	1 ea
Anaeroclip®	1.14226.0001	1 x 25
Anaerocult® C	1.16275.0001	1 x 10
Anaerocult® C mini	1.13682.0001	1 x 25
Crystal violet Certistain®	1.15940.0025	25 g
Plate basket	1.07040.0001	1ea
Thionine (acetate) Certistain®	1.15929.0025	25 g
tri-Sodium citrate dihydrate	1.06448.0500	500 g
Defibrinated blood		
Fuchsin, basic		

Tryptose Broth

Quality control

Test strains	Growth
Streptococcus pyogenes ATCC 12344	good / very good
Streptococcus pneumoniae ATCC 6301	good / very good
Pasteurella multocida ATCC 43137	fair / good
Listeria monocytogenes ATCC 19118	good / very good
Shigella flexneri ATCC 12022	good / very good
Escherichia coli ATCC 25922	good / very good
Staphylococcus aureus ATCC 25923	good / very good

TSC Agar (Tryptose Sulfite Cycloserine Agar), Base

Medium proposed by HARMON et al. (1971) for the isolation and enumeration of the vegetative and spore forms of Clostridium perfringens in foodstuffs, clinical specimens and other materials.

The culture medium complies with the recommendations of the International Organization for Standardization (ISO) 7937 (2004). It also conforms with the APHA recommendations for the examination of foods (1992).

Mode of Action

The superior nutrient base provides optimal conditions for the development of clostridia. Colonies producing hydrogen sulfide are characterized by blackening due to the reaction with sulfite and iron salt. In TSC Agar cycloserine inhibits the accompanying bacterial flora and causes the colonies, which develop, to remain smaller. It also reduces a diffuse and thus disturbing blackening around the Cl. perfringens colonies. SFP Agar contains polymyxin and kanamycin as selective inhibitors of accompanying flora. It is slightly less selective than TSC Agar.

Typical Composition (g/litre)

Tryptose 15.0; peptone from soymeal 5.0; yeast extract 5.0; sodium disulfite 1.0; ammonium iron(III) citrate 1.0; agar-agar 15.0.

Also to be added:

cycloserine 0.4 or polymyxin 0.003; kanamycin 0.012.

Preparation

Suspend 42 g/litre, dispense into suitable vessels, autoclave (15 min at 121 °C). Add the necessary substances, mix, pour plates.

SFP Agar: Prior to autoclaving, add 3 mg polymyxin sulfate/litre and 12 mg kanamycin disulfate/litre to the culture medium base. These antibiotics can also be added to the sterile, liquefied culture medium in the form of filter-sterilized solutions.

TSC Agar: Cool the liquefied culture medium base to approx.

50 °C, add 0.4 g cycloserine/litre (10 ml of filter-sterilized 5 % solution). Alternatively you can use Clostridium perfringens Supplement, Merck Cat. No. 1.00888.0001.

- **Whereas the prepared culture medium base can be stored for several months, the ready-to-use selective culture media must be used within 4 days after preparation. pH:7.6 ± 0.2 at 25 °C.**

Experimental Procedure and Evaluation

Inoculate the medium by the pour-plate method or by spreading the sample material on the surface of the plates.

Incubation: 18-24 hours at 37 °C or 44 °C under anaerobic conditions (e.g. Anaerocult® A, Anaerocult® A mini, or Anaerocult® P).

Cl. perfringens produces black colonies. Further tests should be performed for purposes of identification.

Literature

American Public Health Association: Compendium of methods for the microbiological examination of foods. - 3rd. (1992).

DIN Deutsches Institut für Normung e.V.: Mikrobiologische Untersuchung von Fleisch und Fleischerzeugnissen. Bestimmung von Clostridium perfringens. Plattenguß-Verfahren (Referenzverfahren). - **DIN 10165**.

EMSWILER, B.S., PIERSON, C.J., a. KOTULA, A.W.: Comparative study of two methods for detection of Clostridium perfringens in ground beef. - **Appl.Envir. Microbiol., 33** ; 735-737 (1977).

HARMON, S.M.: Collaborative study of an improved method for the enumeration and confirmation of Clostridium perfringens in foods. - **J.AOAC, 59**; 606-612 (1976).

HARMON, S.M., KAUTER, D.A., a. PEELER, J.T.: Comparison of media enumeration of Clostridium perfringens. - **Appl. Microbiol., 21**; 922-927 (1971).

HAUSCHILD, A.H.W., a. HILSHEIMER, R.: Evaluation and modifications of media for enumeration of Clostridium perfringens. - **Appl. Microbiol., 27**; 78-82 (1974).

HAUSCHILD, A.H.W., HILSHEIMER, R., a. GRIFFITH, D.W.: Enumeration of faecal Clostridium perfringens spores in egg-yolk-free Tryptose-Sulfite-Cycloserine Agar. - **Appl. Microbiol., 27**; 527-530 (1974).

International Organization for Standardization (ISO): Meat and meat products. - Enumeration of Clostridium perfringens (Reference method). - **Working Draft ISO/TC 34/SC 6** (1978).

ORTH, D.S.: Comparison of sulfite-polymyxin-sulfadiazine medium and tryptose-sulfite-cycloserine medium without eggyolk for recovering Clostridium perfringens. - **Appl. Envir. Microbiol., 33** ; 986-988 (1977).

SHAHIDI, S.A., a. FERGUSON, A.R.: New quantitative, qualitative and confirmatory media for rapid analysis food for Clostridium perfringens - **Appl.Microbiol., 21**; 500-506 (1971).

Ordering Information

Product	Merck Cat. No.	Pack size
TSC Agar (Tryptose Sulfite Cycloserine Agar), Base	1.11972.0500	500 g
Anaerobic jar	1.16387.0001	1 ea
Anaeroclip®	1.14226.0001	1 x 25
Anaerocult® A	1.13829.0001	1 x 10
Anaerocult® A mini	1.01611.0001	1 x 25
Anaerocult® P	1.13807.0001	1 x 25
Anaerotest®	1.15112.0001	1 x 50
Clostridium perfringens Supplement	1.00888.0001	16 vials
Plate basket	1.07040.0001	1 ea
UV Lamp (366 nm)	1.13203.0001	1 ea
D-Cycloserine	CN Biosciences	
Kanamycin disulfate	CN Biosciences	
Polymyxin-B-sulfate	CN Biosciences	

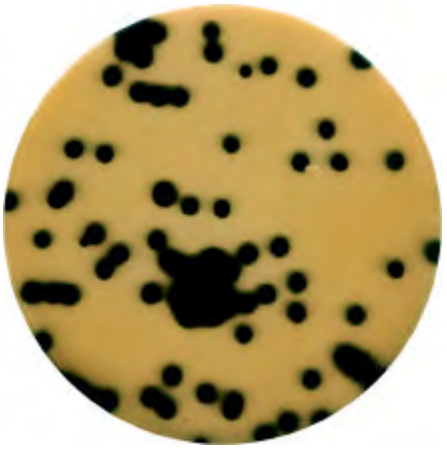
TSC Agar (Tryptose Sulfite Cycloserine Agar), Base

Quality control

Test strains	Growth	Black colonies	Fluorescence*
Clostridium perfringens ATCC 10543	good / very good	+	+
Clostridium perfringens ATCC 13124	good / very good	+	+
Clostridium tetani ATCC 19406	none / fair	-	-
Clostridium novyi ATCC 17861	none / fair	-	-
Pseudomonas aeruginosa ATCC 27853	none / poor	-	-
Bacillus cereus ATCC 11778	none / poor	-	-
Escherichia coli ATCC 25922	none / fair	-	-

*reading if Clostridium Supplement is used

Clostridium perfringens ATCC 10543



Clostridium perfringens ATCC 13124

TSN Agar (Perfringens Selective Agar acc. to MARSHALL)

Tryptone Sulfite Neomycin Agar

Medium proposed by MOSSEL (1959) and MARSHALL et al. (1965) for the detection and enumeration of Clostridium perfringens in foodstuffs and other materials.

This highly selective culture medium provides a rapid quantitative test for Clostridium perfringens.

Mode of Action

TSN Agar (tryptone sulfite neomycin agar) exploits the high neomycin, polymyxin and sulfite tolerances and the strong sulfite-reducing power of Clostridium perfringens and the fact that this organism exhibits optimal growth at 36 °C. The growth of other sulfite-reducing clostridia is almost completely inhibited while that of the accompanying bacterial flora is largely suppressed. Addition of thioglycollate improves and stabilizes anaerobiosis.

Typical Composition (g/litre)

Peptone from casein 15.0; yeast extract 10.0; sodium sulfite 1.0; iron(III) citrate 0.5; Polymyxin B sulfate 0.02; neomycin sulfate 0.05; agar-agar 13.5.

Preparation

Suspend 40 g/litre, autoclave under mild conditions (10 min at 121 °C).

If required, mix in 25 ml of a filter-sterilized, buffered thioglycollate solution (4.0 % di-potassium hydrogen phosphate, 2 % sodium hydrogen carbonate, 4.0 % sodium thioglycollate) to 1 litre of the medium at a temperature of about 50 °C. Avoid subsequent heating.

pH: 7.2 ± 0.2 at 25 °C.

- **The culture medium should be prepared and use the same day.**

The prepared medium is clear and yellowish-brown.

Experimental Procedure and Evaluation

The culture medium is usually inoculated by the mixing in procedure either in test tubes or Petridishes, surface inoculation is not widely used. The thioglycollate solution described above should be added to the medium, if it is poured in Petridishes.

Incubation: at 46 °C for not longer than 18 hours, anaerobically (e.g. using Anaerocult® A, Anaerocult® A mini, or Anaerocult®P).

Clostridium perfringens forms black colonies. The plates should be inspected immediately after opening, otherwise the black colonies become paler in colour due to oxidation of the iron sulfide.

Literature

MARSHALL, R.S., STEENBERGEN, J.F., a. MCCLUNG, L.S.: Rapid technique for enumeration of Clostridium perfringens. – **Appl. Microbiol., 13**; 559-563 (1965).

MOSSEL, D.A.A.: Enumeration of sulfite reducing clostridia occuring in foods. – **J. Sci. Food Agr., 10**; 662-669 (1959).

Ordering Information

Product	Merck Cat. No.	Pack size
TSN Agar (Perfringens Selective Agar acc. to MARSHALL)	1.05264.0500	500 g
Anaerobic jar	1.16387.0001	1 ea
Anaeroclip®	1.14226.0001	1 x 25
Anaerocult® A	1.13829.0001	1 x 10
Anaerocult® A mini	1.01611.0001	1 x 25
Anaerocult® P	1.13807.0001	1 x 25
Anaerotest®	1.15112.0001	1 x 50
di-Potassium hydrogen phosphate	1.05104.1000	1 kg
Plate basket	1.07040.0001	1 ea
Sodium hydrogen carbonate	1.06329.0500	500 g
Sodium thiglycollate	1.06691.0100	100 g

Quality control

Test strains	Growth	Black colonies
Clostridium perfringens ATCC 10543	good / very good	+
Clostridium perfringens ATCC 13124	good / very good	+
Escherichia coli ATCC 25922	none	
Pseudomonas aeruginosa ATCC 27853	none	

Universal Beer Agar (UBA Medium)

Agar for the detection of beer spoilage microorganisms.

Universal Beer Agar is based on the formulation developed by KOZULIS and PHAGE (1968).

Mode of Action

The basal medium is a non-selective agar rich in nutrients that supports the growth and recovery of microorganisms. From a brewer's point of view, only those bacteria and yeasts, which are capable of growing under brewing conditions, are of real significance. The incorporation of beer in the medium adds hop constituents and alcohol which eliminate many airborne contaminants not originating from pitching yeasts, wort or beer, thus minimizing false positive results. Also it stimulates the growth of beer spoilage organisms, such as lactobacilli, pediococci, Acetobacter, Zymomonas spp. and wild yeast strains, which may be found infecting the pitching yeasts, the cooled wort or during fermentation or storage of the finished beer.

For the detection of bacterial contaminants in pitching yeasts, cycloheximide (1 mg/l) may be added.

Typical Composition (g/litre)

Peptonized milk 15.0; yeast extract 10.0; D(+)-glucose 10.0; tomato juice 7.0; dipotassium hydrogen phosphate 0.5; potassium dihydrogen phosphate 0.5; sodium chloride 0.01; iron(II) sulfate 0.01; manganese(II) sulfate 0.01; magnesium sulfate 0.01; agar-agar 12.0

pH 6.3 ± 0.2 at 25 °C.

Preparation

Suspend 55 g in 750 ml demin. water and heat to boiling for approx. 20-35 min until completely dissolved. Add 250 ml beer without degassing to the still hot medium, mix gently and autoclave afterwards at 121 °C for 10 min.

The colour of the prepared basal medium is clear and slightly brown and that of the medium with added beer is determined by the colour of the beer.

Store in the refrigerator and protected from daylight. The shelf-life of prepared plates is approx. 1 week and 2 months for the medium dispensed into bottles when stored at +2-8 °C.

Experimental Procedure

Either direct surface plating, pour plate method (with serial dilutions) or membrane filtration technique can be used.

Plates are incubated at 28-30 °C for 3 days and examined daily, aerobically to detect Acinetobacter and anaerobically to detect microaerophilic lactobacilli, pediococci, and Zymomonas spp.

Interpretation of Results

Examine plate for growth and select identical and typical colonies e.g. via Gram- and catalase testing. Gram-negative and catalase-positive reactions are commonly identified as non-beer-spoilage microorganisms.

Literature

KOZULIS, J.A. AND PAGE, H.E. A new universal beer agar medium for the enumeration of wort and beer microorganisms. Proc. Am. Brew. Chem 52-58, (1968).

Ordering Information

Product	Merck Cat. No.	Pack size
Universal Beer Agar (UBA Medium)	1.00445.0500	500 g
Anaerocult® A	1.13829.0001	1 x 10
Bactident® Catalase	1.11351.0001	1 x 30 ml
Gram-color Staining Set	1.11885.0001	1 pack

Universal Beer Agar (UBA Medium)

Quality control

Test strains	Growth
Lactobacillus fermentum ATCC 9339	good / very good
Pediococcus damnosus ATCC 29358	fair
Saccharomyces cerevisiae ATCC 9763	good / very good
Zymomonas mobilis spp. mobilis ATCC 12568	fair
Acinetobacter baumannii ATCC 19606	good / very good
Escherichia coli ATCC 25922	good / very good

Lactobacillus brevis
ATCC 8287

Pediococcus damnosus
DSMZ 2091

Urea agar Base acc. to CHRISTENSEN

Medium proposed by CHRISTENSEN (1946) for the differentiation of urea-degrading microorganisms.

This culture medium complies with the recommendations of the International Organization for Standardization ISO (1993) and the DIN Norm 10160.

Mode of Action

Urea is hydrolysed to carbon dioxide and ammonia by the enzyme urease. The ammonia formed then causes the medium to become alkaline; this reaction is detected by the indicator phenol red which changes its colour from yellow to purple (see also JEFFRIES, 1964).

Typical Composition (g/litre)

Peptone from meat 1.0; D(+)glucose 1.0; sodium chloride 5.0; potassium dihydrogen phosphate 2.0; phenol red 0.012; agar-agar 12.0.

Also to be added:

Urea 20.0 g/litre.

Preparation

Suspend 21 g/litre, autoclave (15 min at 121 °C). Prior to use, liquefy the medium, cool to 45-55 °C and add 50 ml of a filter-sterilized 40 % urea solution. Prepare slant-agar tubes.

pH: 6.8 ± 0.2 at 25 °C.

The prepared medium is clear and red.

Experimental Procedure and Evaluation

Inoculate the medium massively by spreading the pure culture under investigation on the surface of the agar.

Incubation: 5-48 hours at 35 °C.

Culture medium	Microorganism
Red	Urea-positive: Proteus, Klebsiella, some species of Enterobacter and Citrobacter and others
Yellow	Urea-negative: Shigella, Salmonella, Escherichia, Citrobacter, Enterobacter, Serratia, Providencia and others

Literature

Bundesgesundheitsamt: Amtliche Sammlung von Untersuchungsverfahren nach § 35 LMBG – Beuth Verlag Berlin, Köln

CHRISTENSEN, W.B.: Urea decomposition as means of differentiating Proteus and Paracolon cultures from each other and from Salmonella and Shigella types. – **J. Bact., 52**, 461-466 (1946).

COOK, G.T.: Urease and other biochemical reactions of the Proteus group. - **J.Path. Bact., 60**; 171-181 (1948).

Deutsches Institut für Normung (DIN): Untersuchung von Fleisch und Fleischerzeugnissen – Nachweis von Salmonellen (Referenzverfahren) - **DIN10160**.

International Organization for Standardization (ISO): Detection of salmonellae (Reference method) – **International Standard 6579** (1993).

JEFFRIES, C.D.: Urease activity of intact and disrupted bacteria. - **Arch.Path., 77** ; 544-547 (1964).

STUART, C.A., VAN STRATUM, E., a. RUSTIGIAN, R.: Further studies on urease production by Proteus and related organisms. – **J. Bact., 49**; 437-444 (1945).

THAL, E., a. CHEN, T.H.: Two simple tests for the differentiation of plague and pseudotuberculosis bacilli. – **J. Bact., 69**; 103-104 (1955).

Ordering Information

Product	Merck Cat. No.	Pack size
Urea agar Base acc. to CHRISTENSEN	1.08492.0500	500 g
Urea	1.08487.0500	500 g

Quality control

Test strains	Growth	Clour change to
Escherichia coli ATCC 25922	good / very good	yellow
Shigella flexneri ATCC 12022	good / very good	yellow
Salmonella typhimurium ATCC 14028	good / very good	yellow
Klebsiella pneumoniae ATCC 13883	good / very good	red
Proteus vulgaris ATCC 13315	good / very good	red
Proteus mirabilis ATCC 14153	good / very good	red
Morganella morganii ATCC 25830	good / very good	red

Urea Broth

Differential medium proposed by RUSTIGIAN and STUART (1941) for detecting microorganisms which metabolize urea.

IVD *in vitro diagnosticum –*
For professional use only

See also General Instruction of Use
Warnings and precautions see ChemDAT®
(www.chemdat.info)

Principle

Microbiological method

Mode of Action

This culture medium only supports the growth of microorganisms such as Proteus, which utilize urea as their sole carbohydrate source (STUART et al. 1945, COOK 1948). FERGUSON and HOOK (1943) recommend this medium for differentiating between Proteus and Salmonella; it can also be used to differentiate between bacilli and sarcines. Microorganisms which metabolize urea cause the indicator to change its colour to red and the medium may become turbid as a result of microbial growth.

Typical Composition (g/litre)

Yeast extract 0.1; potassium dihydrogen phosphate 9.1; disodium hydrogen phosphate 9.5; urea 20.0; phenol red 0.01.

Preparation and Storage

Usable up to the expiry date when stored dry and tightly closed at +15 to +25° C. Protect from light.

After first opening of the bottle the content can be used up to the expiry date when stored dry and tightly closed at +15 to +25°C.

Suspend 38.5 g/litre, if necessary heat up to a temperature of 60°C. Sterilize by filtration or dispense aliquots of approx. 3 ml into test tubes and sterilize for 5 minutes in a current of steam under mild conditions.

■ **Do not autoclave.**

pH: 6.8 ± 0.2 at 25 °C.

The broth is clear and orange-red.

If filter sterilization or heat sterilization is not possible, the medium must be inoculated as soon as it has been prepared.

Specimen

e.g. Isolated bacteria from, stool, urine,

Clinical specimen collection, handling and processing, see general instructions of use.

Experimental Procedure and Evaluation

Inoculate the medium massively with the pure culture under investigation.

Incubation: up to 48 hours at 35 °C.

Culture medium	Microorganisms
Red	Urea-positive: Proteus (P. vulgaris, P. mirabilis), Morganella, Rettgerella and others
Yellow	Urea-negative or weakly positive: Shigella, Escherichia, Salmonella, Citrobacter, Enterobacter, Klebsiella, Serratia, Providencia and others

Literature

COOK, G.T.: Urease and other biochemical reactions of the Proteus group. - **J.Path. Bact., 60**; 171-181 (1948).

FERGUSON, W.W., a. HOOK, A.E.: Urease activity of Proteus and Salmonella organisms. – **J. Lab. Clin. Med., 28** ; 1715-1720 (1943).

RUSTIGIAN, R., a. STUART, C.A.: Decomposition of urea by Proteus. - **Proc.Soc. Exptl. Biol. Med., 47**; 108-112 (1941).

STUART, C.A., VAN STRATUM, E., a. RUSTIGIAN, R.: Further studies on urease production by Proteus and related organisms. – **J. Bact., 49**; 437-444 (1945).

Ordering Information

Product	Merck Cat. No.	Pack size
Urea Broth	1.08483.0500	500 g

Quality control

Test strains	Growth	Change to red
Escherichia coli ATCC 25922	poor / fair	-
Salmonella typhimurium ATCC 14028	poor / fair	-
Klebsiella pneumoniae ATCC 13883	poor / fair	-
Proteus vulgaris ATCC 13315	poor / fair	+
Proteus mirabilis ATCC 14153	poor / fair	+
Proteus rettgeri ATCC 29944	poor / fair	+

Urotest® AB

Test system for the detection of antibacterial substances (inhibitors) urine.

IVD *in vitro diagnosticum –*
For professional use only

See also General Instruction of Use
Warnings and precautions see ChemDAT®
(www.chemdat.info)

Mode of Action

Vegetative microorganisms are capable of carrying out a wide range of metabolic functions. The enzymatic transformation of diverse substrates represents the biochemical basis of cellular metabolism.

Spores - the durable form of certain bacteria - do not display any significant metabolic activity. On germination of the spores, metabolism develops i.e. enzyme reactions occur.

Urotest® AB is based on this activation of bacterial metabolism. Germinating spores exhibit metabolic activity. The test organism is provided with a glycoside as a substrate which it then converts to sugar and the corresponding aglycone. In a subsequent reaction 2 aglycone molecules combine to form a stable blue dye.

This reaction sequence can be used to test whether urine samples are free from inhibitors by observing the colour change.

Typical Composition

The reaction zone of an Urotest® AB test strip contains:

Bacillus subtilis	
ATCC 6051	10^7 spores/reaction zone
Nutrient media	1 mg/reaction zone
Indoxyl glycoside	0.04 mg/reaction zone

The special incubation container consists of a "minigrip“ bag made of a special plastic foil.

The special adhesive strip is designed to suit the specific requirements of the test procedure.

Experimental Procedure

The reaction zone of Urotest® AB is wetted with urine, the strip is then placed in the special incubation bag and incubated for 5-24h. It can be used both as a rapid test (incubate for 5-7 h) or for routine testing (incubate for up to 24 h).

See also General Instruction of Use.

Stability

See expiry date.

Remove the number of test strips required from the container and then close immediately taking care that it is tightly sealed. Please store at the specified temperature.

Storage

Store tightly closed in a cool dry place at +2°C to +8°C.

Safe Disposal

All used test strips, special incubation bags and adhesive strips must be disinfected in suitable disinfectant solutions, burnt or autoclaved.

Procedure

After inoculating the immersion culture medium (e.g. Cult-Dip plus Merck®, Cat. No. 1.00777.*), immerse the Urotest® AB test strip in the urine sample under investigation for about 1 sec.

Stroke the Urotest® AB strip against the edge of the sample vesel.

Place the Urotest® AB strip in the special incubation bag and seal carefully.

Label the special adhesive strip with the patient and sample data (not necessary if Cult-Dip plus Merck® is used).

Attach the special adhesive strip to the object to be incubated (immersion culture medium, Petridish) in such a way that the reaction zone is visible.

Incubate at 37°C for 5-24h.

Evaluate the test by comparing the colour change on the test strip with the colour scale on the container for the test strips. The test can be evaluated at any time during incubation (rapid test; routine testing).

Blue = negative, i.e. the urine sample is free from inhibitors.

Beige/white = positive, i.e. the urine sample contains inhibitors.

Evaluation

1. Use a rapid test

Evaluation after 5-7 h** of incubation taking the anamnesis into consideration.

a) BLUE reaction zone

The urine sample is free from inhibitors.

Inhibitor test negative

Anamnesis	Conclusion
Patient not pretreated, no known self medication	./.
Patient pretreated (therapy); known self therapy	Patient does not comply with therapy; anamnesis not correct

Urotest® AB

b) BEIGE/WHITE reaction zone

Urine sample contains inhibitors

Inhibitor test positive

Anamnesis	Conclusion
Patient not pretreated, no known self medication	Anamnesis not correct or incomplete
Patient pretreated (therapy); known self therapy	./.

2. Routine use

Evaluate after 16-24h** of incubation in conjunction with the results obtained after reading the culture medium etc. When interpreting the test, the anamnesis **and** the number of colonies on the culture medium or the measured total microbial count must also be taken into consideration.

a) BLUE reaction zone

The urine sample is free from inhibitors.

Inhibitor test negative

Anamnesis	Microbial growth**	Conclusion
Patient not pretreated, no known self medication	yes	Urinary tract infection; susceptibility test
	no	Take a new sample completed therapy has been successful
Patient pretreated, known self medication	yes	Urinary tract infection patient does not comply with therapy; anamnesis not correct; susceptibility test
	no	Patient does not comply with therapy; anamnesis not correct; take a new sample

b) BEIGE/WHITE reaction zone

Urine contains inhibitors.

Inhibitor test positive

Anamnesis	Microbial growth***	Conclusion
Patient not pretreated, no known self medication	yes	Anamnesis not correct; resistant microorganism or inadequate dosage; susceptibility test
	no	Urinary tract infection and effective drug; anamnesis not correct; take a new sample
Patient pretreated, known self medication	yes	Resistant microorganism; unsatisfactory therapy; susceptibility test
	no	Satisfactory therapy; take a new sample for monitory therapy

*** Availability outside theFederal Republic of Germany on request.

*** The times given are not compulsory. The test can be read and evaluated at any time during the 5-24hours of incubation

*** Mid-stream urine: total microbial count > 5 • 10^4/ml

Notes

1. Ingestion or administration of various substances (e.g. garlic) may produce positive results when using Urotest® AB. This should be clarified when taking the case history.
2. Ingestion or administartion of antibiotics or chemotherapeutic agents produces positive results when using Urotest® AB. It is essential that this problem is carefully dealt with in the case history.
3. Blue spots may occasionally occur on a test zone that does not otherwise change colour and are caused by resistant microorganisms from the urine sample that can act on the substrate. As there is no overall colour change the test should be interpreted as positive.
4. If the inhibitor concentration is close to the detection limit Urotest® AB will display a positive result after 5-7h. It is advisable to re-evaluate the test after 16-24 h if the anamnesis indicates that this is necessary.

Ordering Information

Product	Merck Cat. No.	Pack size
Urotest® AB	1.13194.0001	50 Urotest® AB test strips 50 special incubation bags 50 special adhesive strips
Cult-Dip plus Merck®	1.00777.	

UV Lamp

For the detection of fluoroscent substances.

The UV lamp can be used for the detection of Escherichia coli by means of MUG cleavate in a culture medium containing MUG (Flurocult® or Chromocult® media, page ...) or in conjunction with Bactident® E. coli (Merck, Cat. No. 1.13303.).

Technical Data

Wattage:	4 Watts
Wavelength:	366 nm
Batteries:	5 x 1.5 V baby cells
Weight:	Approximately 400 g
Dimensions:	16 x 9 x 2.5 cm

Ordering Information

Product	Merck Cat. No.	Pack size
UV Lamp	1.13203.0001	

AC
MPF

UVM-Listeria Selective Enrichment Broth, modified

University of Vermont Medium

For the selective enrichment of Listeria in the two-stage procedure according to USDA-FSIS.

Mode of Action

The combination of various peptones, extracts, salts, and buffer substances enable very good growth of Listeria. The selectivity is due to the antiproliferative substances nalidixic acid and acriflavine hydrochloride.

The two-stage enrichment method has demonstrated its value especially with sample materials (meat and meat products) that are characterized by a high level of accompanying flora.

Typical Composition (g/litre)

UVM-I Broth

Tryptose 10.0; meat extract 5.0; yeast extract 5.0; sodium chloride 20.0; disodium hydrogenphosphate 12.0; potassium dihydrogen phosphate 1.35; esculin 1.0; nalidixic acid 20.0 mg; acriflavine hydrochloride 12.0 mg.

UVM-II Broth

Composition identical to UVM-I Broth. In addition, dissolve 13mg acriflavine hydrochloride (= 1 vial of UVM-II Supplement) in 10 ml sterile, distilled water and add to UVM-I Broth which has been previously sterilized and cooled below 50 °C.

Preparation

Suspend 54,4 g in 1 litre of demin. water and autoclave (15 min at 121 °C).

pH: 7.2 ± 0.2 at 25 °C.

The medium is clear to opalescent and yellowish-brown.

1st Enrichment Step: Inoculate the UVM-I broth with sample material (generally 25 g sample material per 225 ml broth) and incubate at 30 °C for 24 hours.

2st Enrichment Step: Inoculate 0.1 ml of UVM-I Broth into 10 ml of UVM-II broth and incubate at 30 °C for further 24 hours aerobically.

Approximately 0.1 ml of the UVM-II Broth is then smeared on the surface of a Listeria-selective agar (e.g. PALCAM Agar, Merck Cat. No. 1.11755. + 1.12122., or Oxford agar, Merck Cat. No.1.07004. + 1.07006.) in such a way to obtain well isolated single colonies.

Literature

DONNELLY, C., BAIGENT, G.: Method for Flow-Cytometric Detection of Listeria Monocytogenes in Milk. – **Appl. Environm. Microbiol.**; 689-695 (1986).

McCLAIN, D., LEE, W.H.: Development of USDA-FSIS Method for Isolation of Listeria monocytogenes from Raw Meat and Poultry. –**J. Assoc. Off. Anal. Chem., 71** (3); 660-664 (1988).

ROLLIER, I., et al.: Comparison of three plating media for enumeration and three media for isolation of Listeria spp. in fermented sausages. - **Arch.Lebensmittelhyg., 42**; 49-76 (1991).

Ordering Information

Product	Merck Cat. No.	Pack size
UVM-Listeria Selective Enrichment Broth, modified	1.10824.0500	500 g
UVM-II Supplement	1.04039.0001	1 vial

Quality control

Test strains	Growth
Listeria monocytogenes ATCC 19114	good / very good
Listeria monocytogenes NCTC 10527	good / very good
Listeria monocytogenes NCTC 7973	good / very good
Listeria ivanovii ATCC 19119	good / very good
Micrococcus luteus ATCC 9341	none / poor
Staphylococcus aureus ATCC 6538	none / poor
Lactobacillus plantarum ATCC 8014	none / fair
Bacillus cereus ATCC 11778	none

UVM-II Supplement

Additive for the preparation of UVM-II-Listeria-Selective Enrichment Broth.

Mode of Action

UVM-II Supplement contains acriflavine hydrochloride. It inhibits the growth of accompanying bacteria in the selective cultivation of Listeria monocytogenes.

Composition (per vial)

Acriflavine hydrochloride 13.0 mg.

Experimental Procedure and Application

Suspend 1 vial in 10 ml sterile, distilled water and add to UVM-Listeria Selective Enrichment Broth (Merck Cat. No. 1.10824), which has been sterilized and cooled to about 50 °C.

Ordering Information

Product	Merck Cat. No.	Pack size
UVM-II Supplement	1.04039.0001	1 x 1 vial

Vitamin B_{12} (Lactobacillus) Assay Broth, Base

For the microbiological assay of vitamins in drugs, foodstuffs, animal feed preparations and other materials.

Certain species of bacteria and some yeasts can only grow in the presence of certain vitamins. If these "test organisms" are transferred to defined culture media which contain all the compounds essential for their growth apart from the vitamin in question, proliferation of the test organisms is totally inhibited or at least drastically reduced. After adding the vitamin the organism can then grow, its growth being dependent on the concentration of the vitamin. The amount of vitamin present can be determined by measuring the turbidity produced as a result of microbial growth or by quantitative assay of a metabolite (e.g. lactic acid). Parallel assays with a pure vitamin preparation of known activity serve as standards.

Typical Composition (g/litre)

D(+)Glucose, anhydrous 40 g; Casein hydrolysate "Vitamin-free" 15 g;DL-Alanine ; L-Asparagine 200 mg; L-Cysteinium chloride 200 mg; L-Cysteine 400 mg; L-Tryptophane 200 mg; Adenine 20mg; Guanosin 40 mg; Uracil 20 mg; Xanthine 20 mg; 4-Aminobenzoic acid 2 mg; L(+)Ascorbic acid 4 g; D(+)Biotin (Vitamin H) 10 µg; Calcium D(+)pantothenate 1 mg; Folic acid 200 µg; Nicotin acid 2 mg; Pyridoxol hydrochloride 4 mg; Pyridoxamine hydrochloride 800 µg; Riboflavin 1 mg; Thiaminium dichloride 1 mg; di-potassium hydrogen phosphate 1 g; Iron(II) sulfate 20 mg; Potassium dihydrogen phosphate 1 g; Magnesium sulfate 400 mg; Manganese(II) sulfate 20 mg; tri-sodium citrate dihydrate ; Sodium acetate, anhydrous 20 g; Sodium chloride 20 mg; To be added: Tween® 80 2 ml; pH at 25°C (± 0.1) 6.0; Quantity per litre (preparation) 83 g

Sample preparation

Vitamin B_{12} Test with Lactobacillus delbrueckii var. lactis

Extraction	If Vitamin B_{12} is freely available in the examination material (e.g. powders, tablets) a simple water extraction is quite adequate. Should the material also contain bound Vit. B_{12}, decomposition either with buffer solution or enzymatic hydrolysis is necessary.
Buffer solution	Homogenize 1 g of examination material in 50 ml of buffer solution (composition: 1.29 g di-sodium hydrogen phosphate, 1.1 g citric acid and 1.0 g sodium disulfite dissolved in 100 ml of demin. water), autoclave for 10 minutes at 121 °C. Adjust pH to 6.0 after cooling, fill up to 100ml with sterile distilled water, filter or centrifuge.
Enzymatic Hydrolysis	Homogenize 1 g of examination material in 80 ml of acetate buffer solution. Add papain, amylase (diastase) and a few drops of toluene or chloroform to the suspension. Maintain at 37°C for about 24 hours, then heat at 100 °C for 30 minutes. After cooling adjust pH to 6.6 with caustic soda solution and fill up to 100 ml with standard acetate buffer solution. The suspension is either filtered or centrifuged. A pre-examination is recommended, if the content of Vit. B_{12} is completely unknown. For this, if possible, a concentrated extract is prepared and examined in a dilution series reducing at the power of 10.
Inoculation Culture	Lactobacillus delbrueckii var. lactis (ATCC 7830) from the type culture of the test organisms is inoculated in Micro-Inoculum-Broth and incubated for 20 hours at 37 °C. Then the culture is centrifuged and rinsed three times with physiological saline and adjusted to a microbial count of 10^8 bacteria/ml.
Calibration	Suspend 100 mg of dried cyanocobalamin (Vitamin B12) in 1 litre of bidistilled water (content: 100 mcg/ml). Before use, this stock solution is diluted to 100 pg/ml to give the reference solution. For calibration a concentration series of 0-25-50-75-100-125-150-200-500 pg cyanocobalamin per 10 ml is made by pipetting 0.0-0.25-0.50-0.75-1.0-1.25-1.5-2.0-5.0 ml of reference solution into test tubes and filling up to 5.0 ml with bististilled water. Test tubes for culture and sterility controls only contain 5 ml of water.
Sample	As with the reference solution, also the sample solution is prepared in a reducing series in test tubes filled up to 5 ml with bidistilled water.
Preparation of test culture medium, inoculation	By briefly boiling, dissolve 83 g of dehydrated Vitamin B_{12} (Lactobacillus) Assay Broth together with 2 ml Tween® 80 in 1 litre bidistilled water. Check the pH and correct if necessary (6.0 at 25°C). Add 5 ml of culture medium to all test tubes with control, sample or reference solution, close with caps and sterilize by autoclaving (10 min at 115 °C). After cooling inoculate the test tubes (apart from sterile controls) with 1 drop of inoculation culture. Incubate for 24 hours at 37°C.
Evaluation	The optical density (OD) of the reference and sample batches is measured photometrically at 546nm against the culture control. A calibration curve is made by applying the turbidity values on the linear ordinate to the corresponding active substance amounts on the logarithmic abscissa. An evaluation is only worthwhile at OD (546 nm, 1 cm) < 0.150 for the control culture measured against water. The sterile controls must not show any growth.

Vitamin B_{12} (Lactobacillus) Assay Broth, Base

Micro-Inoculum Broth

Typical Composition (g/litre)

Proteose peptone 5.0; Yeast extract 20.0; D(+)glucose 10.0; Potassium dihydrogen phosphate 2.0; Tween® 80 0.1

Micro Assay Culture Agar

Preparation

Add 10 g agar-agar to the Micro-Inoculum Broth, autoclave for 15 min at 121 °C.

pH: 6.7 ± 0.1 at 25 °C

Incubation: 24 hours at 35 °C aerobically (both media).

Ordering Information

Product	Merck Cat. No.	Pack size
Vitamin B_{12} (Lactobacillus) Assay Broth, Base	1.11988.0100	100 g
α-Amylase	1.01329.0001	1 g
0.2 N Sodium hydroxide solution	1.09140.1000	1 l
Acetate buffer solution pH 4.66	1.07827.1000	1 l
Agar-agar purified	1.01614.1000	1 kg
Calcium D(+)pantothenate	1.02316.0010	10 g
Chloroform	1.02445.0250	250 ml
Citric acid monohydrate	1.00244.0500	500 g
D(+)Biotin (Vitamin H)	1.24514.0001	1 g
di-sodium hydrogen phosphate	1.06586.0500	500 g
Folic acid for biochemistry	1.03984.0005	5 g
Hydrochloric acid 0.5 N	1.09058.1000	1 l
Nicotinamide	1.06818.0100	100 g
Nicotinic acid	1.06817.0100	100 g
Pancreatin DAB	1.07133.0500	500 g
Papain, water-soluble	1.07144.0025	25 g
Sodium acetate, anhydrous	1.06268.0250	250 g
Sodium chloride	1.06404.0500	500 g
Sodium disulfite	1.06528.0100	100 g
Sodium hydroxide solution 0.1 N	1.09141.1000	1 l
Sodium hydroxide solution 1 mol/l	1.09137.1000	1 l
Sulfuric acid 1.0 N	1.09072.1000	1 l
Toluene	1.08325.1000	1 l
Tween® 80	8.22187.0500	500 ml
Vitamin B_{12} (cyanocobalamin)	1.24592.0100	100 mg

Quality control

Test strains	Inoculation cultures	Growth
Lactobacillus delbrueckii var. lactis ATCC 7830	Adjusted on 50 % T (630 nm, 1 cm cuvette, against 0.9 % NaCl)	Calibration curve shows gradiated growth between 25 to 300 pg cyanocobalamin

Vitamin Biotin Assay Broth

For the microbiological assay of vitamins in drugs, foodstuffs, animal feed preparations and other materials.

Certain species of bacteria and some yeasts can only grow in the presence of certain vitamins. If these "test organisms" are transferred to defined culture media which contain all the compounds essential for their growth apart from the vitamin in question, proliferation of the test organisms is totally inhibited or at least drastically reduced. After adding the vitamin the organism can then grow, its growth being dependent on the concentration of the vitamin. The amount of vitamin present can be determined by measuring the turbidity produced as a result of microbial growth or by quantitative assay of a metabolite (e.g. lactic acid). Parallel assays with a pure vitamin preparation of known activity serve as standards.

Typical Composition (g/litre)

D(+)Glucose, anhydrous 40 g; Casein hydrolysate "Vitamin-free" 12 g; DL-Alanine ; L-Asparagine ; L-Cysteinium chloride ; L-Cysteine 200 mg; L-Tryptophane 100 mg; Adenine 20 mg; Guanosin 40 mg; Uracil 20 mg; Xanthine 10 mg; 4-Amino-benzoic acid 200 µg; L(+)Ascorbic acid ; D(+)Biotin (Vitamin H); Calcium D(+)pantothenate 2 mg; Folic acid ; Nicotin acid 2mg; Pyridoxol hydrochloride 4 mg; Pyridoxamine hydrochloride ; Riboflavin 2 mg; Thiaminium dichloride 2 mg; di-potassium hydrogen phosphate 1 g; Iron(II) sulfate 20 mg; Potassium dihydrogen phosphate 1 g; Magnesium sulfate 400 mg; Manganese(II) sulfate 20 mg; tri-sodium citrate dihydrate ; Sodium acetate, anhydrous 20 g; Sodium chloride 20 mg;
To be added: Tween® 80 ; pH at 25 °C (± 0.1) 6.8; Quantity per litre (preparation) 75 g;

Sample preparation

D-Biotin (Vitamin H) Test

Extraction	To determine the D-biotin content of examination material where the general quantity is known (e.g. pharmaceutical products), the sample under examination is homogenized in water with heating. Pre-examination to establish the general quantity is recommended, if the biotin content is completely unknown. For this, if possible, a concentrated extract is prepared and examined in a dilution series reducing at at the power of 10. If the biotin is bound (e.g. in natural vegetable products) it will be released by acid hydrolysis.
Acid hydrolysis	Homogenize 1 g of examination material in 50 ml 1 N sulfuric acid and then autoclave for 2 hours at 121 °C. After cooling, adjust pH to 4.5, centrifuge and pipette off the supernatant to remove undissolved components. Dilute with distilled water to an optimum concentration for the test. To release biotin from animal material, the autoclavation can be redued to 1 hour at 121 °C, if a stronger acid such as 6 N sulfuric acid is used.
Inoculation culture	Lactobacillus plantarum (ATCC 8014) from the type culture of the test organism is inoculated in Micro-Inoculum Broth and incubated for 24 hours at 37 °C. Then the culture is centrifuged and rinsed three times with physiological saline and adjusted to a microbial count of $3 \cdot 10^8$ bacteria/ml.
Calibration	Suspend 100 mg D-biotin, warming in a steam bath, in 1 litre of bidistilled water (content: 100mcg/ml). Before use, this stock solution is diluted to 1 ng/ml to give the reference solution. For calibration a concentration series of 0.0-0.2-0.4-0.8-1.0-1.5-2.0-2.5-3.0 ng D-biotin per 10ml is made by pipetting 0.0-0.2-0.4-0.8-1.0-1.5-2.0-2.5-3.0 ml of the reference solution into test tubes and filling up to 5.0 ml with bidistilled water. Test tubes for culture and sterility controls only contain 5 ml of water.
Sample	As with the reference solution, also the sample solution is prepared in a reducing series in test tubes filled up to 5 ml with bidistilled water.
Preparation of test culture medium, inoculation	By briefly boiling, dissolve 75 g of dehydrated Vitamin Biotin Assay Broth in 1 litre bidistilled water. Check the pH and if required correct (6.8 at 25 °C). Add 5 ml of the culture medium to all test tubes with control, sample or reference solution, close with caps and sterilize by autoclaving (10 min at 115 °C). After cooling inoculate the test tubes (apart from sterile controls) with 1 drop of inoculation culture. Incubate for 16 to 20 hours at 37 °C.
Evaluation	The optical density (OD) of the reference and sample batches is measured photometrically at 546nm against the culture control. A calibration curve is made by applying the turbidity values on the linear ordinate to the corresponding active substance amounts on the logarithmic abscissa. An evaluation is only worthwhile at OD (546 nm, 1 c) < 0.150 for the control culture measured against water. The sterile controls must not show any growth.

Vitamin Biotin Assay Broth

Micro-Inoculum Broth

Typical Composition (g/litre)

Proteose peptone 5.0; Yeast extract 20.0; D(+)glucose 10.0; Potassium dihydrogen phosphate 2.0; Tween® 80 0.1

Micro Assay Culture Agar

Preparation

Add 10 g agar-agar to the Micro-Inoculum Broth, autoclave for 15 min at 121 °C.

pH: 6.7 ± 0.1 at 25 °C

Incubation: 24 hours at 35 °C aerobically (both media).

Ordering Information

Product	Merck Cat. No.	Pack size
Vitamin Biotin Assay Broth	1.11989.0100	100 g
α-Amylase	1.01329.0001	1 g
0.2 N Sodium hydroxide solution	1.09140.1000	1 l
Acetate buffer solution pH 4.66	1.07827.1000	1 l
Agar-agar purified	1.01614.1000	1 kg
Calcium D(+)pantothenate	1.02316.0010	10 g
Chloroform	1.02445.0250	250 ml
Citric acid monohydrate	1.00244.0500	500 g
D(+)Biotin (Vitamin H)	1.24514.0001	1 g
di-sodium hydrogen phosphate	1.06586.0500	500 g
Folic acid for biochemistry	1.03984.0005	5 g
Hydrochloric acid 0.5 N	1.09058.1000	1 l
Nicotinamide	1.06818.0100	100 g
Nicotinic acid	1.06817.0100	100 g
Pancreatin DAB	1.07133.0500	500 g
Papain, water-soluble	1.07144.0025	25 g
Sodium acetate, anhydrous	1.06268.0250	250 g
Sodium chloride	1.06404.0500	500 g
Sodium disulfite	1.06528.0100	100 g
Sodium hydroxide solution 0.1 N	1.09141.1000	1 l
Sodium hydroxide solution 1 mol/l	1.09137.1000	1 l
Sulfuric acid 1.0 N	1.09072.1000	1 l
Toluene	1.08325.1000	1 l
Tween® 80	8.22187.0500	500 ml
Vitamin B_{12} (cyanocobalamin)	1.24592.0100	100 mg

Quality control

Test strains	Inoculation culture	Growth
Lactobacillus plantarum ATCC 8014	Adjusted on 30 % T (630 nm, 1cm cuvette, against 0.9 % NaCl)	Calibration curve shows gradiated growth between 0.2 to 3 ng biotin

Vitamin Folic Assay Broth, Base

For the microbiological assay of vitamins in drugs, foodstuffs, animal feed preparations and other materials.

Certain species of bacteria and some yeasts can only grow in the presence of certain vitamins. If these "test organisms" are transferred to defined culture media which contain all the compounds essential for their growth apart from the vitamin in question, proliferation of the test organisms is totally inhibited or at least drastically reduced. After adding the vitamin the organism can then grow, its growth being dependent on the concentration of the vitamin. The amount of vitamin present can be determined by measuring the turbidity produced as a result of microbial growth or by quantitative assay of a metabolite (e.g. lactic acid). Parallel assays with a pure vitamin preparation of known activity serve as standards.

Typical Composition (g/litre)

D(+)Glucose, anhydrous 40 g; Casein hydrolysate "Vitamin-free" 12 g; DL-Alanine 400 mg; L-Asparagine 200 mg; L-Cysteinium chloride ; L-Cysteine 200 mg; L-Tryptophane 100 mg; Adenine 20 mg; Guanosin 40 mg; Uracil 20 mg; Xanthine 10 mg; 4-Aminobenzoic acid 200 µg; L(+)Ascorbic acid ; D(+)Biotin (Vitamin H) 0.8 µg; Calcium D(+)pantothenate 400 µg; Folic acid; Nicotin acid 2 mg; Pyridoxol hydrochloride 4 mg; Pyridoxamine hydrochloride ; Riboflavin 2 mg; Thiaminium dichloride 2mg; di-potassium hydrogen phosphate 1 g; Iron(II) sulfate 20 mg; Potassium dihydrogen phosphate 1 g; Magnesium sulfate 400 mg; Manganese(II) sulfate 20 mg; tri-sodium citrate dihydrate 30 g; Sodium acetate, anhydrous 20 g; Sodium chloride 20mg; To be added: Tween® 80 0.4 ml; pH at 25 °C (± 0.1) 6.8; Quantity per litre (preparation) 106 g

Sample preparation

Folic Acid Test

Extraction	Folic acid is extracted from the examination material by water and by adding an equivalent amount of caustic soda solution and heating. Enzymatic pre-treatment is necessary to release bound folic acid. For this, homogenize 1 g of the sample material in 150 ml of 0.05 N phosphate buffer (pH 7.2) and sterilize (15 min at 121 °C). Add 20 mg pancreatin (dried) and incubate for 24hours at 37 °C. Then the suspension is autoclaved (5 min at 121 °C), cooled and filtered.
Inoculation culture	Enterococcus hirae (ATCC 8043) from the type culture of the test organism is inouculated in Micro-Inoculum Broth and incubated for 24 hours at 37 °C. Then the culture is centrifuged and rinsed several times with physiological saline and adjusted to a microbial count of $2 \cdot 10^8$ bacteria/ml.
Calibration	Suspend 50 mg dried folic acid in 30 ml 0.01 N caustic soda solution. Add 300 ml bidistilled water and adjust the pH to 7.8 (± 0.5) filling up to 500 ml (content: 100 mcg/ml). Before use, this stock solution is diluted to 2 ng/ml to give the reference solution. For calibration, a concentration series of 0.0-0.5-1.0-2.0-4.0-8.0-10.0 ng folic acid per 10 ml is made by pipetting 0.0-0.25-0.5-1.0-2.0-4.0-5.0 ml of reference solution into test tubes and filling up to 5.0 ml with bidistilled water. Test tubes for culture and sterility controls only contain 5 ml of water.
Sample	As with the reference solution, also the sample solution is prepared in a reducing series in test tubes filled up to 5 ml with bidistilled water.
Preparation of test culture medium inoculation	By briefly boiling, dissolve 106 g of dehydrated Vitamin Folic Acid Assay Test Broth together with 0.4 ml Tween® 80 in 1 litre bidistilled water. Check the pH and when required corret it (6.8 at 25 °C). Add 5 ml of culture medium to all test tubes with control, sample or reference solution, close with caps and sterilize by autoclaving (10 min at 115 °C). After cooling inoculate the test tubes (apart from sterile controls) with 1 drop of inoculation culture. Incubate for 16 to 20 hours at 37 °C.
Evaluation	The optical density (OD) of the reference and sample batches is measured photometrically at 546nm against the culture control. A calibration curve is made by applying the turbidity values on the linear ordinate to the corrsponding active substance amounts on the logarithmic abscissa. An evaluation is only worthwhile at OD (546 nm, 1 cm) < 0.0150 for the control culture measured against water. The sterile controls must not show any growth.

Vitamin Folic Assay Broth, Base

Micro-Inoculum Broth

Typical Composition (g/litre)

Proteose peptone 5.0; Yeast extract 20.0; D(+)glucose 10.0; Potassium dihydrogen phosphate 2.0; Tween® 80 0.1

Micro Assay Culture Agar

Preparation

Add 10 g agar-agar to the Micro-Inoculum Broth, autoclave for 15 min at 121 °C.

pH: 6.7 ± 0.1 at 25 °C

Incubation: 24 hours at 35 °C aerobically (both media).

Ordering Information

Product	Merck Cat. No.	Pack size
Vitamin Folic Assay Broth, Base	1.11990.0100	100 g
α-Amylase	1.01329.0001	1 g
0.2 N Sodium hydroxide solution	1.09140.1000	1 l
Acetate buffer solution pH 4.66	1.07827.1000	1 l
Agar-agar purified	1.01614.1000	1 kg
Calcium D(+)pantothenate	1.02316.0010	10 g
Chloroform	1.02445.0250	250 ml
Citric acid monohydrate	1.00244.0500	500 g
D(+)Biotin (Vitamin H)	1.24514.0001	1 g
di-sodium hydrogen phosphate	1.06586.0500	500 g
Folic acid for biochemistry	1.03984.0005	5 g
Hydrochloric acid 0.5 N	1.09058.1000	1 l
Nicotinamide	1.06818.0100	100 g
Nicotinic acid	1.06817.0100	100 g
Pancreatin DAB	1.07133.0500	500 g
Papain, water-soluble	1.07144.0025	25 g
Sodium acetate, anhydrous	1.06268.0250	250 g
Sodium chloride	1.06404.0500	500 g
Sodium disulfite	1.06528.0100	100 g
Sodium hydroxide solution 0.1 N	1.09141.1000	1 l
Sodium hydroxide solution 1 mol/l	1.09137.1000	1 l
Sulfuric acid 1.0 N	1.09072.1000	1 l
Toluene	1.08325.1000	1 l
Tween® 80	8.22187.0500	500 ml
Vitamin B_{12} (cyanocobalamin)	1.24592.0100	100 mg

Quality control

Test strains	Inoculation culture	Growth
Enterococcus hirae ATCC 8043	Adjusted on 50 % T (630 nm, 1 cm cuvette, against 0.9 % NaCl)	Calibration curve shows gradiated growth between 200 to 2000 pg folic acid

Vitamin Pantothenic Acid Assay Broth, Base

For the microbiological assay of vitamins in drugs, foodstuffs, animal feed preparations and other materials.

Certain species of bacteria and some yeasts can only grow in the presence of certain vitamins. If these "test organisms" are transferred to defined culture media which contain all the compounds essential for their growth apart from the vitamin in question, proliferation of the test organisms is totally inhibited or at least drastically reduced. After adding the vitamin the organism can then grow, its growth being dependent on the concentration of the vitamin. The amount of vitamin present can be determined by measuring the turbidity produced as a result of microbial growth or by quantitative assay of a metabolite (e.g. lactic acid). Parallel assays with a pure vitamin preparation of known activity serve as standards.

Typical Composition (g/litre)

D(+)Glucose, anhydrous 40 g; Casein hydrolysate "Vitamin-free" 12 g; DL-Alanine ; L-Asparagine ; L-Cysteinium chloride ; L-Cysteine 400 mg; L-Tryptophane 100 mg; Adenine 20 mg; Guanosin 40 mg; Uracil 20 mg; Xanthine ; 4-Aminobenzoic acid 200 µg; L(+)Ascorbic acid ; D(+)Biotin (Vitamin H) 0.8 µg; Calcium D(+)pantothenate ; Folic acid ; Nicotin acid 1 mg; Pyridoxol hydrochloride 800 µg; Pyridoxamine hydrochloride ; Riboflavin ; Thiaminium dichloride 200 µg; di-potassium hydrogen phosphate 1 g; Iron(II) sulfate 20 mg; Potassium dihydrogen phosphate 1 g; Magnesium sulfate 400 mg; Manganese(II) sulfate 20 mg; tri-sodium citrate dihydrate ; Sodium acetate, anhydrous 20 g; Sodium chloride 20 mg;
To be added: Tween® 80 0.4 ml; pH at 25 °C (± 0.1) 6.8;
Quantity per litre (preparation) 75 g

Sample preparation

Pantothenic Acid Test

Extraction	To determine pantothenic acid and its salts in materials where the general quantity is known (e.g. pharmaceutical preparations such as injection solutions, tablets, dragees etc.), an aqueous extraction or solution is diluted so that the expected turbidity values lie in the middle range of the calibration curve. If the content of free vitamin is totally unknown, it is recommended to carry out a pre-examination with a concentrated extract to the material to determine the general quantity. If the vitamin is present in bound form (in natural material such as vegetable or animal samples), it must be previously released completely by enzymatic hydrolysis.
Enzymatic Hydrolysis	Homogenize 1 g of examination material in 80 ml of acetate buffer solution. Add 40 mg papain and 40 mg amylase (diastase) and a few drops of toluene or chloroform to the suspenktsion. Maintain at 37 °C for about 24 hours, then heat the substrate at 100 °C for 30minutes. After cooling adjust pH to 6.8 with caustic soda solution, fill up to 100 ml with standard acetate buffer solution and filter.
Inoculation culture	Lactobacillus plantarum (ATCC 8014) from the type culture of the test organism is inoculated in Micro-Inoculum Broth or in the semi-concentrated (37.5 g/l) culture medium with 20 ng/ml of added calcium pantothenate and incubated for 20 hours at 37 °C. Then the culture is centrifuged and rinsed several times with physiological saline and adjusted to a microbial count of $3 \cdot 10^8$ bacteria/ml.
Calibration	Suspend 50 mg calicum-D-pantothenate in 50 ml of bidistilled water (content: 1 mg/ml). This stock solution is diluted before use by the power of ten down to 20 ng/ml as the reference solution. For calibration a concentration series of 0-10-20-40-60-80-100 ng calcium-D-pantothenate per 10 ml is made by pipetting 0.0-0.5-1.0-2.0-3.0-4.0-5.0 ml of the reference solution into test tubes and filling up to 5.0 ml with bidistilled water. Test tubes for culture and sterility controls only contain 5 ml of water.
Sample	As with the reference solution, also the sample solution is prepared in a deducing series in test tubes filled up to 5 ml with bidistilled water.
Preparation of test culture medium inoculation	By briefly boiling, dissolve 75 g of dehydrated Vitamin Pantothenic Acid Assay Broth together with 0.4 ml Tween® 80 in 1 litre bidistilled water. Check the pH and when required correct (6.8 at 25 °C). Add 5 ml of culture medium to all test tubes with control, sample or reference solution, close with caps and sterilize by autoclaving (10 min at 115 °C). After cooling inoculate the test tubes (apart from sterile controls) with 1 drop of inoculation culture. Incubate for 24 hours at 37 °C.
Evaluation	The optical density (OD) of the reference and sample batches is measured photometrically at 546 nm against the culture control. A calibration curve is made by applying the turbidity values on the linear ordinate to the corresponding active substance amounts on the logarithmic abscissa. An evaluation is only worthwhile at OD (546 nm, 1 cm) < 0.150 for the control culture measured against water. The sterile controls must not show any growth.

Vitamin Pantothenic Acid Assay Broth, Base

Micro-Inoculum Broth

Typical Composition (g/litre)

Proteose peptone 5.0; Yeast extract 20.0; D(+)glucose 10.0; Potassium dihydrogen phosphate 2.0; Tween® 80 0.1

Preparation

Suspend 37.1 g in 1 litre of demin. water, autoclave 15 min at 121 °C.

pH: 6.7 ± 0.2 at 25 °C

The medium is clear and brown.

Micro Assay Culture Agar

Preparation

Add 10 g agar-agar to the Micro-Inoculum Broth, autoclave for 15 min at 121 °C.

pH: 6.7 ± 0.1 at 25 °C

Incubation: 24 hours at 35 °C aerobically (both media).

Ordering Information

Product	Merck Cat. No.	Pack size
Vitamin Pantothenic Acid Assay Broth, Base	1.11993.0100	100 g
α-Amylase	1.01329.0001	1 g
0.2 N Sodium hydroxide solution	1.09140.1000	1 l
Acetate buffer solution pH 4.66	1.07827.1000	1 l
Agar-agar purified	1.01614.1000	1 kg
Calcium D(+)pantothenate	1.02316.0010	10 g
Chloroform	1.02445.0250	250 ml
Citric acid monohydrate	1.00244.0500	500 g
D(+)Biotin (Vitamin H)	1.24514.0001	1 g
di-sodium hydrogen phosphate	1.06586.0500	500 g
Folic acid for biochemistry	1.03984.0005	5 g
Hydrochloric acid 0.5 N	1.09058.1000	1 l
Nicotinamide	1.06818.0100	100 g
Nicotinic acid	1.06817.0100	100 g
Pancreatin DAB	1.07133.0500	500 g
Papain, water-soluble	1.07144.0025	25 g
Sodium acetate, anhydrous	1.06268.0250	250 g
Sodium chloride	1.06404.0500	500 g
Sodium disulfite	1.06528.0100	100 g
Sodium hydroxide solution 0.1 N	1.09141.1000	1 l
Sodium hydroxide solution 1 mol/l	1.09137.1000	1 l
Sulfuric acid 1.0 N	1.09072.1000	1 l
Toluene	1.08325.1000	1 l
Tween® 80	8.22187.0500	500 ml
Vitamin B_{12} (cyanocobalamin)	1.24592.0100	100 mg

Quality control

Test strains	Inoculation culture	Growth
Lactobacillus plantarum ATCC 8014	Adjusted on 50 % T (630 nm, 1 cm cuvette, against 0.9 % NaCl)	Calibration curve shows gradiated growth between 10 to 60 ng Ca-pantothenate

VOGEL-JOHNSON Agar, Base

Medium introducted by ZEBOVITZ et al. (1955) and modified by VOGEL and JOHNSON (1960) for the detection of mannitol-positive staphylococci in clinical specimens and other materials.

This culture medium complies with the recommendations of the United States Pharmakopeia XXVI (2003).

Mode of Action

The growth of accompanying bacteria is almost completely inhibited by tellurite, lithium chloride and a high glycine concentration. The staphylococci may also be slightly inhibited, but this is compensated by the presence of mannitol and glycine. Mannitol also serves as a differentiation reactant as it is degraded to acid by most pathogenic staphylococci; this reaction is indicated by phenol red, which changes its colour to yellow. Pathogenic staphylococci also reduce tellurite to metallic tellurium, thus their colonies turn black in colour.

Typical Composition (g/litre)

Peptone from casein 10.0; yeast extract 5.0; di-potassium hydrogen phosphate 5.0; D(-)mannitol 10.0; lithium chloride 5.0; glycine 10.0; phenol red 0.025; agar-agar 13.0.

Also to be added:

Potassium tellurite 0.24.

Preparation

Suspend 58 g/litre and let stand for 30 minutes. During dissolving, agitate frequently (aprox. every 5 min). Swirl once more prior to autoclaving. Autoclave (15 min at 121 °C). Prior to use, add 0.24 g potassium tellurite/litre in the form of a filter-sterilized solution at a temperature of about 50 °C, mix. Pour plates.

- **Do not heat the complete culture medium.**

pH: 7.2 ± 0.2 at 25 °C.

- **The complete culture medium can be stored in the refrigerator for up to 1 week, whereas the prepared culture medium base can be kept for several months.**

The plates are clear and red.

Experimental Procedure and Evaluation

Inoculate the plates massively.

Incubation: up to 48 hours at 35 °C aerobically.

Pathogenic staphylococci usually grow within the first 24 hours.

Appearance of Colonies	Microorganisms
Small, black, sourrounded by yellow zones	Staphylococcus aureus
Small, gray-black, not surrounded by zones	Staph. epidermidis, Proteus hauseria (usually completely inhibited) and others

Literature

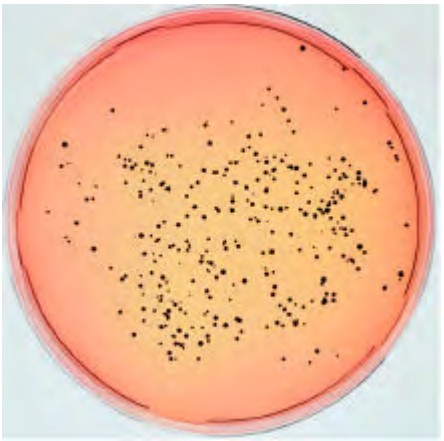
Unted States Pharmacopeia XXVI, Chapter "Microbial Limit Tests" (2003).

VOGEL, R.A., a. JOHNSON, M.: A modification of the tellurite-glycine medium for the use in the identification of Staphylococcus aureus. - **Publ.Hlth. Lab., 18**; 131-133 (1960).

ZEBOVITZ, E., EVANS, J.B., a. NIVEN, C.F.: Tellurite-glycine agar, a selective plating medium for the quantitative detection of coagulase positive staphylococci. - **J. Bact., 70**; 686-690 (1955).

Ordering Information

Product	Merck Cat. No.	Pack size
VOGEL-JOHNSON Agar, Base	1.05405.0500	500 g
Potassium tellurite trihydrate	1.05164.0100	100 g



VOGEL-JOHNSON Agar,
black colonies; change of pH indicated by colour change to yellow

VOGEL-JOHNSON Agar, Base

Quality control

Test strains	Growth / Recovery rate %	Black colonies	Colour change to yellow
Staphylococcus aureus ATCC 25923	≥ 40	+	+
Staphylococcus aureus ATCC 6538	≥ 40	+	+
Staphylococcus epidermidis ATCC 12228	poor / fair	±	-
Enterococcus faecalis ATCC 11700	none / fair	±	-
Proteus mirabilis ATCC 29906	fair / good	+	-
Micrococcus luteus ATCC 10240	none / poor		
Escherichia coli ATCC 25922	none / poor		
Pseudomonas aeruginosa ATCC 27853	none		

VRB Agar (Violet Red Bile Agar)

Selective agar proposed by DAVIS (1951) for the detection and enumeration of coliform bacteria including E. coli in water, milk, ice-cream, meat and other foodstuffs.

This medium complies with the recommendations of the American Public Health Association (1992), the International Dairy Federation FIL-IDF (Internationaler Milchwirtschaftsverband 1985), the Institute for Food Technology and Packaging (Institut für Lebenmitteltechnologie und Verpackung) (1974) and the EUROGLACE (KLOSE 1968 a, b).

Mode of Action

Crystal violet and bile salts inhibit growth primarily of the Gram-positive accompanying bacterial flora. Degradation of lactose to acid is indicated by the pH indicator neutral red, which changes its colour to red, and by precipitation of bile acids.

Typical Composition (g/litre)

Peptone from meat 7.0; yeast extract 3.0; sodium chloride 5.0; lactose 10.0; neutral red 0.03; bile salt mixture 1.5; crystal voilet 0.002; agar-agar 13.0.

Preparation

Suspend 39.5 g in 1 litre of demin. water and heat to boiling with frequent stirring until completely dissolved. Afterwards do not boil more than 2 minutes.

■ **Do not autoclave. Do not overheat!**

pH: 7.4 ± 0.2 at 25 °C.

The prepared medium is clear and dark-red.

Experimental Procedure and Evaluation

Culture medium is usually inoculated by the pour-plate procedure.

Incubation: 24 ± 2hours at 30 ± 1 °C (IDF-FIL) respectively according to recommended procedures.

Appearance of Colonies	Microorganisms
Red, surrounded by reddish precipitation zones, diameter 1-2 mm	Lactose-positive Enterobacteriaceae: coliform bacteria, E. coli
Pink pin-point colonies	Enterococci, possibly Klebsiella
Colourless	Lactose-negative Enterobacteriaceae

Literature

American Public Health Association: Compendium of Methods for the microbiological Examination of Foods. - 3rd ed. (1992).

American Public Health Association: Standard Methods for the Examination of Dairy Products. - 15th ed. (1995).

DAVIS, J.G.: Milk Testing - **Dairy Industries Ltd., London**, 1951.

Institut für Lebensmitteltechnologie und Verpackung der TU München: Merkblatt 19: Bestimmung der Gesamtkeimzahl, der Anzahl an Schimmelpilzen und Hefen und der Anzahl an coliformen Keimen in Flaschen und vergleichbaren enghalsigen Behältern. - **Milchwiss., 29**; 602-606 (1974).

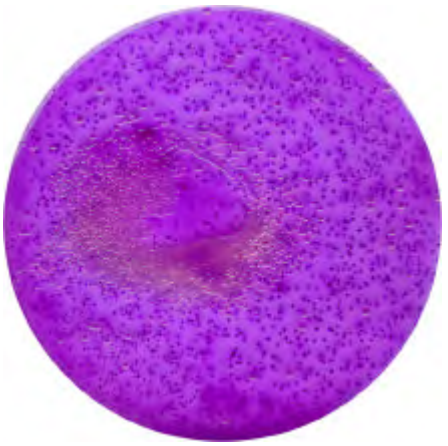
Internationaler Milchwirtschaftsverband: Zählung coliformer Bakterien in Milch und Milchprodukten. - **Internationaler Standard FIL-IDF 73 A: 1985**.

KLOSE, J.: Harmonisierung des Speiseeisrechtes in der EWG. - **Süßwaren, 14**; 778-780 (1968a).

KLOSE, J.: Entwurf einer Richtlinie zur Angleichung der Rechtsvorschriften für Speiseeis in den Mitgliedsstaaten der EWG. Neufassung des AnhangsIII zum Entwurf vom 19.12.1966. - **Süßwaren, 14**; 780-782 (1968b).

Ordering Information

Product	Merck Cat. No.	Pack size
VRB Agar (Violet Red Bile Agar)	1.01406.0500	500 g
VRB Agar (Violet Red Bile Agar)	1.01406.5000	5 kg



Escherichia coli ATCC 11775

VRB Agar (Violet Red Bile Agar)

Quality control (spiral plating method)

Test strains	Inoculum (cfu/ml)	Recovery rate %	Colony colour	Precipitate
Escherichia coli ATCC 11775	10^3-10^5	≥ 30	red	+
Salmonella gallinarum NCTC 9240	10^3-10^5	≥ 30	colourless-reddish	-
Shigella flexneri ATCC 29903	10^3-10^5	≥ 30	colourless	-
Yersinia enterocolitica ATCC 9610	10^3-10^5	≥ 30	colourless	-
Staphylococcus aureus ATCC 6538	> 10^5	≤ 0.01		
Micrococcus luteus ATCC 9341	> 10^5	≤ 0.01		
Lactococcus lactis spp. lactis ATCC 19435	> 10^5	≤ 0.01		
Bacillus cereus ATCC 11778	> 10^5	≤ 0.01		
Lactobacillus plantarum ATCC 14917	> 10^5	≤ 0.01		

VRBD (Violet Red Bile Dextrose) Agar acc. to MOSSEL

Selective agar proposed by MOSSEL et al. (1962, 1963) for the isolation and enumeration of all Enterobacteriaceae species in foodstufs.

This medium complies with the recommendations of the International Organization for Standardization (ISO) (1977) and the German Minister of Health (Bundesminister für das Gesundheitswesen) (1967) and largely conforms with the European Pharmacopeia II. HECHELMANN et al. (1973) obtained good results with this culture medium. The medium also complies with the German recommendations according § 35 LMBG for food examination.

Mode of Action

Crystal violet and bile salts inhibit the accompanying bacterial flora. Degradation of glucose is accompanied by production of acid, which is indicated by a colour change to red and by zones of precipitated bile acids surrounding the colonies. All Enterobacteriaceae are detected as they all degrade glucose to acid. The culture medium is not, however, absolutely specific for these organisms as some other accompanying bacteria (e.g. Aeromonas) also show these reactions.

Typical Composition (g/litre)

Peptone form meat 7.0; yeast extract 3.0; sodium chloride 5.0; D(+)glucose 10.0; bile salt mixture 1.5; neutral red 0.03; crystal violet 0.002; agar-agar 13.0.

Preparation

Suspend 39.5 g in 1 litre of demin. water and heat to boiling with frequent stirring until completely dissolved. Afterwards do not boil more than 2 minutes.

■ **Do not autoclave! Do not overheat!**

pH: 7.3 ± 0.2 at 25 °C.

The prepared medium is clear and dark red.

Incubation: for 24 hours at 35 °C aerobically.

The identity of suspected Enterobacteriaceae colonies should be confirmed by further tests.

Appearance of Colonies	Microorganisms
Red, surrounded by reddish precipitation zones	Enterobacteriaceae and others
Colourless	No Enterobacteriaceae present

Literature

Bundesgesundheitsamt: Amtliche Sammlung von Untersuchungsverfahren nach § 35 LMBG. - Beuth Verlag Berlin, Köln.

Bundesminister für das Gesundheiswesen: "Allgemeine Verwaltungsvorschrift für das Verfahren bei der amtlichen Untersuchung von vorbehandelten Eierprodukten". - **Bundesanzeiger, 96**; 2-3 (1967) [s. auch **Dtsch. Lebensmitt.-Rdsch., 63**; 245-249 (1967)].

European Pharmacopeia II, Chapter VIII, 10.

HECHELMANN, H.; ROSSMANITH, E., PERIC, M., u. LEISTNER, L.: Untersuchung zur Ermittlung der Enterobacteriaceae-Zahl bei Schlachtgeflügel. - **Fleischwirtsch., 53**; 107-113 (1973).

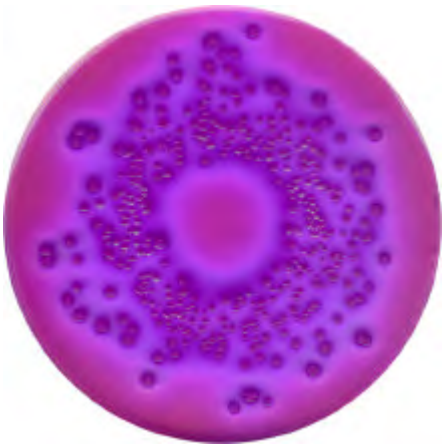
International Organization for Standardization: Meat and meat products - detection and enumeration of Enterobacteriaceae (Reference methods). - **Draft International Standard ISO/DIS 5552** (1977).

MOSSEL, D.A.A., MENGERINK, W. HJ., a. SCHOLTS, H.H.A.: Use of a modified MacConkey agar medium for the selective growth and enumeration of all Enterobacteriaceae. - **J. Bact., 84**; 381 (1962).

MOSSEL, D.A.A., a. CORNELISSEN, A.M.R.: The examination of foods for Enterobacteriaceae using a test of the type generally adopted for the detection of Salmonellae. - **J. Appl. Bact., 26**; 444-452 (1963).

Ordering Information

Product	Merck Cat. No.	Pack size
VRBD (Violet Red Bile Dextrose) Agar acc. to MOSSEL	1.10275.0500	500 g



Escherichia coli ATCC 8739

Shigella flexneri ATCC 29903

VRBD (Violet Red Bile Dextrose) Agar acc. to MOSSEL

Quality control (spiral plating method)

Test strains	Inoculum (cfu/ml)	Recovery rate %	Colony colour	Preicpitate
Escherichia coli ATCC 8739	10^3-10^5	≥ 30	red	+
Salmonella gallinarum NCTC 9240	10^3-10^5	≥ 30	red	+
Salmonella typhimurium ATCC 14028	10^3-10^5	≥ 30	red	+
Shigella flexneri ATCC 29903	10^3-10^5	≥ 30	red	+
Yersinia enterocolitica ATCC 9610	10^3-10^5	≥ 30	red	+
Staphylococcus aureus ATCC 6538	> 10^5	≤ 0.01		
Micrococcus luteus ATCC 9341	> 10^5	≤ 0.01		
Lactococcus lactis spp. lactis ATCC 19435	> 10^5	≤ 0.01		
Bacillus cereus ATCC 11778	> 10^5	≤ 0.01		
Lactobacillus plantarum ATCC 14917	> 10^5	≤ 0.01		

WL Nutrient Agar

For the enumeration and cultivation of yeasts and bacteria in the microbiological control carried out in the brewing and other fermentation industries (GREEN and GRAY 1950, 1951; GRAY 1951).

GRAY (1951) has given a detailed description of the use of WL Nutrient Agar and WL Differential Agar in the microbiologial quality control employed in the beer-brewing industry.

Mode of Action

WL Nutrient Agar has a pH of 5.5, which is optimal for the enumeration of brewers' yeast. If bakers' or distillers' yeast is to be examined, the pH should be adjusted to 6.5 (better yields). When cultivating microorganisms from an alcoholic mash, tomato juice should be added to the medium. WL differential agar contains cycloheximide to suppress yeasts and any moulds which may be present; this medium allows reliable counting of all bacteria which may be encountered in the tests performed in brewery laboratories.

Typical Composition (g/litre)

Yeast extract 4.0; casein hydrolysate 5.0; D(+)glucose 50.0; potassium dihydrogen phosphate 0.55; potassium chloride 0.425; calcium chloride 0.125; magnesium sulfate 0.125; iron(III) chloride 0.0025; manganese sulfate 0.0025; bromocresol green 0.022; agar-agar 17.0

Preparation

Suspend 77 g/litre, if required dissolve the medium in a mixture of 400 ml clarified tomato juice and 600 ml demineralized water, adjust the pH to 6.5 if necessary, autoclave (15 min at 121 °C), pour plates.

pH: 5.5 ± 0.2 at 25 °C.

The plates are clear and blue-green.

Experimental Procedure and Evaluation

Dilute the sample material, spread 0.1 ml on WL Nutrient Agar and, if necessary, on WL differential agar.

Incubation: up to 2 weeks at 25 ° and, if applicable, at 30 °C aerobically. WL Differential Agar should be incubated both aerobically and anaerobically.

Count the number of colonies per plate and calculate the microbial count. Acetic acid bacteria, flavobacteria, thermobacteria, Proteus bacteria and other species grow on WL Differential Agar under aerobic conditions whereas lactobacilli and pediococci proliferate under anaerobic conditions.

Literature

GRAY, P.P.: Some advances in microbiological control for beer quality. - **Wallerstein Lab. Comm., 14**; 169-183 (1951).

GREEN, S.R., a. GRAY, P.P.: Paper read at Am. Soc. of Brewing Chemists Meeting; - **Wallerstein Lab. Comm., 12**; 43 (1950).

GREEN, S.R., a. GRAY, P.P.: A differential procedure applicable to bacteriological investigation in brewing. - **Wallerstein Lab. Comm., 13**; 357-366 (1950).

GREEN, S.R., a. GRAY, P.P.: A differential procedure for bacteriological studies useful in the fermentation industries. - **Wallerstein Lab. Comm., 14**; 289-295 (1951).

Ordering Information

Product	Merck Cat. No.	Pack size
WL Nutrient Agar	1.10866.0500	500 g
Anaeroclip®	1.14226.0001	1 x 25
Anaerocult® A mini	1.01611.0001	1 x 25
Anaerocult® P	1.13807.0001	1 x 25
Anaerotest®	1.15112.0001	1 x 50

Quality control

Test strains	Growth
Candida albicans ATCC 10231	good / very good
Saccharomyces cerevisiae ATCC 9763	good / very good
Lactobacillus acidophilus ATCC 4356	fair / good
Leuconostoc mesenteroides ATCC 9135	good / very good
Enterococcus faecalis ATCC 11700	good / very good
Escherichia coli ATCC 11775	good / very good
Proteus mirabilis ATCC 29906	good / very good

Wort Agar

For the cultivation, isolation and enumeration or enrichment of fungi, especially of yeasts.

According to RAPP (1974), addition of certain indicator dyes to Wort Agar allows differentiation between yeast and bacterial colonies.

Mode of Action

The accompanying bacterial flora is weakly suppressed by the pH value of 5.0 and largely by a pH of 3.5.

Typical Composition (g/litre)

Malt extract 15.0; universal peptone 0.75; maltose 12.75; dextrin 2.75; glycerol 2.35; potassium dihydrogen phosphate 0.4; ammonium chloride 1.0; agar-agar 20.0.

Preparation

Suspend 55 g/litre by heating briefly, dispense into suitable vessels, autoclave (15 min at 121 °C).

■ **Do not overheat. If possible do not reliquefy.**

pH: 5.0 ± 0.2 at 25 °C.

The prepared medium is clear and brownish.

pH: 3.5: Cool to about 50 °C, add approximately 12 ml of filter-sterilized 10 % lactic acid/litre, mix.

■ **Do not reheat.**

Experimental Procedure and Evaluation

Inoculate Wort Agar by the pour-plate method or by spreading the sample onto the surface of the medium. Further steps depend on the purpose for which the medium is used.

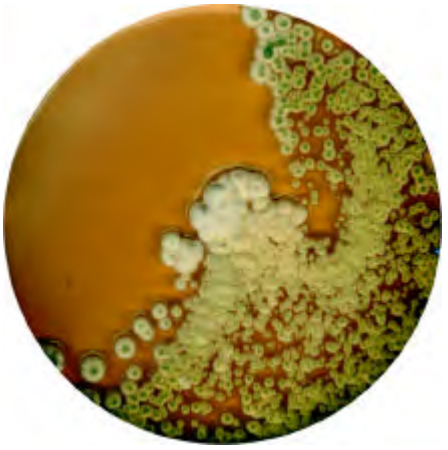
Incubation: up to 7 days at 28 °C aerobically.

Literature

RAPP, M.: Indikatorzusätze zur Keimdifferenzierung auf Würze- und Malzextrakt-Agar. – Milchwiss., 29; 341-344 (1974).

Ordering Information

Product	Merck Cat. No.	Pack size
Wort Agar	1.05448.0500	500 g
Wort Agar	1.05448.5000	5 kg
Glycerol (about 87 %)	1.04094.0500	500 ml
L(+)-Tartaric acid	1.00804.0250	250 g
Lactic acid about 90 % purified	1.00366.0500	500 ml



Penicillium commune
ATCC 10428

Rhodotorula mucilaginosa
DSMZ 7043

Wort Agar

Quality control

Test strains	Growth
Geotrichum candidum DSMZ 1240	good / very good
Penicillium commune ATCC 10428.	good / very good
Aspergillus niger ATCC 16404	good / very good
Trichophyton ajelloi ATCC 28454	fair / good

Quality control (spiral plating method)

Test strains	Inoculum (cfu/ml)	Recovery rate %
Candida albicans ATCC 10231	10^3-10^5	≥ 70
Saccharomyces cerevisiae ATCC 9763	10^3-10^5	≥ 70
Saccharomyces cerevisiae ATCC 9080	10^3-10^5	≥ 70
Rhodotorula mucilaginosa DSMZ 70403	10^3-10^5	≥ 70

Wort Broth, Base

For the cultivation, isolation and enumeration or enrichment of fungi, especially of yeasts.

According to RAPP (1974), addition of certain indicator dyes to Wort Agar allows differentiation between yeast and bacterial colonies.

Mode of Action

The accompanying bacterial flora is weakly suppressed by the pH value of 5.0 and largely by a pH of 3.5.

Typical Composition (g/litre)

Malt extract 15.0; universal peptone 0.75; maltose 12.75; dextrin 2.75; potassium dihydrogen phosphate 0.75; ammonium chloride 1.0.

Also to be added:

glycerol 2.5 ml.

Preparation

Suspend 33 g/litre together with 2.5 ml glycerol/litre, if desired dispense into suitable containers, autoclave (15 min at 121 °C).

pH: 5.0 ± 0.2 at 25 °C.

The prepared broth is clear and yellowish-brown.

Experimental Procedure and Evaluation

Inoculate Wort Broth. Further steps depend on the purpose for which the medium is used.

Incubation: up to 7 days at 28 °C aerobically.

Literature

RAPP, M.: Indikatorzusätze zur Keimdifferenzierung auf Würze- und Malzextrakt-Agar. – **Milchwiss., 29** ; 341-344 (1974).

Ordering Information

Product	Merck Cat. No.	Pack size
Wort Broth, Base	1.05449.0500	500 g
Glycerol (about 87 %)	1.04094.0500	500 ml
L(+)-Tartaric acid	1.00804.0250	250 g
Lactic acid about 90 % purified	1.00366.0500	500 ml

Quality control

Test strains	Growth
Candida albicans ATCC 10231	good / very good
Saccharomyces cerevisiae ATCC 9763	good / very good
Saccharomyces cerevisiae ATCC 9080	good / very good
Geotrichum candidum DSMZ 1240	good / very good
Rhodotorula mucilaginosa DSMZ 70403	good / very good
Penicillium commune ATCC 10428	good / very good
Aspergillus niger ATCC 16404	good / very good
Trichophyton ajelloi ATCC 28454	good / very good

XLD (Xylose Lysine Deoxycholate) Agar

Medium proposed by TAYLOR (1965), TAYLOR and HARRIS (1965, 1967) and TAYLOR and SCHELHART (1967) for the isolation and differentiation of pathogenic Enterobacteriaceae, especially of Shigella and Salmonella species.

This culture medium complies with the recommendations of the ISO 6579.

Mode of Action

Degradation of xylose, lactose and sucrose to acid causes phenol red to change its colour to yellow. Production of hydrogen sulfide is indicated by thiosulfate and iron(III) salt, which react to form a precipitate of black iron sulfide in the colonies. Bacteria which decarboxylate lysine to cadaverine can be recognized by the appearance of a purple colouration around the colonies due to an increase in pH.

These reactions can proceed simultaneously or successively, this may cause the pH indicator to exhibit various shades of colour or it may change its colour from yellow to red on prolonged incubation. The culture medium is weakly inhibitory.

Typical Composition (g/litre)

Yeast extract 3.0; sodium chloride 5.0; D(+)xylose 3.75; lactose 7.5; sucrose 7.5; L(+)lysine 5.0; sodium deoxycholate 1.0; sodium thiosulfate 6.8; ammonium iron(III) citrate 0.8; phenol red 0.08; agar-agar 14.5.

Preparation

1. Weigh out 55 g of XLD Agar.
2. Add 50 ml of demin. water to a flask
3. Transfer 55 g of XLD Agar gently to flask with swirling.
4. Mix thoroughly, add remaining 950 ml demin. water, until completely suspended. Check for lumps. If present repeat mixing.
5. Heat to boiling to dissolve completely.
6. Immediately cool the medium to about 47-50 °C in a waterbath set at this temperature. Agitate flask to cool rapidly.
7. Pour plates.
8. Dry plates and check for sterility prior to use.

Note: preparation of large volumes, overheating and prolonged storage in water bath (47-50 °C) should be avoided.

■ **Do not autoclave.**

pH: 7.4 ± 0.2 at 25 °C.

The plates are clear and red.

Crystalline precipitate of salts may occur. To avoid this, the liquid medium needs to be filtered through a flute-formed filter.

Experimental Procedure and Evaluation

Inoculate by spreading the material thinly on the surface of the plates.

Incubation: up to 48 hours at 35 °C aerobically.

Further tests should be performed in order to identify the colonies.

Appearance of Colonies	Microorganisms
Yellow, surrounded by yellow zones, opaque with precipitation zones	Escherichia coli, Enterobacter, Aeromonas
Yellow, surrounded by yellow zones, opaque, mucoid with precipitation zones	Klebsiella
Yellow, surrounded by yellow zones, opaque, sometimes with a black centre	Citrobacter (lactose-positive strains)
Yellow, surrounded by yellow zones, opaque,	Serratia, Hafnia
Yellow, surrounded by yellow zones, translucent, black centre	Proteus vulgaris, most Proteus mirabilis
Colonies have the same colour as the culture medium, translucent, sometimes with a black centre	Salmonella
Colonies have the same colour as the culture medium, translucent	Shigella, Providencia, Pseudomonas
Orange, slightly opaque	Salmonella typhosa (xylose-positive strains)

Literature

American Public Health Association. Compendium of Methods for the microbiological Examination of Foods. – 3rd ed. (1992).

BHAT, P., a. RAIAN, D.: Comparative evaluation of deoxycholate citrate medium and xylose lysine deoxycholate medium in the isolation of shigellae. – **Am. J. Clin. Pathol**., 64; 99-404 (1975).

DUNN, C., a. MARTIN, W.J.: Comparison of media for isolation of Salmonellae and Shigellae from fecal specimens. – **Appl. Microbiol., 22**; 17-22 (1971).

European Pharmacopeia II, Chapter VIII, 10.

ROLLENDER, W., BECKFORD, O., BELSKY, R.D., a. KOSTROFF, B.: Comparison of xylose lysine deoxycholate agar and MacCONKEY Agar for the isolation of Salmonella and Shigella from clininal specimens. – **Am. J. Clin. Pathol., 51/2**; 284-386 (1969).

TAYLOR, W.J.: Isolation of Shigellae. I. Xylose lysine agars: new media for isolation of enteric pathogens. – **Am. J. Clin. Path., 44**; 471-475 (1965).

TAYLOR, W.J., a. HARRIS, B.: Isolation of Shigellae. II. Comparison of plating media and enrichment broths. – **Am. J. Clin. Path., 44X** 476-479 (1965).

TAYLOR, W.J., a. HARRIS, B.: Isolation of Shigellae. III. Comparison of new and traditional media with stool specimens. – **Amer. J. Clin. Pathol., 48**; 350-355 (1967).

TAYLOR, W.J., a. SCHELHART, D.: Isolation of Shigellae. IV. Comparison of plating media with stools. – **Amer. J. Clin. Pathol., 48** ; 356-362 (1967).

TAYLOR, W.J., a. SCHELHART, D.: Isolation of Shigellae. V. Comparison of enrichment broth with stools. – **Appl. Microbiol., 16**; 1383-1386 (1968).

United States Pharmacopeia XXVI, Chapter "Microbioal Limit Tests", 2003

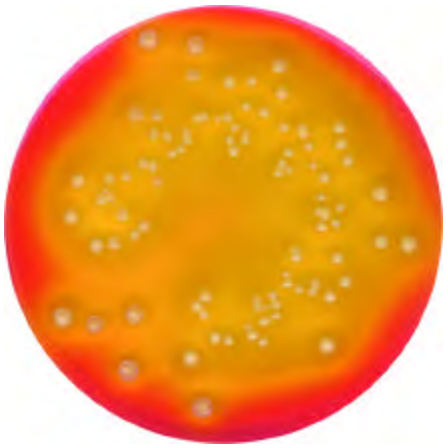
XLD (Xylose Lysine Deoxycholate) Agar

Ordering Information

Product	Merck Cat. No.	Pack size
XLD (Xylose Lysine Deoxycholate) Agar	1.05287.0500	500 g

Quality control (spiral plating method)

Test strains	Inoculum (cfu/ml)	Recovery rate %	Colony colour	Black centre	Colour change of medium
Escherichia coli ATCC 25922	> 10^5	not limited	yellow	-	yellow + precipitate
Enterobacter cloacae ATCC 13047	10^3-10^5	≥ 30	yellow	-	yellow + precipitate
Klebsiella pneumoniae ATCC 13883	10^3-10^5	≥ 30	yellow	-	yellow + precipitate
Shigella flexneri ATCC 12022	10^3-10^5	≥ 10	colourless	-	
Shigella sonnei ATCC 11060	10^3-10^5	≥ 10	colourless	-	
Salmonella typhimurium ATCC 14028	10^3-10^5	≥ 30	colourless	+	-
Salmonella enteritidis NCTC 5188	10^3-10^5	≥ 30	colourless	+	-
Proteus mirabilis ATCC 14273	10^3-10^5	≥ 30	yellow	+	yellow / orange
Enterococcus faecalis ATCC 11700	> 10^5	≤ 0.01		-	



Klebsiella pneumoniae ATCC 13883

Salmonella enteritidis NCTC 5188

DA

XLT4 Agar, Base

Medium for the isolation and identification of pathogenic Enterobacteriaceae, especially Salmonella spp, according to MILLER and TATE (1990).

Mode of Action

The selection of suitable nutrients and vitamins (peptones, yeast extract) allow optimal growth of salmonellae. At the same time the surfactant NIAPROOF-4 (formerly Tergitol-4/Sodiumtetradecylsulfate) largely inhibits the accompanying flora.

Salmonellae, due to H_2S-formation (thiosulfate and iron(III)-ions), can be easily detected as black colonies on a red-violet background and differentiated from the residual accompanying flora. E. coli, in contrast, will show yellow colonies on a yellow background due to acidification of the medium (pH-indicator: phenol-red). Other accompanying organisms, like Shigella, due to a missing H_2S-formation and acidification, will grow colourless on a red background.

Typical Composition (g/litre)

Proteose peptone No. 3 1,6; yeast extract 3.0; L-lysine 5.0; xylose 3.75; lactose 7.5; sucrose 7.5; ammonium-iron(III) citrate 0.8; sodium thiosulfate 6.8; sodium chloride 5.0; phenol-red 0.08; agar-agar 18.0.

Preparation

Suspend 59 g in 1 litre of demin. water, add 4.6 ml XLT4 Agar Supplement solution and heat the medium in a boiling water-batch (not on a heating-plate!). Cool to approx. 50 °C and pour plates.

■ **Do not overheat, do not autoclave.**

The medium should not be kept longer than 45 minutes at 50 °C to avoid possible precipitates.

pH: 7.4 ± 0.2 at 25 °C.

The plates are clear and red.

Experimental Procedure

Spread sample material from an enrichment on the surface of the culture medium.

Incubation: 18-24 hours at 35 °C aerobically. If this will neither result in black colonies nor in visible growth continue incubation up to 48 hours.

Evaluation

Black or black centred colonies on a red-violet background indicate the presence of H2S-positive salmonellae. Further tests should be performed in order to identify the colonies.

Literature

MILLER, R.G., C.R. TATE. 1990. XLT4: A highly selective plating medium for the isolation of Salmonella. **The Maryland Poultryman, April: 2-7 (1990).**

Ordering Information

Product	Merck Cat. No.	Pack size
XLT4 Agar, Base	1.13919.0500	500 g
XLT4 Agar Supplement (Sodium tetradecylsulfate solution 26-28 %)	1.08981.0100	100 ml

Quality control

Test strains	Growth	Colony color
Salmonella typhimurium ATCC 14028	good / very good	black center
Salmonella enteritidis ATCC 13076	good / very good	black center
Salmonella anatum ATCC 9270	good / very good	black center
Shigella sonnei ATCC 11060	good / very good	colourless
Shigella flexneri ATCC 12022	good / very good	colourless
Enterobacter aerogenes ATCC 13048	fair / good	yellow
Citrobacter freundii ATCC 8090	fair / good	yellow
Proteus mirabilis ATCC 14273	none / poor	-
Escherichia coli ATCC 25922	none / fair	yellow to colourless

Yeast Extract Agar

For the cultivation of yeasts and moulds from various materials, especially from milk and dairy products.

Typical Composition (g/litre)

Yeast extract 5.0; glucose 10.0; agar-agar 20.0.

Preparation

Suspend 35 g/litre, autoclave (15 min at 121 °C).

pH: 6,5 ± 0.2 at 25 °C.

The plates are clear and yellowish.

Experimental Procedure and Evaluation

Inoculate the culture medium by the pour-plate method or by spreading the material on the surface. The subsequent procedure depends on the purpose for which the medium is used.

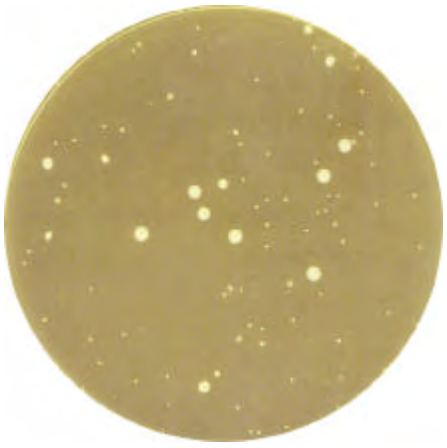
Incubation: up to 7 days at 28 °C aerobically.

Ordering Information

Product	Merck Cat. No.	Pack size
Yeast Extract Agar	1.03750.0500	500 g

Quality control

Test strains	Growth
Staphylococcus aureus ATCC 25923	good / very good
Escherichia coli ATCC 25922	good / very good
Candida albicans ATCC 10231	good / very good
Geotrichum candidum DSMZ 1240	good / very good
Aspergillus niger ATCC 16404	good / very good
Penicillium commune ATCC 10428	good / very good
Rhodotorula mucilaginosa DSMZ 70403	good / very good



Escherichia coli ATCC 25922

Penicillium commune ATCC 10428

Yeast Extract Agar acc. to ISO 6222

Nutrient medium for the determination of total microbial count in water.

Yeast extract agar is a medium rich in nutrients which permits the recovery of a wide spectrum of bacteria, yeast and moulds.

The medium conforms with the ISO norm 6222 and the Swedish Standard SS 028171 for the examination of water.

Mode of Action

Water can contain a large number of microorganisms coming in particular from the earth and vegetation.

The combination of a culture medium rich in nutrients with incubation temperatures of 36°C and 22°C allows the detection of a large number of these organisms.

Typical Composition (g/litre)

Peptone from casein 6.0; yeast extract 3.0; agar-agar 15.0.

Preparation

Suspend 24.0 g in 1 litre demin. water and heat in a boiling water bath or steam jet until the medium is completely dissolved. Then autoclave medium for 15 min. at 121 °C and cool to 45±1°C. The culture medium should not be kept in the water bath for longer than 4 h at 45 °C.

pH: 7.2 ± 0.2 at 25°C

The prepared medium is clear and yellow-brown in colour.

The prepared medium remains stable for 1 week at 4 ± 2 °C.

Experimental Procedure

The determination of the total microbial count is carried out by the pour plate method.

15 - 20 ml of culture medium (45°C) are added to 1 ml of sample and mixed well.

Each sample is incubated both at 36 ± 2 °C for 44 ± 4 h as well as 22 ± 2 °C for 68 ± 4 h.

Evaluation

The colonies per plate are counted for each incubation temperature and the microbial count/ml is calculated.

Literature

International Organization for Standardization: Water Quality – Enumeration of culturable microorganisms – Colony count by inoculation in a nutrient agar culture medium, International Standard ISO 6222 (1999).

Ordering Information

Product	Merck Cat. No.	Pack size
Yeast Extract Agar acc. to ISO 6222	1.13116.0500	500 g

Quality control

Test strains	Inoculum cfu/ml	Growth 36 ± 2 °C / 48 h	Growth 22 ± 2 °C / 72 h
Escherichia coli ATCC 25922	ca. 100	+	
Pseudomonas fluorescens ATCC 13525	ca. 100		+
Enterococcus faecalis ATCC 11700	ca. 100	+	
Candida albicans ATCC 10231	ca. 100	+	
Aspergillus niger ATCC 16404	ca. 100		+

Yersinia Selective Agar Base acc. to SCHIEMANN (CIN-Agar)

Medium proposed by SCHIEMANN (1979) for the selective cultivation of Yersinia, particularly Y. enterocolitica and Y. pseudotuberculosis, from clinical specimens, foodstuffs, water etc.

IVD *in vitro diagnosticum –*
For professional use only

See also General Instruction of Use
Warnings and precautions see ChemDAT®
(www.chemdat.info)

The medium complies with the recommendations of the APHA (1992) for food examination.

Principle

Microbiological method

Mode of Action

The accompanying flora is largely inhibited by a mixture of antibiotics [Yersinia Selective Supplement (CIN)], crystal violet and bile salts. The growth of Yersinia is, however, promoted by pyruvate and a superior nutrient base. Yersinia degrade the present mannitol to form acid; the colonies therefore turn red due to a change in the colour of the indicator neutral red.

Typical Composition (g/litre)

Peptone from casein 10.0; peptone from meat 10.0; yeast extract 2.0; D(-)mannitol 20.0; sodium pyruvate 2.0; sodium chloride 1.0; magnesium sulfate 0.01; bile salt mixture 1.0; neutral red 0.03; crystal violet 0.001; agar-agar 12.5.

Preparation and Storage

Cat. No. 1.16434. Yersinia Selective Agar Base acc. to SCHIEMANN (CIN-Agar) (500 g)

Usable up to the expiry date when stored dry and tightly closed at +15 to +25° C. Protect from light.

After first opening of the bottle the content can be used up to the expiry date when stored dry and tightly closed at +15 to +25° C.

Suspend 58.5 g/litre autoclave (15 min at 121 °C), cool to 45-50 °C. Add the contents of one vial of Yersinia Selective Supplement (CIN) to 500 ml culture medium and mix under sterile conditions. Pour plates.

pH: 7.4 ± 0.2 at 25 °C.

The plates are clear and red.

Specimen

e.g. Stool, smears of infected tissue.

Clinical specimen collection, handling and processing, see general instructions of use.

Experimental Procedure and Evaluation

Inoculate the plates with sample material from an enrichment culture, Yersinia Broth acc. to OSSMER, by the streak-plate method.

Incubation: 24-48 hours at 28 °C aerobically.

Yersinia grows to produce colonies that have a dark red centre and a transparent periphery. The size of the colonies, the width of their edges and their surface structure may vary depending on the serotype.

Certain accompanying microorganisms (e.g. some Enterobacteriaceae and Pseudomonas) may also sometimes exhibit scanty growth.

Literature

American Public Health Association: Compendium of Methods for the microbiologica Examination of Foods. – 3rd ed. (1992).

BERINGER, T.: Erfahrungen mit einem neuen Yersinia-Nährboden. **Ärztl. Lab., 30**, 327-330 (1984).

PRIMAVESI, C.A., u. LORRA-EBERTS, A.: Erfahrungen mit einem neu entwickelten Selectiv-Agar nach Schiemann zum Nachweis von Yersinia enterocolitica. **- Lab. med., 7**; 59-61 (1983).

SCHIEMANN, D.A.: Synthesis of a selective agar medium for Yersinia enterocolitica. **- Canad. J. Microbiol., 25**; 1298-1304 (1979).

Ordering Information

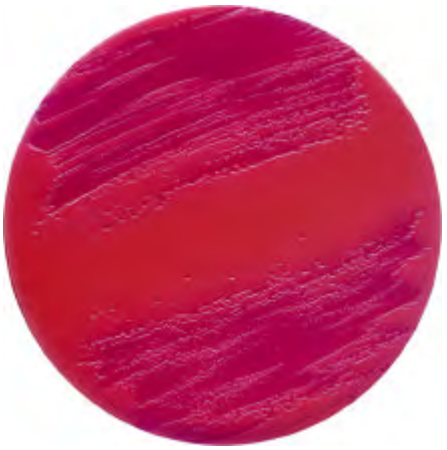
Product	Merck Cat. No.	Pack size
Yersinia Selective Agar Base acc. to SCHIEMANN (CIN-Agar)	1.16434.0500	500 g
Yersinia Selective Enrichment Broth acc. to OSSMER	1.16701.0500	500 g
Yersinia Selective Supplement (CIN)	1.16466.0001	1 x 16 vials
Merckoplate® Yersinia Selective agar acc. to Schiemann (CIN-agar)	1.13578.0001	1 x 20 plates

Yersinia Selective Agar Base acc. to SCHIEMANN (CIN-Agar)

Quality control

Test strains	Growth	Red centre
Yersinia enterocolitica ATCC 9610	good / very good	+
Yersinia enterocolitica ATCC 35669	good / very good	+
Escherichia coli ATCC 25922	none	
Salmonella typhimurium ATC 14028	none	
Enterobacter cloacae ATCC 13047	none / poor	
Staphylococcus aureus ATCC 25923	none	

Yersinia enterocolitica ATCC 35669-4-6o



Yersinia enterocolitica ATCC 9610-orig-2o

Yersinia Selective Supplement (CIN)

Supplement for preparing Yersinia Selective Agar acc. to SCHIEMANN (MERCK, Cat. No. 1.16434.)

in vitro diagnosticum –
For professional use only

See also General Instruction of Use
Warnings and precautions see ChemDAT®
(www.chemdat.info)

Principle

Microbiological method

Mode of Action

Yersinia Selective Supplement (CIN) consists of a lyophilized mixture of three different inhibitors. If largely inhibits the accompanying flora encountered in the cultivation of Yersinia, particularly in the case of Y. enterocolitica.

Typical Composition (g/litre)

Cefsulodin 7.5 mg; Irgasan 2.0 mg; novobiocin 1.25 mg.

Experimental Procedure

Suspend the lyophilisate in the vial by adding 1 ml of sterile, distilled water and 1 ml ethanol. Mix the contents of one vial uniformly with 500 ml of the sterile, still liquid Yersinia Selective Agar Base, cooled to a temperature of 45-50 °C.

Storage

Usable up to the expiry date when stored dry and tightly closed at +2 to +8 °C.

After first opening of the bottle the content should be used completely.

Ordering Information

Product	Merck Cat. No.	Pack size
Yersinia Selective Supplement (CIN)	1.16466.0001	1 x 16 vials
Ethanol absolute	1.00983.1000	1 l

Yersinia Selective Enrichment Broth acc. to OSSMER

For the selective enrichment of Yersinia, especially Yersinia enterocolitica.

Mode of Action

By selecting specific peptones and substances the rapid growth of Yersinia is enhanced. Growth of accompanying flora is largely inhibited by the addition of Irgasan and Bacitracin.

Typical Composition (g/litre)

Peptone 10.0; L-asparaginic acid 20.0; sodium pyruvate 2.5; Bacitracin 0.15; Irgasan 0.01, Tween® 80 0.5; MOPS/TRIS 5.5.

Preparation

Suspend 38.7 g in 1 litre demin. water and autoclave (15 min at 121 °C). In case the broth is poured into suitable vessels prior to autoclavation make sure that ingredients like Irgasan are completely dissolved.

pH: 7.2 ± 0.2 at 25 °C.

The prepared broth is clear and yellowish-brown and can be stored at +4 to +8 °C for about 6 months.

Experimental Procedure

The sample material is - based on the grade of contamination - in ratios 1:10 or 1:100 mixed with the broth.

After a 24 hour aerobic incubation at 30 °C, the material is plated on a selective agar (e.g. Yersinia Selective Agar acc. to SCHIEMANN (CIN-Agar or SSDC Agar acc. to ISO).

Literature

SCHIEMANN, D.A.: Synthesis of a selective agar medium for Yersinia enterocolitica. **- Canad. J. Microbiol., 25**; 1298-1304 (1979).

WAUTERS, G.: Improved methods for the isolation and the recognition of Yersinia enterocolitica. **- Contro. Microbiol. Immunol., 2**; 68-70 (1973).

Ordering Information

Product	Merck Cat. No.	Pack size
Yersinia Selective Enrichment Broth acc. to OSSMER	1.16701.0500	500 g
SSDC Agar acc. to ISO	1.16724.0500	500 g
Yersinia Selective Agar Base acc. to SCHIEMANN (CIN-Agar)	1.16434.0500	500 g
Yersinia Selective Supplement	1.16466.0001	1 x 16 vials

Quality control

Test strains	Inoculum % / Mixed culture	Growth after 24 hours
Yersinia enterocolitica ATCC 23715	5 – 10 %	≥ 90 %
Yersinia enterocolitica ATCC 23715	5 – 10 %	≥ 80 %
Escherichia coli ATCC 25922	90 – 95 %	≤ 10 %
Pseudomonas aeruginosa ATCC 27853	90 – 95 %	≤ 20 %

YGC Agar
(Yeast Extract Glucose Chloramphenicol Agar FIL-IDF)

Selective agar for isolating and counting yeasts and moulds in milk and milk products.

This culture medium complies with the recommendations of the International Dairy Federation (Internationaler Milchwirtschaftsverband) (1990), the International Organization for Standardization (ISO), the DIN Norm 10186 for the examination of milk and the German recommendations for food examination acc. to § 35 LMBG.

Mode of Action

The culture medium contains chloramphenicol to suppress accompanying bacterial flora. Unlike other similar culture media, which contain antibiotics (e.g. Oxytetracycline Glucose Yeast Agar), it has the advantage of being fully autoclavable. Once prepared, it is stable for a long period of time - at least 4 months according to ENGEL (1982).

Typical Composition (g/litre)

Yeast extract 5.0; D(+)glucose 20.0; chloramphenicol 0.1; agar-agar 14.9.

Preparation

Suspend 40 g/litre and autoclave (15 min at 121 °C).

pH: 6.6 ± 0.2 at 25 °C.

The plates are clear and yellowish.

Application and Evaluation

The culture medium is usually inoculated using the poured plate method and incubated aerobically for up to 5 days at 25 °C aerobically. The yeast and mould colonies are then counted.

Literature

Bundesgesundheitsamt: Amtliche Sammlung von Untersuchungsverfahren nach § 35 LMBG. - Beuth Verlag Berlin, Köln.

DIN Deutsches Institut für Normung e.V.: Mikrobiologische Milchuntersuchung. Bestimmung der Anzahl von Hefen und Schimmelpilzen. Referenzverfahren. **- DIN 10186**.

International Organization for Standardization (ISO): Milk and milk products - Enumeration of yeast and moulds - Colony count technique at 25 °C. **- International Standard ISO/DIS 6611** (1992).

Internationaler Milchwirtschaftsverband: Milch und Milchprodukte - Zählung von Hefen und Schimmelpilzen - Koloniezählung bei 25 °C. **- Internationaler IDF-Standard 94** (1990).

Ordering Information

Product	Merck Cat. No.	Pack size
YGC Agar (Yeast Extract Glucose Chloramphenicol Agar FIL-IDF)	1.16000.0500	500 g

Aspergillus niger ATCC16404

Saccharomyces cerevisiae ATCC9080

YGC Agar
(Yeast Extract Glucose Chloramphenicol Agar FIL-IDF)

Quality control

Test strains	Growth
Geotrichum candidum DSMZ 1240	good / very good
Penicillium commune ATCC 10428.	good / very good
Aspergillus niger ATCC 16404	good / very good

Quality control (sprial plating method)

Test strains	Inoculum (cfu/ml)	Recovery rate %
Candida albicans ATCC 10231	10^3-10^5	≥ 70
Saccharomyces cerevisiae ATCC 9763	10^3-10^5	≥ 70
Saccharomyces cerevisiae ATCC 9080	10^3-10^5	≥ 70
Escherichia coli ATCC 25922	> 10^5	≤ 0.01
Bacillus cereus ATCC 11778	> 10^5	≤ 0.01
Staphylococcus aureus ATCC 25923	> 10^5	≤ 0.01

Ready-To-Use Culture Media

Merckoplate®

Ready-to-use culture media for every application

Merck offers ready-to-use culture media for all sorts of applications. On the following pages you will find a list showing our extended range of cultue media. It will help you answer your questions concerning our microbiological products.

Tradition creates confidence

Merck looks back on 100 years of experience in the development and production of culture media. Already in 1878 Merck produced peptones that were mainly used as food additives.

In 1885 Merck established a department for microbiology and started selling peptones, gelatines and culture media specifically suited for culturing microorganisms. Only a few years later the industrial production of culture media was fully set up and running. Until Merck once again set new standards in the 1950's by being the first company to consider health aspects and, as a result, started to produce granulated culture media.

Today, Merck is the only pharmaceutical company among the producers of culture media. And you can profit from this combination of innovation and experience.

Quality garantees safety

Preparing culture media can be an error-prone process and therefore requires specially trained personnel. Merckplate® ready-to-use culture media are produced by means of the latest technology and are subject to strict quality controls carried out by qualified microbiologists. Avoid unnecessary risks by taking advantage of the consistent, high quality of Merckoplate® ready-to-use culture media.

Ready-to-use plates can improve cost-effectiveness

Merckoplate® culture media are ready-to-use, thus eliminating the time consuming production process. Benefit from this potential saving of cost of space, staff and equipment.

In addition to this we feature custom-made culture media matching your needs. For more information visit **www.microbiology.merck.de** or send us an e-mail: **mibio@merck.de.**

The item number and the expiry date are imprinted on every Merckplate® culture media for safe and easy product identification and monitoring of shelf life.

Merckoplate®

Enumeration and isolation of microorganisms

Product	Cat. No.	Shelf life in month	Filling quantity in ml per plate	Plates per pack
R2A-Agar	1.00073.0020	6	20	20
R2A-Agar*	1.00073.0480	6	20	480
DEV Nutrient Agar	1.00075.0020	6	18	20
Standard-I-Nutrient Agar with neutralizer	1.00417.0020	6	18	20
Standard-I-Nutrient Agar with neutralizer	1.00417.0480	6	18	480
Brolacin-Agar	1.10411.0001	6	18	20
Plate-Count-Agar	1.13108.0001	6	18	20
CASO (TSA) Agar	1.13499.0001	6	18	480
CASO (TSA) Agar	1.13582.0001	6	18	20
Sabouraud 4%-Glucose-Agar with neutralizer	1.18359.0001	2	20	20
CASO (TSA) Agar with neutralizer	1.18360.0001	6	18	20
CASO (TSA) Agar with neutralizer	1.18363.0001	6	18	480

* Media are only produced in special order.

Detection of Bacillus cereus

Product	Cat. No.	Shelf life in month	Filling quantity in ml per plate	Plates per pack
Cereus-Selective Agar acc. to Mossel	1.00830.0020	5	18	20

Detection of Campylobacter

Product	Cat. No.	Shelf life in month	Filling quantity in ml per plate	Plates per pack
Campylobacter-Selective Agar	1.13579.0001	2	18	20

Detection of Clostridium perfringens

Product	Cat. No.	Shelf life in month	Filling quantity in ml per plate	Plates per pack
TSC Agar	1.00078.0020		18	20

Detection and enumeration of E.coli/Coliform

Product	Cat. No.	Shelf life in month	Filling quantity in ml per plate	Plates per pack
Lactose TTC Agar with Tergitol®	1.00074.0020	6	18	20
Chromoplate® Coliform Agar	1.10156.0001	6	18	20
ENDO-Agar	1.15156.0001	3	18	20
VRBD-Agar	1.18351.0001	6	18	20

Merckoplate®

Detection and isolation of Enterococci

Product	Cat. No.	Shelf life in month	Filling quantity in ml per plate	Plates per pack
m-Enterococci Agar acc. to Slanetz	1.00076.0020	6	18	20
Bile-Aesculin-Azid Agar	1.00077.0020	4	18	20
CATC-Agar	1.00831.0020	4	18	20
Barnes-Agar	1.13576.0001	6	18	20

Detection of Legionella

Product	Cat. No.	Shelf life in month	Filling quantity in ml per plate	Plates per pack
Legionella BCYE-Agar	1.10097.0020	3	18	20
Legionella GVPC-Selectiv Agar	1.10098.0020	3	18	20

Detection of Listeria

Product	Cat. No.	Shelf life in month	Filling quantity in ml per plate	Plates per pack
Chromoplate® Listeria Selectiv Agar nach Ottaviani and Agosti	1.00420.0020	3	18	20

Detection of Pseudomonas

Product	Cat. No.	Shelf life in month	Filling quantity in ml per plate	Plates per pack
Cetrimide-Agar	1.00851.0020	6	18	20

Detection of Staphylococcus aureus

Product	Cat. No.	Shelf life in month	Filling quantity in ml per plate	Plates per pack
Mannitol-Salt-Penolred-Agar	1.10749.0001	6	18	20
Baird-Parker-Agar	1.00881.0020	3	16	20

Detection of Yersinia

Product	Cat. No.	Shelf life in month	Filling quantity in ml per plate	Plates per pack
Yersinia-Selective Agar	1.13578.0001	5	18	20

Merckoplate®

Detection of Salmonella

Product	Cat. No.	Shelf life in month	Filling quantity in ml per plate	Plates per pack
BPLS-Agar (USP)	1.00855.0020	6	18	20
BPLS-Agar modified*	1.00928.0020	4	18	20
MacConkey-Agar	1.10748.0001	6	18	20
MacConkey-Agar	1.15276.0001	6	18	480
Gassner-Agar	1.13580.0001	6	18	20
Rambach-Agar	1.13999.0001	6	18	20
Rambach-Agar	1.15999.0001	6	18	480
BPLS-Agar	1.15164.0001	6	18	20
Hektoen-Entero-Agar*	1.15171.0001	2	18	20
Leifson-Agar	1.15175.0020	3	18	20
Leifson-Agar	1.15175.0480	3	18	480
SS-Agar	1.15178.0001	3	18	20
XLD-Agar	1.15184.0001	3	18	20

* Media are only produced on special order.

Detection of yeasts and moulds

Product	Cat. No.	Shelf life in month	Filling quantity in ml per plate	Plates per pack
Wort Agar with neutralizer	1.00412.0020	6	20	20
Wort Agar with neutralizer	1.00412.0480	6	20	480
Dichloran-Glycerine (DG18)-Agar	1.00755.0020	7	20	20
Sabouraud 2%-Glucose-Agar	1.10413.0001	7	20	20
Sabouraud 2%-Glucose-Agar*	1.15504.0001	7	20	480
Sabouraud 2%-Glucose-Agar with neutralizer	1.18368.0020	4	20	20
Sabouraud 2%-Glucose-Agar with neutralizer*	1.18368.0480	4	20	480
Sabouraud 4%-Glucose Agar	1.18358.0020	7	20	20
Sabouraud 4%-Glucose Agar	1.18358.0480	7	20	480
Sabouraud 4%-Glucose Agar with neutralizer	1.18364.0001	2	18	480
Standard-I-Nutrient Agar	1.10416.0001	6	18	20
Maltextract-Agar with Chloramphenicol and Gentamycine	1.13423.0020	7	20	20
Maltextract-Agar with Chloramphenicol and Gentamycine	1.13423.0480	7	20	480
Maltextract-Agar	1.13573.0001	7	20	20

* Media are only produced on special order.

Merckoplate®

Detection of pathogenic yeasts and dermatophytes

Product	Cat. No.	Shelf life in month	Filling quantity in ml per plate	Plates per pack
Candida-Agar acc. to Nickerson	1.10412.0001	7	20	20
Fluroplate® Candida Agar	1.11011.0001	6	18	20
Selective Agar for pathogenic fungi	1.10415.0001	6	20	20
Fungi-Agar acc. to Kimmig	1.10421.0001	7	20	20
Dermatophytes-Selective Agar	1.10422.0001	7	20	20
Rice Extract Agar	1.10424.0001	6	20	20

Cultivation and isolation of fastidious microorganisms

Product	Cat. No.	Shelf life in month	Filling quantity in ml per plate	Plates per pack
Schaedler-Agar	1.13051.0001	2	18	20
Schaedler-KV-Agar	1.13575.0001	2	18	20
Blood-Agar	1.13414.0001	2	18	20
Blood-Agar	1.13421.0001	2	18	480
Chocolate Agar	1.15177.0001	5	18	20

Testing of sensitivity of microorganisms towards antibiotics

Product	Cat. No.	Shelf life in month	Filling quantity in ml per plate	Plates per pack
ASS-Agar	1.10410.0001	6	18	20
Mueller-Hinton-Agar	1.10414.0001	7	20	20
Mueller-Hinton-Agar	1.13405.0001	7	20	480
Mueller-Hinton-Agar with blood	1.13571.0001	2	18	20
Isotonic-Sensi-Agar	1.13574.0001	6	18	20

Examination of chemical components for toxic/cancerogenic properties

Product	Cat. No.	Shelf life in month	Filling quantity in ml per plate	Plates per pack
Minimal-Glucose-Agar for AMES test	1.15198.0001	6	18	20
Minimal-Glucose-Agar for AMES test	1.13496.0001	6	18	480

Review of Culture Media Base Materials


Perfectly prepared.
Based on raw material of high-quality.
Microbiology
Gallé Aesculin Azid Agar, nach ISO 7899-2
Zum Nachweis und zur Keimzahlbestimmung von Enterokokken
(enthält Natriumazid)
Bile Aesculin Azide Agar, acc. to ISO 7899-2
For the detection and enumeration of intestinal enterococci
(contains Sodium azide)
Gélose à la bile et à l'esculine selon l'ISO 7899-2
Pour la recherché et le dénombrement des entérocoques intestinaux
(contient Sodium azide)
Agar Bilis Sal Esculina, acc. a ISO 7899-2
Para la detección y enumeración de enterococos intestinales
(contiene Azida sódica)
MERCK
1.00072.

Culture Media Ingredients

Peptones

What are peptones?

Peptone comes from the Greek and means to digest. Peptone are a mixture of water soluble polypeptides, peptides, amino acids and other substances remaining after the digestion of protein material.

Simple proteins contain only amino acids. They produce on hydrolysis amino acids and no major other organic or inorganic products. They usually contain about 50% carbon, 7 % hydrogen 23% oxygen 16% nitrogen and 0-3% sulfur. Water soluble peptones have a Mol weight between 200 to 6000. The protein materials from which peptones are commonly produced include: bovine-, porcine-, or poultry meat, milk protein (casein), soybean, sunflower seed, gelatin, and yeast.

Peptones are termed after the origin of raw material and often the process of hydrolysis is also used. The digestion of the protein material occurs enzymatic or by acid treatment. For the enzymatic hydrolysis of protein material proteolytic enzymes such as pepsin, papain, pancreatin which contains trypsin and chymotrypsin or trypsin. The enzymes are of animal (pancreatin or pepsin) or vegetable origin (papain from papaya), or proteases from microbial origin.

The manufacturing of peptones

Protein is broken down in a digestion or hydrolysis process to polypeptides of various lengths and amino acids. The quality of the raw material employed, their storage, and the digestion process parameters determine the quality of the peptones. Raw material must be stored under conditions that avoid growth of spoilage organisms. Fresh meat is chilled stored up to digestion, whereas frozen meat is thawed shortly before processing.

The first step in the manufacturing of peptones is the digestion or hydrolysis of the raw material. In a hydrolysis vessel the raw material is dispersed in water to which the digesting agent is added.

In the second step of the process the digest is centrifuged, so that fat and oil can be removed. Thereafter, the digest is filtered, the liquid concentrated in a vacuum heat exchanger to a syrup which is in the final step spray dried to powder.

Filtration reduces drastically the bioburden, particularly of peptic and tryptic digests that are kept over long periods at about 40°C. After filtration the bioburden is commonly low and concentration to syrup contributes to the preservation.

The production of high quality peptones requires much more than a standardisation of the digestion process parameters. A total quality management system must be in place with emphasis on raw material specification, tracibility, non conmingly practices hygiene and cleaning and disinfection. Clearly in minimizing the risk of TSE the enzymes employed for digestion must be taken in account. They must be of non bovine origin.

The growth characteristics of peptones varies with the composition of raw materials and the digestion process parameters. Raw materials employed, such as meat or vegetables, vary in the concentration of fermentable carbohydrates. The carbohydrate concentration in the final peptone should be taking into account when assessing the growth characteristics.

Acid hydrolysis

The digesting agent in a complete or total acid hydrolysis is commonly hydrochloric acid (6-8N). The hydrochloric acid concentration in the digestion vessel is about 15%. The digestion occurs at a temperature of ca. 110°C and takes about 18-24h. Acid hydrolysis is a crude process that cleaves all peptide bonds. Acid hydrolysis results commonly to a total hydrolysis of protein to amino acids. The process destroys glutamine, asparagines, tryptophan, cysteine, serine, threonine, lysine, aspartic acid, proline racemises amino acids and completely destroys vitamins. The degree of destruction with time of hydrolysis and varies from one protein to another. Decolourisation of the digest using activated charcoal and the filtration contribute to a further loss of water soluble amino acids and vitamins. Low yields of serine and glutamic acid often occur when HCl is removed from acid hydrolysates by dessication. When carbohydrates are present cyteine and cystine yields are low. The stability of peptide bonds formed by valine, isoleucine and leucine often leads to low yields of these amino acids. Special conditions of hydrolysis increase the yields of the most labile derivatives.

The neutralisation step with sodium hydroxide makes that the salt (sodium) content of acid hydrolysate is high. This high salt content makes that the N content per gram peptone is relatively low. Acid hydrolysis gives a poor peptone and an addition of enzymatic digest or meat extract is required to give a good growth of microorganisms.

Enzymatic hydrolysis

Proteolytic enzymes hydrolyse peptide bonds formed by specific amino acids. It produces high yields of peptides and/or amino acids. Enzymatic hydrolysis is a process under mild conditions.

Peptic digestion results in relative high molecular peptides. If the neutralisation of the digest occurs with sodium hydroxide the ash content is increased. This neutralisis reduces the N content per gram.

Pancreatic digestion produces a balanced mixture of amino acids, including essential amino acids, in a optimal ration and low molecular peptides.

Papain digestion is most widely used in the production of peptone. Papain is sulphydryl protease from *Carcia papaya* latex. Papain has a wide specificity and degrades most protein substrates more extensively than trypsin, pepsin, chymotrypsin or pancreatric proteases.

Tryptic peptone is excellent nutrient base for the growth of microorganisms. Figure 1 shows the difference in the growth performance of peptic peptone as compared to Merck tryptic peptone.

Bacterial proteases (e.g. from Aspergillus, Bacillus subtilus or Streptomyces griseus) provide an excellent means of degrading small polypeptides into smaller peptides and amino acids. The extensive hydrolysis is comparable to that of a mild acid hydrolysis. Unlike acid hydrolysis bacterial proteolytic hydrolysis does not destruct amino acids and vitamins. Bacterial protease, notably *S. griseus* produces a more extensive hydrolysis than papain.

Extracts

An extract is the concentrate produced from the infusion. An infusion is the water soluble fraction obtained by soaking of a substrate in water for a period of time and the following filtration to clear solution. The substrates are often digested by a weak proteolysis with pancreatin (porcine origin) before being filtered and concentrated.

Selection of peptones/extracts

Composition data versus microbial growth criteria

Peptones are employed in a wide range microbiological applications. They are used for the production of antibiotics, enzymes, toxin-toxoids, vaccines starter cultures, in cell cultures as a replacement of serum and in a great variety of microbiological culture media. For each application there is a different demand to the peptone. A peptone suitable for an optimal growth of an organism is not necessary satisfactory for another organisms , the production of a desired end product, or cell culture.

A range of visual, physical, biochemical (USP), and composition data characterise peptones. The composition data commonly provide only information that is useful for a gross preliminary selection. The composition information allow to select a peptone with specific nutritive characteristics, for example, a peptone that should be free of carbohydrates, or has a high concentration of peptides and free amino acids, or has a low vitamin content, or has a high concentration of minerals and phosphates, or is rich on vitamin B. However, some times a discrepancy is seen between the physical and chemical values and the biological test results. The composition, appearance, biochemical, chemical and physical test parameters may be satisfactory, but if a biological test gives aberrant results the peptone is not suitable. A high total N and aminonitrogen content can be accompanied by poor growth promoting properties. In these cases the biological (microbial) growth characteristics tests are conclusive.

In selection, or optimising the performance of cell culture or fermentation applications, it is often necessary to test an assortment of peptones at a variety of concentrations. Also the lot-to-lot variation should be taken in account. Evaluations of multiple lots may be necessary to reproduce growth characteristics of a given peptone producing a high yield.

The USP describes for the selection of peptones and extracts biochemical characteristics, such as bioburden (<500Cfu/g), endotoxin level (< 500EU/g), indole-, acetylmethylcarbinol-, H_2S production and fermentable sugar presence.

Physical characteristics

Colour of dehydrated material

The colour of peptones may vary from white to cream; beige to light brown. The colour of a peptone depends on the processing and raw material. A papaic digest of liver has a light brown colour, whereas an acid hydrolysate of casein is white. It is not necessary that the whiter the colour is, the better the peptone. A white colour indicates commonly that a peptone is processed (activated charcoal and filtering). Decolourisation of the digest using activated charcoal and filtration contributes to a loss of water soluble amino acids and vitamins.

A dark brown colour may indicate a deterioration. In case of peptic or pancreatic peptones a dark brownish colour occurs when the filtered digest is hold for too long at temperatures >60°C. This causes non enzymatic browning.

The colour of the peptone is informative when various batches of a given peptone from a given manufacture are compared. A darkening of a given colour indicates a deterioration.

The colour must lie within an accepted range; dark powders yield dark solutions and are not acceptable.

Structure

The structure of dry material must be homogenous.

No conglomerates, foreign materials or structure modifications are permitted. Bottles of peptones that are clumped can not be used and should be discarded.

Odours

Abnormal odours are not permitted.

pH

A peptone solution of 2 or 5 % is prepared. The pH should be as specified. Changes in pH from specified values give information about the deterioration of peptones of extracts during storage or heating.

Colour in solution

A peptone solution of 1, 2 or 5% is prepared. The cold, boiled and autoclaved solutions are measured photometrically against standard controls and distilled water. The colour-corrected absorption must not exceed the given values.

Clarity-solubility

Clarity in different fluids after autoclaving is eliminatory. A clear medium after autoclaving allows the observation of microbial growth. A peptone solution of 1, 2 or 5% is prepared, checked on clarity and solubility. Thereafter it is autoclaved and checked again on clarity and solubility.

Compatibility with other ingredients

Peptones are mostly used in complex media. Their physical compatibility must be tested with other frequently used ingredients. The use of a peptone may not result in precipitation and produce the standard colour, clarity and biological (growth) performance.

Biochemical characteristics (USP)

Bioburden

Bioburden gives information of the microorganism contamination. The bioburden is determined by the quality of raw materials, plant hygiene and the digestion process. Peptic and tryptic digests are highly nutritious solutions that are kept over long periods at temperatures allowing growth of microorganisms (ca. 40°C). Filtration will drastically reduce the bioburden whereas of the digest concentration to syrup acts preservative.

Indole presence/production

Indole production gives information on the presence of tryptophan. It indicates the usefulness of peptone or extract as ingredient in media for indole testing.

Hydrogen sulfide (H_2S)

Hydrogen sulfide production gives information on the presence of sulfur amino acids.

Fermentable sugars

The test informs about the presence of fermentable sugars. It is intended to verify that a peptone does not give false positives in studies on sugar fermentation.

Chemical characteristics

Loss on drying

It is determined as the loss in weight due to incubation at 105°C for 4 h. Loss on drying gives information on the moisture content of a peptone. The moisture content is indicative for the stability and shelf life. For optimal storage a peptone has a moisture content of less than 5-7 %, whereas for a agar-agar this less than 12 %.

If the moisture is too high, there is an increased risk of bacteriological deterioration.

Biuret reaction

The biuret reaction informs on the presence of peptides. It gives information on the type of hydrolysis (acid or enzymatic).

Proteoses

Proteoses give information on the presence of peptides of high molecular weight.

Coagulable proteins

Coagulable proteins must be absent in peptones. The high Mol. weight (>6000) proteins have no nutritive value. They affect the clarity of peptone solutions adversely.

Total nitrogen

Total nitrogen content can be detemined by Kjeldahl digestion and titration. The total nitrogen content can be used to estimate the protein content.

Amino-nitrogen

Amino nitrogen content is more important. It gives information on the nutritional value. The increase in the amino acid concentration gives an indication on the hydrolysis of the raw material. A high amino acid concentration indicates a high degree of hydrolysis.

Amino acid pattern

Amino acid analysis gives information on the presence of amino acids and their levels.

Mol weight pattern

Oligopeptides and amino acids provide a ready available source of amino-nitrogen for microbial nutrition. A Sephadex G25 gel filtration of a peptone solution gives information the distribution of polypeptides in peptones. It helps to blend peptones to obtain the widest spectrum of peptides. In combination with microbial growth data it is a useful instrument in the selection and blending of peptones.

Peptides with Mol Weight greater than 6000 are denaturated by autoclaving at 121°C for 15min. These have no nutritive value and should have been filtered out. Small peptides MW1000-5000 are taken up by microorganism have a great nutritive value, because they are to microorganisms a valuable source of amino acids.

Nitrites

Nitrites form an indication of microbial contamination. Nitrites should be absent.

Total carbohydrates

The total carbohydrate level gives information about the presence of sugars (hexoses, disaccharides, polysaccharides etc.).

Ash

Ash is the inorganic residue remaining after the water and organic matter have been removed by heating in the presence of oxidizing agents. The ash content is a measure of the total amounts of minerals present. The ash content gives information on the level of sodium chloride, phosphates, sulphates, silicates, and metal oxides.

Chlorides

The sodium chloride or salt content is related to the ash content. It is high in acid and peptic digested peptones.

Phosphates

Phosphate content gives an information on the buffering capacity. Phosphates are expressed in % of P_2O_5.

Ca^{++}, Mg^{++} and Iron

Ca^{++}, Mg^{++} Iron give information on the nutritive value of peptones and extracts. Accurate Ca^{++} and Mg^{++} are required for sensitivity testing media. These ions are the principle cause of turbidity when they react with phosphates and carbonates. Iron content is critical for toxin production.

Toxic heavy metals

As, Ag and Pb are toxic elements and their value must be kept as low as possible. Toxic heavy metals should be absent or below 0.001%.

Inhibitors

Colloidal sulfur may act as an inhibitor.

Non saturated fatty acids colloidal sulfur, serine, valine and heavy metal like cupper and lead, zinc and arsen are if in appropriate concentrations present act as microbial growth.

Biological (microbial) characteristics

Bioburden

The quantitative and qualitative microbiological load of a peptone is tested. The presence of heat resistant and thermophilic spores is tested. The USP limit of less than 5000cfu per g should apply.

Furthermore heat resistant organisms and thermophilic spores should be absent.

Antagonistic activity

Testing for antagonists is performed using quantitative sensitivity test on Mueller Hinton agar against known standards devoid of such antagonists, usually by the substitution method.

Antagonists should be absent.

Growth performance

The growth performance of a selection of ATCC strains is tested in different concentrated peptone solutions and in a complex media. The growth promoting properties are measured in terms of turbidity, log phase or generation time.

A set of inoculated, a golden standard peptone and a previous batch are included in the growth promoting properties testing.

The complex media are prepared omitting the ingredient to be tested. The peptone on test is added to the incomplete complex medium in various concentration (above and under the concentration acc. to the formulation).

Biological reactivity

The biological reactivity includes testing of peptones on USP tests but it also can concern the effect of peptones on bacterial haemolysin production, influence on pigmentation, protective action against chemical disinfection etc.

Agar-Agar

Agar-agar is the polysaccharide that structures the cellular cohesion of seaweed. The species containing agar-agar include Gelidium, Gracilaria, Pterocladia and Anpheltia. The best quality of agar-agar for bacteriological purpose is obtained from Gelidium sesquipedal. Agar-agar forms a gel with a gelation temperature of 40-45°C after cooling from heating to a fusion temperature of 80-90°C.

Merck's agar-agars are manufactured from Gelidium sesquipedal seaweeds cultivated in coastal areas of Marocco or Spain.

Selection of agar-agar

Agar-gar for bacteriological purposes differ particularly in gel strength, mineral content and acid content. Ultra purification of agar-agar produces an agar-agar with a very low mineral and nitrogen content.

A good agar-agar for microbiological purposes must be free of foreign substances, thermoduric bacteria and any substances inhibitory to growth of microorganisms. The inhibitory substances, include bleaching agents as used in the production of food grade agar-agar, fatty acid residues, or toxic heavy metals such as copper or tin. Also the calcium, magnesium and iron ion content of agar-agar must be low. These salts react with soluble phosphates in peptones, meat and yeast extract to form insoluble phosphates (precipitates) when agar media are autoclaved or re-melted. Bacteriological agar is clarified and free of pigments. However, the clarification process may not have reduced calcium magnesium and phosphate levels to avoid "floc" appearing after.

Bacteriological Agar-Agar should have only a mechanical function. It should not be a source of nutrients or other chemically active substances.

Agar-agar is commonly characterised by the moisture content (loss on drying at 105°C for 4h), sulphated ash (600°C), the presence of heavy toxic metals (expressed as Pb), calcium and magnesium content, gel strength and solidification point. Gel strength, setting temperature, and clarity are key criteria for agar-agar.

Appearance

Colour

The colour of agar-agar is white to a pale tan.

An overheating of agar-gar is recognized by a brownish colour due to fall in pH and caramelisation of sugars.

Clarity

A molten solution of agar-agar is clear and free from deposits. Clarity after autoclaving is also measured. And the transmission at 560nm and 60 °C should be greater than 95%.

Physical characteristics

Loss on drying

It is determined as the loss in weight due to incubation at 105°C for 4h. Loss on drying gives information on the moisture content of agar-agar. The moisture content is indicative for the stability and shelf life. For optimal storage an agar-agar has a moisture content of less than 12%.

Compatibility with other ingredients

Agar-agar is used in combination with other ingredients. Its physical compatibility must be tested with other frequently used ingredients. The use of agar-agar may not result in precipitation and produce the standard colour, clarity and biological (growth) performance.

Gel strength

The gel strength of agar-agar is graded. At Merck determined with a Gelomat (Figure). The gel strength criterium for Merck agar agar measured with Gelomat is >50g. Agar-agar can be used in concentrations of 1-1.5%. The gel strenght of agar-agar has an influence to the flow of nutrients to micoorganisms and the efflux of toxic metabolites. High gel strength agar produces small colonies, whereas low strength agar-agar produces large colonies.

Figure:
Measurement of gel strength with Costin's Gelomat

Setting point

The setting point gives the temperature where Agar-Agar after heating to boiling (fusion) become solid. The setting point of agar-agar is determined by the Glass-tube/glass-ball test (Figure) with at least two concentrations. A good agar-agar remains perfectly fluid at 40°C for at least 12 h. The setting point should be tested with several liquids besides deionised water. Miscibility with biological fluids (milk, serum blood and egg yolk) must be tested regularly.

Gelling limit

The minimal concentration at which geling occurs should be less than 0.25 % agar-agar.

Melting point

The meting point of 1.2 % agar-agar should be higher than 85°C.

Diffusion rate

The diffusion of a 1% Safranin CI 50240 solution in agar-agar is tested. In a 1.2% agar-agar solution the diffusion zone should be greater than 25 mm after 25 h at 25°C.

Chemical characteristics

Ash

The ash content of agar-agars may vary from 1-4%. The lower the ash content the higher the quality of the agar-agar.

Sulphate

The sulphate content is a measure of the agropectins present in agar-agar. The greater the sulphate level the higher the amount of agropectin contamination. Acid agaropectin in agar-agar interferes with polymixin B and aminoglycoside antibiotics.

Chlorides

The sodium chloride increases the gel strength. A good agar-gar has a sodium chloride level of less than 1%.

Ca^{++} and Mg^{++}

Ca^{++} and Mg^{++} react with phosphates and produces in water insoluble precipitates. High levels occur in not sufficiently washed agar-gar or are indicative for the addition of lime at the first stage of the extraction.

The calcium concentration should be less than 0.05 % and that of magnesium lees than 0.01 %.

Iron

Iron enhances the pigment and acts as a growth factor in culture media. Iron contamination can result from rust of the processing equipment.

Phosphate

The phosphate content should be less than 0.5%.

Toxic heavy metals

As, Ag and Pb are toxic elements and their value must be kept as low as possible. Toxic heavy metals should be absent or below 0.0005 %.

Biological (Microbial) characteristics

Bioburden

The presence of heat resistant and thermophilic spores is tested. The USP limits should apply.

Thermophilic spores and these should be absent.

Antimicrobial activity

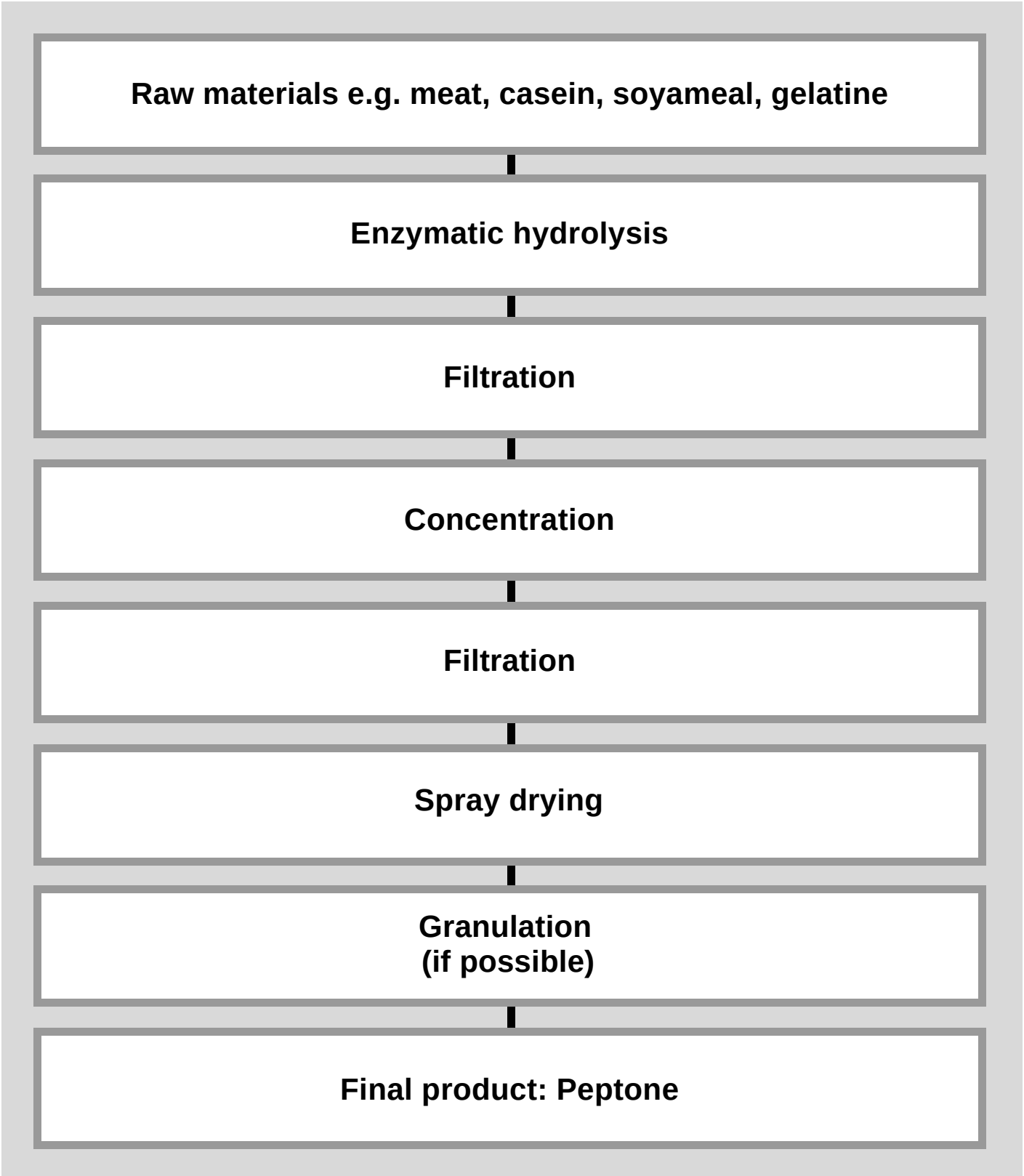
Agar-agar is substituted in a complex medium for a standard ingredient devoid of inhibitors and compared with the non-inhibitory complex medium.

Paper discs are impregnated with various concentrations of agar-agar. The impregnated disk are placed on nutrient agar plates seeded with different specific organisms. The development of the inhibition zone is recorded and compared to the stndard value.

Growth performance

The growth performance of a selection of ATCC strains in 1.2% agar-agar is tested in complex media such as for example Blood agar base, Tryptic Soy Agar, Wort Agar and Baird Parker Agar.

General Flow-chart for the Production of Peptones



Typical Potential Applications

Merck Peptones

	Merck Cat. No.	Culture Media	Vaccines	Hormones	Starter Cultures	Biomass	Enzymes	Antibiotics	Tissue Cultures
Peptones from Meat									
Peptone from Meat (pancreatic)	1.07214.	✓	✓		✓			✓	
Peptone from Meat (peptic)	1.07224.	✓	✓		✓			✓	
Peptone from Poultry (peptic)	1.10245.	✓	✓		✓			✓	
Peptone from Gelatin (pancreatic)	1.07284.	✓							
Peptone Mixtures									
Proteose Peptone	1.07229.	✓	✓						
Tryptose (tryptic)	1.10213.	✓	✓						✓
Vegetable Peptone									
Peptone from Soya (papainic)	1.07212.	✓					✓		
Casein and other milk-derived Peptones									
Lactalbumin hydrolysate	1.12523.	✓						✓	✓
Casein hydrolysate, acid hydr.	1.02245.	✓	✓					✓	
Peptone from Casein (pancr.), Typtone	1.11931.	✓	✓		✓			✓	
Peptone from Casein (pancr.), Tryptone	1.07213.	✓	✓		✓			✓	
Peptone from Casein (pancreatic)	1.02239.	✓	✓		✓			✓	
Extracts									
Meat Extract	1.03979.	✓	✓			✓	✓	✓	
Malt Extract	1.05391.	✓							
Yeast Extract	1.11926.	✓	✓	✓				✓	
Yeast Extract	1.03753.	✓	✓	✓				✓	

Typical Analysis: Chemical Composition

Merck Peptones

	Merck Cat. No.	Total Nitrogen (%)	Sulphated ash (%): (600-800 °C)	Amino nitrogen N (%)	Amino-N/Total-N	Loss on drying (%): (105 °C, 4 h)	NaCl (%)	Calcium (%)	Iron (%)	Nitrites	Indole (%)	pH: (2% aqueous sol.)	Total carbohydrates (%)
Peptones from Meat													
Peptone from Meat (pancreatic)	1.07214.	>11.00	≤17.00	4.5-6.5	0.46	≤6.00	<5.00	0.30	0.006		-	6.5-7.5	3.60
Peptone from Meat (peptic)	1.07224.	>12.00	≤15.00	4.0-6.0	0.42	≤6.00	<3.50	0.05	0.004		-	6.5-7.5*	1.10
Peptone from Poultry (peptic)	1.10245.	>11.00	≤10.00	2.5-3.5	0.27	≤7.00						5.8-6.3	
Peptone from Gelatin (pancreatic)	1.07284.	>13.50	≤15.00	2.5-4.5	0.25	≤6.00	0.70	≤0.2	0.001	-	-	6.5-7.5*	0.30
Peptone Mixtures													
Proteose Peptone	1.07229.	>12.00	≤15.00	2.5-3.5	0.21	≤10.00						6.5-7.5	
Universal Peptone M 66	1.07043.	>12.00	≤15.00	≥3.5	0.24	≤5.00						6.5-7.5	
Tryptose (tryptic)	1.10213.	>11.00	≤15.00	3.0-5.0	0.35	≤6.0	<2.00					6.5-7.5*	
Vegetable Peptone													
Peptone from Soya (papainic)	1.07212.	>9.03	≤15.00	1.8-3.2	0.25	≤6.00	<2.00	0.20	0.005	-	-	6.5-7.5*	24.00
Casein and other milk-derived Peptones													
Casein hydrolysate, acid hydr.	1.02245.	>7.0-8.0	≤58.00	5.0-6.5	>0.73	≤6.00	<45.0	0.20	0.002	-	-	4.7-7.0	0.10
Lactalbumin hydrolysate	1.12523.	12.50	≤10.00	5.0-6.0	0.44	≤7.00		≤0.20			1.0-3.0	6.5-7.5	
Peptone from Casein (pancr.); Tryptone	1.07213.	>12.00	≤15.00	3.0-5.0	0.32	≤6.00	<1.00	≤0.10	0.003	-	-	6.7-7.7	0.50
Peptone from Casein (pancreatic)	1.02239.	>13.80	≤5.00	4.7-6.7	0.39	≤6.00	<1.80	0.20	0.002	-	-	5.0-6.0*	0.40
Extracts													
Meat Extract	1.03979.	>11.5	≤18.00	3.5-4.5	0.35	≤6.00	<10.0	0.07	0.004		-	6.0-7.0	6.90
Malt Extract	1.05391.	1.00	≤3.00			≤5.00					-	5.0-6.0	80.00
Yeast Extract	1.03753.	>10.50	≤17.00	4.7-5.7	0.50	≤5.00	≤5.0	≤0.05	0.006	-	-	5.5-7.2	7.0-13.0

pH*: (5% aqueous sol.)

Typical Amino Acid Analysis (% W/W)

Merck Peptones

	Merck Cat. No.	Alanine	Arginine	Aspartic Acid	Cystine	Glutamic acid	Glycine	Histidine	Isoleucine	Leucine	Lysine	Methionine	Phenylalanine	Proline	Serine	Threonine	Tryptophane	Tyrosine	Valine
Peptones from Meat																			
Peptone from Meat (pancreatic)	1.07214.	6.00	4.10	6.80		10.20	8.90	1.80	2.80	5.40	5.40	1.20	3.20	4.70	3.10	2.80		1.00	4.60
Peptone from Meat (peptic)	1.07224.	5.70	4.00	6.90		12.30	7.30	2.50	3.30	6.50	7.60	1.50	3.90	5.90	2.90	3.00		1.10	5.60
Peptone from Poultry (peptic)	1.10245.	5.67	4.97	n.d.		10.90	9.66	1.33	1.99	3.99	4.35	0.79	1.97	5.57	2.37	2.30		0.89	2.50
Peptone from Gelatin (pancreatic)	1.07284.	6.25	5.50	6.50		12.40	13.90	1.52	2.60	4.70	5.20	1.30	2.80	10.00	3.40	2.40		0.70	4.35
Peptone Mixtures																			
Tryptose (tryptic)	1.10213.	3.40	3.20	6.90		17.20	2.80	2.40	4.50	7.40	6.80	1.20	4.20	6.90	3.70	3.20		1.40	5.60
Vegetable Peptone																			
Peptone from Soya (papainic)	1.07212.	2.90	4.20	7.20		13.20	2.90	1.90	3.00	5.20	4.60	0.80	3.40	4.20	3.40	2.50		1.30	4.60
Casein and other milk-derived Peptones																			
Casein hydrolysate, acid hydr.	1.02245.	2.00	2.20	4.40		12.50	1.20	1.80	2.40	3.40	5.60	1.20	2.50	6.10	2.70	2.20		0.60	3.90
Peptone from Casein (pancreatic)	1.11931.	3.10	3.10	6.30		18.40	1.80	2.30	4.10	8.00	6.80	2.30	4.10	9.20	4.40	3.60		2.00	5.30
Peptone from Casein (pancr.); Tryptone	1.07213.	3.10	3.20	6.90		18.50	3.20	2.90	4.90	8.10	7.60	2.40	4.90	9.00	4.10	3.30	1.50	1.50	8.10
Peptone from Casein (pancreatic)	1.02239.	2.55	3.05	6.35		17.40	1.85	2.70	4.25	7.15	6.45	2.35	4.05	8.35	4.20	3.40		1.95	5.10
Extracts																			
Meat Extract	1.03979.	5.40	3.80	7.50		9.60	7.40	1.90	3.00	6.00	7.00	0.90	3.70	4.30	3.00	3.00		1.20	4.80
Malt Extract	1.05391.	0.40	0.50	0.90		1.60	0.40	0.60	0.50	0.60	0.60	0.20	0.70	0.60	0.40	0.40		0.30	0.60
Yeast Extract	1.11926.	8.80	5.10	9.90	0.90	16.30	4.80	2.10	5.50	7.60	8.00	1.40	3.70	4.00	4.60	4.30	1.30	2.40	5.90
Yeast Extract	1.03753.	8.80	5.10	9.90		16.30	4.80	2.10	5.50	7.60	8.00	1.40	3.70	4.00	4.60	4.30		2.40	5.90

Agar-Agar, granulated

A granulated high quality solidifying agent that is essentially free of impurities. It is used as gelling medium for culture media, auxotrophic studies bacterial and yeast transformation studies and bacterial genetics applications

Mode of Action

Agar-agar is a water-soluble polygalactoside which is obtained from marine harvested Gelenium sesquipedal. Agar-agar remains firm at growth temperatures for many microorganisms and is generally resistant to a breakdown by bacterial enzymes.

Preparation

Agar is a gel at room temperature, remaining firm at temperatures as high as 65°C. Agar melts at approximately 85°C, a different temperature from that at which it solidifies, 32-40°C.

Agar-agar is used in a final concentration of 1-1.5% (1.0-1.5g/100ml) for solidifying culture media. Smaller quantities are used in media for motility studies (0.5% or 0.05g/100ml) and for growth of anaerobes (0.1% or 0.01g/100ml) and microaerophiles. If the culture medium has a pH <5.0 the working strength should be 2% (2g /100ml).

Autoclave a completely dissolved medium 121°C for 15 min.

Typical Analysis

Colour granules	brownish-yellow
Appearance	Light Free flowing granules
Colour in solution	Light amber
Appearance in solution	clear
pH (5% in water)	5.0-6.0
Loss on drying (Moisture)	<12%
Ash	12%
Heavy (toxic) metals (as Pb)	0.0005%
Ca	0.5%
Mg	0.1%
Solidification point	32-36°C
Melting point	>85 °C
Working strength	1-1.5%

Literature

United States Pharmacopoeia 26 2003 The National Formulary 20 United States Pharmacopoeia Covention Inc. Rockville Md.

Ordering Information

Product	Merck Cat. No.	Pack size
Agar Agar, granulated	1.01614.1000	1 kg
Agar Agar, granulated	1.01614.5000	5 kg

Quality control

Test strains	Growth[1] after 24 hours
Escherichia coli ATCC 25922	+
Strept. pyogenes ATCC 21059	+
Stapyhlococcus aureus ATCC 25923	+
Shigella sonnei ATCC 29930	+
Erysipelothrix rhusiopathiae ATCC 19414	+
Streptococcus agalactiae ATCC 13813	+
Streptococcus equinus DSM 20062	+
Streptococcus pneumoniae ATCC 6301	+

[1] in Standard I Nutrient broth

Agar-Agar ultra pure, granulated

A granulated, highly pure solidifying agent that is essentially free of impurities

Mode of Action

Agar-agar ultra-pure granulated is an ultra purified agar with a high agar strength, low ash and mineral content that is essentially free of impurities. It is free of toxic pollutants, non agar gums, nitrogenous compounds insoluble salts, thermoducic bacteria and dead bacteria.

Its advantages include excellent clarity, controlled gelation temperature, controlled melting temperature, good diffusion characteristics, absence of toxic bacterial inhibitors and relative absence of metabolically useful minerals and compounds.

It is used for nutritional studies, molecular genetic testing and for antibiotic M.I.C. testing, electrophoresis and diffusion tests. The low ash and mineral content of agar-agar ultra pure limits the interference of agar in antibiotic M.I.C. studies and diffusion tests. A low mineral content prevents the inhibition of the migration of chemotherapeutic acid inantibiotic diffusion test.

Preparation

Agar-agar ultra pure is used in a final concentration of 1-1.5% (10-15g/100ml) for solidifying culture media. Smaller quantities are used in media for motility studies (0.5% or 0.5g/100ml) and for growth of anaerobes (0.1% or 0.1g/100ml) and microaerophiles.

Adjust the pH, if necessary, so that it is 7.0 ± 0.2 at 25°C. Autoclave completely dissolved solution 121°C for 15min.

Typical Analysis

Colour granules	brownish-yellow
Appearance	Free flowing granules
Colour in solution	Light amber
Appearance in solution	Opalacent
pH (5% in water)	5.0-6.0
Loss on drying (Moisture)	≤10%
Sulfated ash	≤5%
Heavy (toxic) metals (as Pb)	0.0005%
Ca	≤0.1%
Mg	≤0.05%
Solidification point	32-36 °C
Melting point	>85 °C
Working strength	1-1.5%

Ordering Information

Product	Merck Cat. No.	Pack size
Agar Agar ultar pure, granulated	1.01613.1000	1 kg

Quality control

Test strains	Growth[1] after 24 hours
Escherichia coli ATCC 25922	+
Strept. pyogenes ATCC 21059	+
Stapyhlococcus aureus ATCC 25923	+
Shigella sonnei ATCC 29930	+
Erysipelothrix rhusiopathiae ATCC 19414	+
Streptococcus agalactiae ATCC 13813	+
Streptococcus equinus DSM 20062	+
Streptococcus pneumoniae ATCC 6301	+
Suitability for microbiology	+

[1] in Standard I Nutrient broth

Caseinhydrolysate (acid hydrolyzed)

Caseinhydrolysate is suitable for the production of vaccines, industrial fermentations with yeasts and fastidious Bacillus spp.

Mode of Action

Caseinhydrolysate is produced by the digestion of casein with hydrochloric acid. The digestion conditions are such that a proportion of vitamins and growth promoting substances are retrained. Tryptophane is destroyed by the digestion. The inorganic salts level is high due to neutralisation of the digestive acid.

Caseinhydrolysate is especially suited for for the large-scale cultivation of diphteria bacteria, tetanus bacilli and streptococci, of toxins and streptase. In industrial fermentations it favours a high yield of biomass, particularly for certain yeasts and fastidious bacilli.

Typical Analysis

Colour powder	Light yellow-beige
pH (5% water)	4.7-7.0
Loss on drying (105 °C)	≤6.0%
Sulfated ash (800 °C)	≤58%
Amino-nitrogen (N_α) (as N)	5.0-6.5%
Nitrogen (N_T) (Kjeldahl)	7.0-8.5%
Amino acid specification	See table page 542

Ordering Information

Product	Merck Cat. No.	Pack size
Caseinhydrolysate (acid hydrolyzed)	1.02245.0500	500 g

Quality control

Test strains	Growth
Staphylococcus aureus ATCC 25923	+
Staphylococcus aureus ATCC 6538P	+
Enterococcus faecalis ATCC 11700	+
Listeria monocytogenes ATCC 19113	+
Escherichia coli ATCC 8739	+
Klebsiella pneumoniae ATCC 13883	+
Salmonella typhimurium ATCC 14028	+

Gelatin

Gelatin is used as substrate for the detection of gelatin degrading microorganisms and the microbial count in water

Mode of Action

Microorganisms like Enterobacteriaceae, *Aerococcus*, Pseudomonadaceae, *Bacilli*, *Clostridium*, *Pediococcus*, and *Vibrio spp* which degrade gelatin cause liquefaction of gelatin causing a clear halo around the colony or inoculation streak.

Preparation

As a gelling agent gelatin is used in the concentration of 15% (15g /100ml). Gelatin melts at a temperature of about 28 °C. Gelatin is heat sensitive. Gelatin culture media should be sterilised at 115°C for 10min.

Typical Analysis

Colour powder	Light yellow to beige
Colour in solution	yellow-beige
Solubility	total
pH (1% in water)	5.0-6.0
Loss on drying (105 °C)	≤15%
Sulfated ash (800 °C)	≤2%
Sulfuroixde (SO_2)	≤0.005%
Peroxide (as H_2O_2)	≤001%
Heavy toxic metals (as Pb)	≤0.001

Literature

LEVINE, M. & CARPENTER, D.C. 1923 Gelatin liquefaction by bacteria. **Journal of Bacteriology 8**, 297-306

FISCHER, G.W. & KELTER, N. 1957 Zur Gelatineverflüssung bei 37°C und bei Zimmertemperatur. Acrchives für Hygiene 41, 368-372

Ordering Information

Product	Merck Cat. No.	Pack size
Gelatin	1.04070.0500	500 g

Quality control

Test strains	Growth
Staphylococcus aureus ATCC 25923	+
Staphylococcus aureus ATCC 6538P	+
Enterococcus faecalis ATCC 11700	+
Listeria monocytogenes ATCC 19113	+
Escherichia coli ATCC 8739	+
Klebsiella pneumoniae ATCC 13883	+
Salmonella typhimurium ATCC 14028	+

Lactalbumin hydrolysate

Lactalbumin hydrolysate is suitable for the production of vaccines, fermentations, and bacterial, insect, mammalian and virus cell culture media

Mode of Action

Lactalbumin hydrolysate is the pancreatic hydrolysed protein portion of milk whey. It is a mixture of peptides, amino acids e.g. tryptophan, and carbohydrates, and has high nutritional properties.

Typical Analysis

Colour powder	Cream
Colour in solution	yellow-beige
pH (2% water)	6.5-7.5
Loss on drying (105 °C)	≤7%
Amino-nitrogen (N_α) (as N)	5-6%
Assay protein (ex. N calc. on dried substance)	≥80%
Sulfated ash (600 °C)	≤10%
Phosphorus compounds (as P)	≤1.5%
Calcium	≤0.2%
Magnesium	≤0.1%
Heavy metals (as Pb)	<0.003%
Tryptophane	1-3%
Amino acid specification	See table page 542

Ordering Information

Product	Merck Cat. No.	Pack size
Lactalbumin hydrolysate	1.12523.1000	1 kg

Quality control

Test strains	Growth
Staphylococcus aureus ATCC 25923	+
Staphylococcus aureus ATCC 6538P	+
Enterococcus faecalis ATCC 11700	+
Listeria monocytogenes ATCC 19113	+
Escherichia coli ATCC 8739	+
Klebsiella pneumoniae ATCC 13883	+
Salmonella typhimurium ATCC 14028	+

Malt Extract

Malt Extract is used as nutritive substrate in mycological media for the propagation of yeasts and moulds and test strains in vitamin assays

Mode of Action

Malt extract is the water-soluble portion of malted barley.

It contains a high content of reduced sugars and to a lesser extend nitrogeneous constituents. The carbohydrates comprises mainly the disaccharide maltose and fractions of hexoses such as glucose and fructose, the disaccharide sucrose and other carbohydrates. The nitrogenous components include peptides, amino acids purines and vitamins. Malt extract favours the sporulation of moulds such as Aspergillus and Penicillium.

Typical Analysis

Colour powder	Brownish yellow
Colour in solution	yellow-beige
pH (2% in water)	4.0-6.0
Loss on drying (105 °C)	≤5%
Sulfated ash (800 °C)	≤3%

Ordering Information

Product	Merck Cat. No.	Pack size
Malt Extract	1.05391.0500	500 g

Quality control

Test strains	Growth
Staphylococcus aureus ATCC 25923	+
Staphylococcus aureus ATCC 6538P	+
Enterococcus faecalis ATCC 11700	+
Listeria monocytogenes ATCC 19113	+
Escherichia coli ATCC 8739	+
Klebsiella pneumoniae ATCC 13883	+
Salmonella typhimurium ATCC 14028	+

Meat Extract

Meat Extract is used as nutritive substrate. It complies with the USP specification of meat extract paste

Mode of Action

Meat extract is prepared from selected animal tissues which is free from fat and sinew. It is digested by a weak proteolysis with pancreatin (porcine origin) before being extracted.

In culture media meat extract is usually employed in concentrations of 0.3 to 1.0%. Meat extract is particularly suitable for cultivating of lactic acid bacteria.

Specification

Colour granules	yellow-light brown
Colour in solution	yellow-beige
pH (5% in water)	6.0-7.0
Nitrite (NO_2)	Absent
Loss on drying (105 °C)	≤6.0%
Sulfated ash (800 °C)	≤18.0%
Amino-nitrogen (N_α)	3.5-4.5%
Nitrogen (N_T)	11.5-12.5%
Total carbonhydrates	2%
Amino acid specification	See table page 542

Literature

Cote. 1999. In Flickinger and Drew (ed.), Encyclopedia of bioprocess technology: fermentation, biocatalysis, and bioseparation. John Wiley & Sons, Inc., New York, N.Y.

3. U.S. Food and Drug Administration. 1995. Bacteriological analytical manual, 8th ed. AOAC International, Washington, D.C.

Ordering Information

Product	Merck Cat. No.	Pack size
Meat Extract	1.03979.0500	500 g
Meat Extract	1.03979.2500	2,5 kg

Ox bile, dried

Ox bile is used in the preparation of selective media for the detection and enumeration of Gram negative bacteria particularly Enterobacteriaceae, Salmonella and Gram- positive enterococci

Mode of Action

Ox bile is prepared by concentration, purification and spray drying of fresh bile.

Ox bile is a complex mixture of bile free and conjugated bile salts. The bile salts in fresh bile are mainly totally conjugated as peptides formed from bile acid, glycine or taurine. The selective activity of fully conjugated bile acids is less than that of free acids. Deoxycholic acid is the most active of the bile acids. Bacterial enzymes hydrolyse bile conjugates to the more inhibitory free bile acids. The inhibitory activity of ox bile increases in the presence of phosphate or citrate. These substances chelate magnesium. Magnesium chloride can decrease the selectivity.

Ox bile inhibit most Gram-positive bacteria without affecting the growth of Gram-negative enteric bacilli.

Ox bile is also used for the differentiation of pneumococcci (bile soluble) from streptococci (bile insoluble).

Typical Analysis

Colour powder	light beige
Colour in solution	yellow-beige
Solubility (2% in BGB Broth)	total
pH (5% in water)	5.5-7.5
Bile salts (cal. as cholic acid USP)	≥45%
Total ash (800 °C)	≤15%
Water (acc. to K. Fischer)	≤5%
Unsoluble matter (in 80% ethanol)	≤1%

Ordering Information

Product	Merck Cat. No.	Pack size
Ox bile, dried	1.03756.0500	500 g

Quality control

Test strains	Growth
Staphylococcus aureus ATCC 25923	+
Staphylococcus aureus ATCC 6538P	+
Enterococcus faecalis ATCC 11700	+
Listeria monocytogenes ATCC 19113	+
Escherichia coli ATCC 8739	+
Klebsiella pneumoniae ATCC 13883	+
Salmonella typhimurium ATCC 14028	+

Peptone from Casein (Tryptone), pancreatic, free from sulfonamide antagonists

This peptone is obtained by pancreatic degradation of casein and is used to prepare media for the cultivation of fastidious microorganisms. It contains negligible amounts of sulfonamide antagonists (p-aminobenzoic acid) and is thus utilized especially for the preparation of culture media used for testing the sensitivity of infectious microorganisms towards sulfonamides.

A BSE-free certificate is available upon request.

Typical Analysis

Total nitrogen (ex N; calc. on dried substance)	13.8-15.8 %
Amino nitrogen (as N)	4.7-6.7%
Sulfated ash (800 °C)	≤5%
Loss on drying (105 °C; 4 h)	≤6%
pH (5 % solution)	5.0-6.0
Total nitrogen (as N)	13.8-15.8%

Ordering Information

Product	Merck Cat. No.	Pack size
Peptone from Casein, (Tryptone), pancreatic, free from sulfonamide antagonists	1.02239.0500	500 g

Quality control

Test strains	Growth
Staphylococcus aureus ATCC 25923	+
Staphylococcus aureus ATCC 6538P	+
Enterococcus faecalis ATCC 11700	+
Listeria monocytogenes ATCC 19113	+
Escherichia coli ATCC 8739	+
Klebsiella pneumoniae ATCC 13883	+
Salmonella typhimurium ATCC 14028	+

Peptone from Casein, pancreatic, granulated

Tryptone, pancreatic, granulated

Tryptone, granulated is suited for the preparation of media for fastidious microorganisms, fungi, bacterial vaccine production, and the elaboration of indole testing

Mode of Action

Tryptone is a pancreatic digestion of casein. It fulfils the USP criteria for tryptone.

Tryptone is a nitrogen source containing a high level of free amino acids, particularly tryptophane and the absence of detectable carbohydrates.

It is usually employed in concentrations of 1.0%.

Typical Analysis

Colour granules	Beige
pH (5% in water)	6.7-7.7
Loss on drying (105 °C)	≤6%
Sulfated ash (800 °C)	≤15%
Amino-nitrogen (N_α)	3.0-5.0%
Assay protein (ex. N calc. on dried substance)	≥80%
Nitrogen (N_T) (Kjeldahl)	12-13%
Calcium	≤0.1%
Amino acid specification	See table page 542

Ordering Information

Product	Merck Cat. No.	Pack size
Peptone from Casein, pancreatic, granulated	1.07213.1000	1 kg
Peptone from Casein, pancreatic, granulated	1.07213.2500	2.5 kg
Peptone from Casein, pancreatic, granulated	1.07213.9025	25 kg

Quality control

Test strains	Growth
Staphylococcus aureus ATCC 25923	+
Staphylococcus aureus ATCC 6538P	+
Enterococcus faecalis ATCC 11700	+
Listeria monocytogenes ATCC 19113	+
Escherichia coli ATCC 8739	+
Klebsiella pneumoniae ATCC 13883	+
Salmonella typhimurium ATCC 14028	+

Peptone from Gelatin (pancreatic)

Gelatin Peptone, pancreatic

Gelatin peptone is used in cell cultures, bacterial fermentation requiring high hydroxyproline content, low carbohydrates, cystine and tryptophan levels and the cultivation of non fastidious microorganisms

Mode of Action

Gelatin peptone is a pancreatic digest of gelatin. Gelatin is extracted from collagen, which is the fibrous protein in bone, cartilage and connective tissue.

As a basic nutrient, gelatin peptone is suitable for preparing media for organisms that are not particularly fastidious in their nutritional requirements.

Typical Analysis

Colour powder	Light yellow to beige
Colour in solution	Clear, yellow-beige
pH (5% in water)	6.5-7.5
Loss on drying	≤6.0%
Sulfated ash (800 °C)	≤15%
Amino-nitrogen (N_α)	2.5-4.5%
Nitrogen (N_T)	13.5-16.5%
Calcium	≤0.2%
Amino acid specification	See table page 542

Ordering Information

Product	Merck Cat. No.	Pack size
Peptone from Gelatin (pancreatic)	1.07284.1000	1 kg

Quality control

Test strains	Growth
Staphylococcus aureus ATCC 25923	+
Staphylococcus aureus ATCC 6538P	+
Enterococcus faecalis ATCC 11700	+
Listeria monocytogenes ATCC 19113	+
Escherichia coli ATCC 8739	+
Klebsiella pneumoniae ATCC 13883	+
Salmonella typhimurium ATCC 14028	+

Peptone from Meat (peptic), granulated

Meat Peptone (peptic), granulated

Granulated Meat Peptone is used as nutritive substrate in fermentation and culture media. It complies with meat peptone peptic in USP

Mode of Action

It is prepared from selected animal tissues of which fat and sinew removed.

The meat peptone peptic complies with the USP specification for peptic digest of animal tissue.

Meat peptone provides nitrogen, vitamins, amino acids and carbon in microbiological culture media. Its high sulfur compound content makes meat peptone, peptic suited for the detection of bacteria (*Clostridia spp.*, *Salmonella spp.*) producing hydrogen sulfide.

As an ingredient In culture media meat peptone, peptic is usually employed in concentrations of 0.3 to 1.0%. It is used for the cultivation of yeast and moulds, enterobacteria, and staphylococci.

Typical Analysis

Colour granules	Light yellow-beige
Colour in solution	yellow-beige
pH (5% in water)	6.5-7.5
Loss on drying (105 °C)	≤6.0%
Sulfated ash (800 °C)	≤17.0%
Amino-nitrogen (N_α)	4.5-6.5%
Total nitrogen (Kjeldahl)	12.0-13.0%
Nitrite (NO_2)	absent
Amino acid specification	See table page 542

Literature

Cote. 1999. In Flickinger and Drew (ed.), Encyclopedia of bioprocess technology: fermentation, biocatalysis, and bioseparation. John Wiley & Sons, Inc., New York, N.Y.

3. U.S. Food and Drug Administration. 1995. Bacteriological analytical manual, 8th ed. AOAC International, Washington, D.C.

Ordering Information

Product	Merck Cat. No.	Pack size
Peptone from Meat (peptic), granulated	1.07224.1000	1 kg
Peptone from Meat (peptic), granulated	1.07224.2500	2.5 kg

Quality control

Test strains	Growth
Staphylococcus aureus ATCC 25923	+
Staphylococcus aureus ATCC 6538P	+
Enterococcus faecalis ATCC 11700	+
Listeria monocytogenes ATCC 19113	+
Escherichia coli ATCC 8739	+
Klebsiella pneumoniae ATCC 13883	+
Salmonella typhimurium ATCC 14028	+

Peptone from Meat (pancreatic), granulated

Meat Peptone (pancreatic), granulated

Granulated Meat Peptone is used as nutritive substrate in fermentation and culture media

Mode of Action

It is prepared from selected animal tissues of which fat and sinew is removed.

Meat peptone provides nitrogen, vitamins, amino acids and carbon in microbiological culture media. Its high sulfur compound content makes meat peptone, peptic suited for the detection of bacteria (*Clostridia spp.*, *Salmonella spp.*) producing hydrogen sulfide.

As an ingredient in culture media meat peptone, pancreatic is usually employed in concentrations of 0.3 to 1.0%. It is used for the cultivation of yeast and moulds, enterobacteria and staphylococci.

In blood agar formulations meat peptone favours the growth of streptococci and the production of a characteristic haemolysis.

Typical Analysis

Colour granules	Light yellow-beige
Colour in solution	yellow-beige
pH (5% in water)	6.5-7.5
Loss on drying (105 °C)	≤6.0%
Sulfated ash (800 °C)	≤17.0%
Amino-nitrogen (N_α)	4.5-6.5%
Total nitrogen (Kjeldahl)	12.0-13.0%
Nitrite (NO_2)	absent
Amino acid specification	See table page 542

Ordering Information

Product	Merck Cat. No.	Pack size
Peptone from Meat (pancreatic), granulated	1.07214.1000	1 kg
Peptone from Meat (pancreatic), granulated	1.07214.2500	2.5 kg

Quality control

Test strains	Growth
Staphylococcus aureus ATCC 25923	+
Staphylococcus aureus ATCC 6538P	+
Enterococcus faecalis ATCC 11700	+
Listeria monocytogenes ATCC 19113	+
Escherichia coli ATCC 8739	+
Klebsiella pneumoniae ATCC 13883	+
Salmonella typhimurium ATCC 14028	+

Peptone from Poultry (peptic)

Poultry Peptone, peptic

Poultry peptone is used as nutritive substrate in fermentation and culture media

Mode of Action

Poultry peptone is produced by peptic digestion of selected animal tissues of which fat and sinew is removed.

Poultry peptone has been developed to minimise the Bovine Spongiform Encephalopathy (BSE) risk.

It is an alternative to meat peptone peptic and has similar growth support. It is a nutritious peptone that is very suitable for growth support of fastidious microorganisms.

As an ingredient in culture media Poultry Peptone, peptic can be used in concentrations of 0.3 to 1.0%.

Typical Analysis

Colour powder	yellow-beige
pH (2% in water)	5.8-6.3
Nitrites (NO_2)	absent
Loss on drying (105 °C)	≤7%
Sulfated ash (800 °C)	≤10%
Peptone content (ex. N calc. on dried substrate)	≥75%
Amino acid specification	See table page 542
Bioburden yeast and moulds	≤100 cfu/g
Bioburden aerobe bacteria	≤10000 cfu/g
E. coli	absent in 1 g
Salmonella	absent in 25 g
Staphylococcus aureus	absent in 1 g
Enterobacteriacae and Gram-negative bacteria	absent in 1 g

Ordering Information

Product	Merck Cat. No.	Pack size
Peptone from Poultry (peptic)	1.10245.1000	1 kg

Quality control

Test strains	Growth
Staphylococcus aureus ATCC 25923	+
Staphylococcus aureus ATCC 6538P	+
Enterococcus faecalis ATCC 11700	+
Listeria monocytogenes ATCC 19113	+
Escherichia coli ATCC 8739	+
Klebsiella pneumoniae ATCC 13883	+
Salmonella typhimurium ATCC 14028	+

Peptone from Soyameal (papainic), granulated

Soya Peptone (papainic)

Soya peptone is used in cell culture, molecular genetic media, for microbiological assay procedures and as nutritive substrate for cultivation and resuscitation of a large variety of microorganisms

Mode of Action

Defatted soya meal is digested with papainic enzyme into amino acids and peptides.

Soy peptone is a non animal peptone that contains a broad range of nutrients. It is characterised by its high concentration of vitamins and carbohydrates. Its nitrogen content combined with the naturally occurring vitamins and high carbohydrate content facilitate rapid and profuse growth of fastidious microorganisms and for the resuscitation of sublethally injured microorganisms.

Its high content of fermentable sugars makes soya peptone unsuitable for fermentation studies, such as for example, in media used for the identification of microorganisms on basis of sugar fermentation.

Typical Analysis

Colour powder	Light yellow-beige
Colour in solution	yellow-beige
pH (5% in water)	6.5-7.5
Loss on drying (105°C)	≤6%
Sulfated ash (800 °C)	≤15.0%
Amino-nitrogen (N_α)	1.8-10.7%
Nitrogen (N_T)	9.3-10.7%
Amino acid specification	See table page 542

Ordering Information

Product	Merck Cat. No.	Pack size
Peptone from Soyameal (papainic), granulated	1.07212.0500	500 g

Quality control

Test strains	Growth
Staphylococcus aureus ATCC 25923	+
Staphylococcus aureus ATCC 6538P	+
Enterococcus faecalis ATCC 11700	+
Listeria monocytogenes ATCC 19113	+
Escherichia coli ATCC 8739	+
Klebsiella pneumoniae ATCC 13883	+
Salmonella typhimurium ATCC 14028	+

Proteose Peptone

Proteose peptone is used as nutritive substrate in media for production of enzymes, toxin production, cell culture fermentation and the cultivation of fastidious pathogenic microorganisms

Mode of Action

Proteose peptone is a special mixture of peptones as defined in the USP. Proteose peptones are proteins from animal sources that have been hydrolysed under different digestion conditions into low molecular weight peptides and free amino acids.

Proteose peptone is also an excellent nutrient for the cultivation of pathogenic organisms that require a highly nutritious substrate, such as for example, Corynebacterium, Haemophilus, Histoplasma, Gonococcus, Neisseria, Pasteurella, Pneumococcus Salmonella, Staphylococcus, Streptococcocus and others.

Typical Analysis

Colour powder	Light yellow-beige
Colour in solution	yellow-beige
pH (2% in water)	6.5-7.5
Loss on drying (105 °C)	≤10%
Sulfated ash (800 °C)	≤15%
Nitrogen (N_T)	≥12.0%
Heavy metals (as Pb)	≤0.001%

Ordering Information

Product	Merck Cat. No.	Pack size
Proteose Peptone	1.07229.1000	1 kg

Quality control

Test strains	Growth
Staphylococcus aureus ATCC 25923	+
Staphylococcus aureus ATCC 6538P	+
Enterococcus faecalis ATCC 11700	+
Listeria monocytogenes ATCC 19113	+
Escherichia coli ATCC 8739	+
Klebsiella pneumoniae ATCC 13883	+
Salmonella typhimurium ATCC 14028	+

Skim milk powder

Skim milk powder is used as nutritive substrate in media for the growth of fastidious lactobacilli and in bacteriological media for dairy products

Mode of Action

Skim milk powder is a spray dried skimmed cow milk that is free of inhibitors (e.g. antibiotics).

It is used as substrate in media for the bacteriological examination of dairy products and for the identification of Clostridia spp. The latter can be differentiated based on their ability to proteolytically break downs proteins to peptones (peptonisation) or to coagulate milk.

As an ingredient in culture media Skim milk powder is usually employed in concentrations of 0.1 to 1.0%.

Typical Analysis

Colour powder	Cream
Bioburden	<10000 cfu/g
Solubility (5%)	Total
pH (2% in water)	6.0-7.0
Water (Karl Fischer)	≤10%
Sulfated ash (800 °C)	≤10%
Assay protein (ex. N calc. on dried substance)	≥35%
Fat	≤1.5%
Free acids (as lactic acid)	≤2%
Lactose	50-55%
Amino acid specification	See table page 542

Ordering Information

Product	Merck Cat. No.	Pack size
Skim milk powder	1.15363.0500	500 g

Quality control

Test strains	Growth
Staphylococcus aureus ATCC 25923	+
Staphylococcus aureus ATCC 6538P	+
Enterococcus faecalis ATCC 11700	+
Listeria monocytogenes ATCC 19113	+
Escherichia coli ATCC 8739	+
Klebsiella pneumoniae ATCC 13883	+
Salmonella typhimurium ATCC 14028	+

Tryptose

Tryptose is used in cell culture, molecular genetic media, and as nutritive substrate for cultivation and resuscitation of a large variety of fastidious microorganisms

Mode of Action

Tryptose is a mixture of tryptic digested proteins. It is suitable nutrient base material for applications in biotechnology and pharmaceutical industry.

It is recommended for use in media for the cultivation of a wide range of fastidious bacteria such as *Brucella*, *Streptococcoccus*, *Pneumococcus*, *Meningococcus spp.*, etc.

Typical Analysis

Colour powder	Brownish yellow-beige
Colour in solution	Beige
Solubitlity (5%)	Total
pH (5% in water)	6.5-7.5
Loss on drying (Moisture)	≤10%
Sulfated ash (800 °C)	≤15%
Amino-nitrogen (N_α)	3.0-5.0%
Nitrogen (N_T)	11-13%
Amino acid specification	See table page 542

Ordering Information

Product	Merck Cat. No.	Pack size
Tryptose	1.10213.1000	1 kg

Quality control

Test strains	Growth
Staphylococcus aureus ATCC 25923	+
Staphylococcus aureus ATCC 6538P	+
Enterococcus faecalis ATCC 11700	+
Listeria monocytogenes ATCC 19113	+
Escherichia coli ATCC 8739	+
Klebsiella pneumoniae ATCC 13883	+
Salmonella typhimurium ATCC 14028	+

Universal Peptone M 66

Universal peptone is a poly peptone with well balanced nutritional characteristics. It can be employed in cell culture, fermentation, molecular genetic media, and as nutritive substrate for cultivation and resuscitation of a large variety of fastidious microorganisms

Mode of Action

Universal M 66 peptone is a mixture of casein peptone and meat peptone.

Universal peptone M66 combines the nutritive characteristics of casein and meat peptone. The well balanced peptone mix containing a representative mix of low and high molecular peptones, a broad range of free amino acids in growth supporting concentrations, vitamins and other growth factors.

Universal peptone M 66 can be used as nutrient, for example as replacement of serum albumin, in cell culture and in fermentations. As an ingredient in culture media Universal Peptone M66 can be employed in media for the cultivation of a wide range of fastidious bacteria.

Typical Analysis

Colour powder	Brownish yellow-beige
Colour in solution	Beige
pH (2% in water)	6.0-7.0
Loss on drying (105 °C)	≤5%
Sulfated ash (800 °C)	≤15%
Amino-nitrogen (as N)	≥3.5%
Peptone content (ex. N calc. on dried substance)	≥80%
Heavy (toxic) metals (as Pb)	≤0.001%
Amino acid specification	See table page 542

Ordering Information

Product	Merck Cat. No.	Pack size
Universal Peptone M 66	1.07043.1000	1 kg

Quality control

Test strains	Growth
Staphylococcus aureus ATCC 25923	+
Staphylococcus aureus ATCC 6538P	+
Enterococcus faecalis ATCC 11700	+
Listeria monocytogenes ATCC 19113	+
Escherichia coli ATCC 8739	+
Klebsiella pneumoniae ATCC 13883	+
Salmonella typhimurium ATCC 14028	+

Yeast extract, granulated

Yeast Extract is a non animal and low endotoxin peptone suitable for use in fermentations, cell culture, molecular genetic media, and culture media for cultivation and resuscitation of a large variety of fastidious microorganisms and antibiotics assays

Mode of Action

Yeast Extract is defined in the USP as a water soluble peptone like derivative of yeast (Saccharomyces) cells. Yeast Extract is prepared by proteolytic autolyse of the cells. The autolytic activity is stopped by a heating step. The autolysis is carefully controlled to preserve the naturally occurring B-complex vitamins.

Yeast Extract is employed in fermentations and bacterial mammalian and insect cell cultures. It is as a versatile and growth enhancing nutritive substrate and can replace in cell cultures serum. It has an endotoxin value of less than 500 EU/g.

Typical Analysis

Colour granules	Brownish-yellow
Colour in solution	Beige
pH (2% in water)	5.5-7.2
Loss on drying (50 °C; 20 Pa, 3 h)	≤5.0%
Sulfated ash (800 °C)	≤17%
Nitrogen (ex. N calc. on dried substance)	≥10.5%
Chlorides (expressed as NaCl)	≤5.0%
Total phosphor (as P)	≤2.5%
Calcium	≤0.05%
Magnesium	≤0.10%
Heavy (toxic) metals (as Pb)	≤0.005%
Amino acid specification	See table page 542

Ordering Information

Product	Merck Cat. No.	Pack size
Yeast extract, granulated	1.03753.0500	500 g
Yeast extract, granulated	1.03753.9025	25 kg

Quality control

Test strains	Growth
Staphylococcus aureus ATCC 25923	+
Staphylococcus aureus ATCC 6538P	+
Enterococcus faecalis ATCC 11700	+
Listeria monocytogenes ATCC 19113	+
Escherichia coli ATCC 8739	+
Klebsiella pneumoniae ATCC 13883	+
Salmonella typhimurium ATCC 14028	+

FERMTECH tailor made culture media

We deliver any special mixture on request! You don't like making your own nutrient mixtures and checking its suitability? Merck's special mixtures offer all the advantages!

In the age of biotechnology, the users of fermentation processes are confronted with new and sophisticated demands. Producers of vaccines, antibiotics, tissue cultures, and microorganisms as well as research laboratories are dependent on a constant, defined quality and composition of the respective culture media for their research, production, and other applications. Problems often arise in the provision and coordination of the individual components for the very special required culture media.

Being a major producer of culture media and user of fermentation processes, we have gained extensive experience in the past 100 years. As your competent partner, you are welcome to count on this expertise and experience. The analytical possibilities of our laboratory guarantee fast and high-quality solutions to your problems.

Our services include:

- All products from the same source
- **We'll take care of all R+D work up to the finished culture media**
- **Quality controls and guarantees according to your test standards**
- **Specification and further development according to your wishes**
- **We work in accordance with DIN/ISO 9001**
- **Production of up to 10 tons in one batch is possible**
- **Production in pharmaceutical and food plants according to GMP guidelines**
- **Constant quality and low costs due to bulk purchase**
- **Questions and production requests will naturally be kept confidential (CDA on request)**

You save:

- **The search for suppliers and suitable materials**
- **Personnel and material costs**
- **Laboratory costs because you draw on the knowledge of our laboratories which are equipped in keeping with the latest technological developments.**

Ordering Information

Product	Merck Cat. No.	Pack size
Agar-agar	1.11925.1000	1 kg
Yeast extract	1.11926.1000	1 kg
Yeast extract	1.11926.9025	25 kg
Peptone from casein (Tryptone)	1.11931.1000	1 kg
Peptone from casein (Tryptone)	1.11931.9025	25 kg

Base materials for culture media in biotechnology

FERMTECH/Designation	Culture media	Antibiotic production	Fermentation	Vaccine production
Agar-agar	x			
Yeast extract	x	x	x	x
Peptone from casein (Tryptone)	x	x	x	x

Abbreviations

In this book the following abbreviations will be used for organisations and standards.

AOAC	Association of Analytical Communities (AOAC International)
APHA	American Public Health Association
API	American Petroleum Institute
ATCC	American Type Culture Collection
BAM	Bacteriological Analytical Manual
BGA	Umweltbundesamt, Berlin German Public Health Authority
DAB	Deutsches Arzneibuch (German Pharmacopoeia)
DEV	Deutsches Einheitsverfahren zur Wasser-, Abwasser- und Schlammuntersuchung (German Methods for the Examination of Water, Waste Water and Sludge)
DIN	Deutsches Institut für Normung e.V.* (German Institute of Standardization)
Eiprod.-Verordng.	Eiprodukte-Verordnung (German Egg Product Regulations)
EP	European Pharmacopoeia
EPA	United States Environmental Protection Agency
FIL-IDF	Fédération Internationale de Laiterie International Dairy Federation (Internationaler Milchwirtschaftsverband)
FIBG	Deutsches Fleischbeschaugesetz (German Meat Inspection Law)
ISO	International Organization for Standardization
LMBG	Lebensmittel- und Bedarfsgegenständegesetz (German Food and Consumer Goods Law)
Merkblätter-Packmittel	Arbeitsgruppen des Instituts für Lebensmitteltechnologie und Verpackung der Technischen Universität München: Merckblätter für die Prüfung von Packmitteln (Institute for Food Technology and Packing, Technical University of Munich: Instruction Leaflets for the Examination of Packaging Materials)
Methodenbuch-Milch	Handbuch der landwirtschaftlichen Versuchs- und Untersuchungsmethodik (Methodenbuch) (Methodology Handbook for Agricultural Experiments and Studies)
NCA	National Canners Association
NCCLS	National Committee of Clinical Laboratory Standards
SMDP	Standard Methods for the Examination of Diary Products
SMWW	Standard Methods for the Examination of Water and Wastewater
USDA	United States Department of Agriculture
USP	United States Pharmacopoeia
WHO	World Health Organization

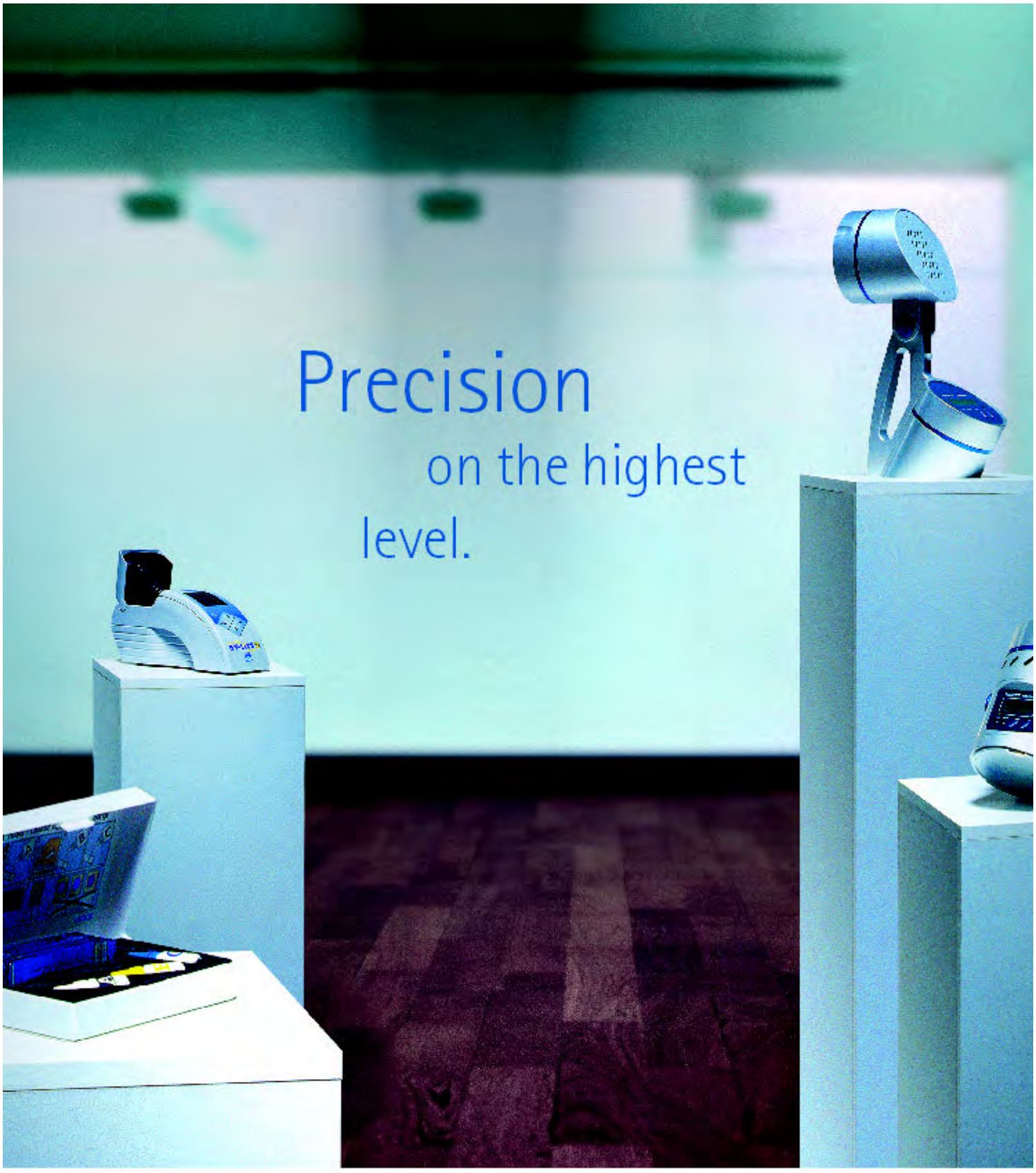
* DIN norm standards and standards "Amtliche Sammlung von Untersuchungsverfahren acc. to § 35 LMBG" can be obtained from Beuth-Verlag, D-10787 Berlin 30, 6 Burggrafenstr. Phone 030/26011

List of additives and auxiliary agents

Cat. No.	Product	Application
1.15931.	Acridine orange	fluorescent staining
1.00846.	Adonitol	reactant for biochemical identification
1.06776.	Alizarin yellow 2 G	culture media additive
1.01890.	Ammonium bismuth citrate	culture media additive
1.01492.	L(+)Arabinose	reactant for biochemical identification
1.01301.	Auramine	fluorescent staining
1.09211.	Azur II	staining of smears and sections
1.04054.	Bile salt mixture	culture media additive
	Bismarck brown see Vesuvine	
1.01310.	Brilliant green	culture media additive
1.03025.	Bromocresol purple	culture media additive; pH indicator
1.03026.	Bromothymol blue	culture media additive; pH indicator
1.15940.	Crystal violet	staining of smears and sections; culture media additive
1.03067.	N,N-Dimethyl-p-phenylenediammonium dichloride	for detection of cytochrome oxidase
1.05990.	Dulcitol	reactant for biochemical identification
1.15934.	Eosin bluish	staining of smears and sections
1.15935.	Eosin yellowish	staining of smears and sections, culture media additive
1.15936.	Erythrosine B	staining of smears and sections
1.00842.	Esculin	culture media additive; reactant for biochemical identification
1.05323.	D(-)Fructose	reactant for biochemical identification
1.04062.	D(+)Galactose	reactant for biochemical identification
	Gentiana violet see Methyl violet	
1.09203.	Giemsa's azur eosin methylene blue	staining of smears and sections
1.09204.	Giemsa's azur eosin methylene-blue solution	staining of smears and sections
1.08342.	D(+)Glucose (monohydrate)	culture media additive; for biochemical identification
1.04094.	Glycerol (about 87 %)	reactant for biochemical identification
1.08238.	Glycerol triacetate	for detection of lipolytic enzymes
1.01958.	Glycerol tributyrate	for detection of lipolytic enzymes
1.00327.	Hydrochloric acid in ethanol	staining of smears and sections acc. to Ziehl-Neelsen
1.04728.	myo-Inositol	reactant for biochemical identification
	Iodine/potassium iodide solution see Lugol's solution	
1.13741.	Lactophenol blue solution	for staining fungi (see page 366)
1.07657.	Lactose (monohydrate)	culture media additive and reactant for biochemical identification
1.15941.	Light green SF	staining of smears and sections
1.01287.	Löffler's methylene-blue solution	staining of smears
1.09261.	Lugol's solution	Gram staining
1.01398.	Malachite-green oxalate	culture media additive and staining of smears
1.05910.	Maltose (monohydrate)	reactant for biochemical identification
1.05982.	D(-)Mannitol	culture media additive; reactant for biochemical identification

List of additives and auxiliary agents

Cat. No.	Product	Application
1.05984.	D(+)Mannose	reactant for biochemical identification
1.01352.	May-Grünwald's eosin methylene blue	staining of smears and sections
1.01424.	May-Grünwald's eosin methylene blue solution modified	staining of smears and sections
1.15943.	Methylene blue	staining of smears and culture media additive
1.15944.	Methyl green	staining of smears and sections
1.06223.	1-Naphthol	for preparing oxidase reagent
1.09028.	Nessler's ammonium reagent	for detecting ammonia formation
1.01369.	Neutral red	culture media additive; pH indicator
1.04041.	New fuchsin (NB powder)	staining of smears and sections
1.15924.	Nigrosine	contrast picture of bacteria
1.05164.	Potassium tellurite	culture media additive
1.05169.	Potassium tetrathionate	culture media additive
1.07518.	Pyronin® G	fluorescent staining of smears and sections
1.07549.	Raffinose (pentahydrate)	reactant for biochemical identification
1.04736.	L(+)Rhamnose (monohydrate)	reactant for biochemical identification
1.15948.	Safranin O	staining of smears and sections
1.07665.	Salicin	reactant for biochemical identification
1.06340.	Sodium hydrogen selenite	culture media additive
1.06691.	Sodium thioglycollate	anaerobic culture media additive
1.07758.	D(-)Sorbitol extra pure	culture media additive; reactant for biochemical identification
1.01252.	Starch soluble	reactant for biochemical identification
1.07651.	Sucrose	reactant for biochemical identification
	Tellurite see Potassium tellurite	
	Tetrathionate see Potassium tetrathionate	
1.00697.	Thioglycollic acid about 80 %	anaerobic culture media nutrient additive
1.08353.	Trehalose (dihydrate)	reactant for biochemical identification
1.08380.	2,3,5-Triphenyltetrazolium chloride	culture media additive (TTC culture medium)
1.08487.	Urea	culture media additive
1.08689.	D(+)Xylose	reactant for biochemical identification; culture media additive
1.09215.	Ziehl-Neelsen's carbol-fuchsin solution	staining of tubercle bacilli and for counterstaining in Gram staining


Precision
on the highest
level.

Hygiene and Air Monitoring

Introduction to hygiene monitoring

Why hygiene monitoring?

Food production plants have to be as clean as possible. If not, quality problems, complaints or even image loss will follow.

The traditional methods used to test cleanliness, do have severe disadvantages: Visual checks are not sufficient, as dirt and food residues may be invisible, and microbiology takes day.

New and rapid test methods based on the detection of typical molecules like ATP (Adenosine triphosphate) have improved the situation and fit into hygiene or HACCP plans as required in different industries.

What is ATP?

Adenosine triphosphate (ATP) is a substance found in all animal and vegetable matter, including food and food debris, in addition to bacteria, fungi and other microorganisms.

How can ATP-bioluminescence be used as a detection method?

Levels of ATP can be used to indicate the amount of such matter on surfaces that could come into contact with food, thus giving a measure of cleanliness.

By precisely measuring the light released by this reaction, HY-LiTE®2 allows an accurate determination of the quantity of ATP present, by counting photons out of the bioluminescence light reaction.

The intensity of the light emitted from a sample measured with the HY-LiTE® System is indicated on the display in relative light units (RLU). This RLU value is directly proportional to the quantity of ATP in the sample tested and therefore also directly proportional to any contamination with biological material.

Why measuring "total ATP" for hygiene monitoring?

All sources of ATP in an environment do contribute to "total ATP". This is a measure for the dirt or uncleanliness of a tested area.

HY-LiTE®, by measuring total ATP, is therefore a completely new method to measure cleanliness, and can therefore not be compared directly to traditional microbiology or to visual checks.

Microbiology detects living microorganisms, which are forming colonies on specific nutrient media. Other cellular food residues, which contribute to dirt and thus are a potential source for growth of microorganisms are not detected. Another diadvantage is the time consuming procedure.

Visual checks are extremely easy to perform, but the low detection limit and extremely low repeatability does not allow any comparison to the other methods.

Where in a food or beverage production site should HY-LiTE® be used?

The cleanliness of critical points in food production which conceal a quality risk should be examined regularly with HY-LiTE® before the start of production.

Measuring points may be filling equipment, storage tanks, cutting tables, conveyor belts, CIP (Cleaning In Place) rinse water samples and all points of the equipment with which the product has direct contact.

Examinations should be made either after cleaning or before the start of production. A regular, routine examination of critical points will provide consistent records for internal and/or external audit purposes. In addition to this the tailor-made data analysis package TREND 2, will provide easily understandable documentation for analysis, and communication of results in both table and graphical formats.

In which industries should HY-LiTE® be used?

HY-LiTE® should be used routinely in all areas, where confirmation of proper sanitation is required:

- Meat and Fish processing industry
- Frozen Food, Pizza, Ice cream
- Vegetables and Potato processing
- High care areas for Baby Food
- Soft drink industry
- Beer industry
- Catering and large Kitchens

Which official requirements for hygiene monitoring do exist?

Equipment for the production of foods should be as clean as possible. If this is not the case, quality problems or complaints may occur.

Beyond this, statutory requirements such as the HACCP concept or the European directive 93/43/EEC are also applicable in most countries. This may include a requirement for a method which allows a rapid examination and which can verify and document the cleanliness of the equipment - before the start of production.

Procedures to control and ensure proper cleanliness already before production starts have been missing up to now!

Visual examination is insufficient, because invisible food residues are not detected. Classical microbiological methods only provide evidence of microorganisms and are too slow to allow a reaction before the start of production.

Which arguments do count for HY-LiTE®?

- Rapid method, which covers basic HACCP requirements. Quick results allow confirmation of cleanliness or corrective action before production starts.
- On-site results, without transfer of samples to laboratory and without waiting for results.
- Detection of food residues as major source of unhygienic conditions. Food residues are the main reason for rapid increase of microorganisms in facilities, which have to be cleaned.
- Easy detection of residues also in liquid samples like CIP (Cleaning in place) rinse waters. This is a major issue in beverage industry and testing of tanks, pipelines and filling heads. The HY-LiTE® Pen can be used directly, without requiring a pipette.
- Ready prepared test Pens and easy handling.
- Direct effect of rapid test results on motivation, education and improvement of cleaning and production personnel.
- "HACCP-secure" documentation allows tracking and comparison of all data.

Which detection principle is used?

The HY-LiTE® System is a luminometer. Photons, produced by the firefly reaction, are detected with a extremely light-sensitive, built-in photo multiplier tube.

Can I trust in the reliability of the system?

HY-LiTE® is the only available Luminometer, with a built-in calibration self check against an internal light standard. The system uses automatically the following self-diagnosis features: (A) self-check against a built-in standard reference light source when the instrument is switched on. (B) correction of the background signal before each measurement. (C) temperature compensation during each measurement. (D) in case any deviation of the performance is detected, an error message is indicated on the display.

How reproducible is the pen?

The pen is produced in very large batches in an automated production line. The conditions are similar to pharmaceutical production, to ensure a sterile and ATP free environment.

Due to the standardized production, the pen to pen variation for HY-LiTE® is very low.

Could HY-LiTE® results be influenced by sanitizer or chemical residues?

The firefly reaction could be disturbed by chemical residues, picked up with the sample. The HY-LiTE® Pen therefore has two unique features, to ensure minimum interference with possible residues:

- A dilution step between swabbing and sample uptake into the pen cuvette reduces any chemicals significantly.
- The patented buffer solution equalizes any extreme pH values of residues and contains neutralizers to ensure proper biochemical conditions, which are required for the enzymatic firefly reaction.

Why temperature compensation?

The firefly reaction is an enzymatic reaction and therefore shows a dependance on pH value (as described) and ambient temperature. Any portable ATP system therefore has to calculate and compensate different possible conditions. A major advantage of the HY-LiTE® luminometer is the built-in temperature compensation.

Under conditions between 5 and 35°C the HY-LiTE® System can be used without any problems. Other luminometers do not have this broad range of use.

How sensitive is the HY-LiTE® test?

The detection limit is 1.4 x 10-14 Mol ATP per sample. Compared to an average food residue, this allows to detect invisible residues of only about 1 mg cellular residues per sample!

Can bacteria be detected?

Bacteria itself are usually below the detection limit. But studies have shown, that bacteria do never appear alone, but only together with other food residues. And: bacterial growth is only possible, if food residues have been left on not properly cleaned areas. HY-LiTE® therefore has been designed, to detect food residues as a measure of "uncleanliness" in food production. The philosophy is: The more ATP is detected with HY-LiTE® = the more food residues have been left = the higher the risk for bacterial contamination!

Why not measuring bacterial ATP?

Total ATP consists of all types: somatic ATP, microbial ATP and free ATP. All ATP sources together are indicating the cleanliness. This leads to a strictly proportional relationship: if cleaning has been done twice as good, then the amount of total ATP is reduced by factor two.

Detecting microbial ATP only, would not lead to a sufficient picture of cleanliness.

Does HY-LiTE® replace Microbiology?

HY-LiTE® will reduce the amount of microbiological tests significantly, but standard microbiology may still be required, in case single microorganisms shall be detected and identified. Microbiology results are available only several days after the production has been finished, and (in terms of HACCP) do not allow immediate action! Microbiological results are only of historical or retrospective relevance.

HY-LiTE® in contrary allows rapid sanitation checks before production starts as a basis for installation of any HACCP plan!

Is there any evaluation, comparing HY-LiTE® with Microbiology?

A meat trial has been performed in the Netherlands and published by Prof. de Zutter, to compare HY-LiTE and microbiological methods for sanitation monitoring. The result is: HY-LiTE® is an outstanding method to establish the efficiacy of cleaning and gives substantially more information than traditional microbiological methods such as RODAC and microbial count!

Why should sanitation not simply be checked visually?

Invisible residues can not be detected. The question "Is it really clean?" remains.

Documentation according to HACCP is poor - if at all possible.

Visual check does not provide any repeatability!

Which level of ATP is acceptable?

This depends on factors like: types of raw materials, processes applied, material of the surfaces measured. Also the "risk" associated with the product is important: will it be eaten as it is, or cooked/re-heated prior to eating. Are consumers healthy adults or babies, geriatrics or immuno-compromised, etc.

It is therefore not possible to give absolute standards for what Pass and Fail limits should be. However, below is a list of suggested limits for major groups of food products which can be used as an initial guideline. The list has been composed based on several years experience with HY-LiTE® applied in a variety of food industries, but it must be stressed that the values are only a rough guideline:

	Suggested RLU values:	
Production environment	**Pass**	**Fail**
Raw Milk	100	300
Raw Meat / Fish / Egg	300	1000
Raw Vegetable / Fruit	500	1500
Processed Milk / Milk products	70	200
Processed Meat / Fish / Egg	70	200
Processed Vegetable / Fruit	200	600
Mass Catering / Flight Catering Facilities	500	1500
Beverages	50	100

PASS / FAIL Limit setting: which procedure is recommended?

Ideally, limits should be set based on HY-LiTE® results obtained after routine cleaning of the equipment.

Experience shows that it is appropriate to make a total of at least 40-50 measurements at the selected, cleaned measuring points and to sort these according to the measured values. The middle value of this list of ascending values (not the average value, but the median) is then taken as the pass limit and three times this pass limit is defined as the fail limit.

Which control or test points should be monitored with HY-LiTE®?

Principally all sites, which have to be cleaned routinely, should be monitored. According HACCP, a team of responsible persons should decide, which points in detail require sanitation monitoring. Typical sites are:

Cutting tables, boards, tools, slicing machines, transport belts, filling heads, tanks etc.

The sites usually can be divided in two areas:

Surfaces, where samples are taken by swabbing with the Swab of the HY-LiTE® Pen, and on the other hand Cleaning In Place (CIP) sites, where samples from rinse water samples are simply taken by dipping with the sampling stick of the HY-LiTE® Pen.

Which area should be swabbed?

Usually an area of 10 x 10 cm is swabbed. It is important, to define the size of the area in the HACCP plan, so that sampling can always be done under the same conditions. Personnel should be trained to do swabbing under repeatable conditions, concerning size, pressure etc.

Why has the HY-LiTE® swab to be moistened?

Reproducible sample uptake depends significantly on the moisture of the swab. Premoistened swabs do have a very limited shelflive, as they tend to dry out. Manual moistening of the HY-LiTE® swab takes only a second, but ensures, that the swab is always really wet!

Second, if surfaces are still wet from sanitation or rinse water, it is possible to leave the swab unmoistened.

Third, the swab does NOT leave any residues or extractant (as some competitor systems do it) on the surface!

How can results be documented with HY-LiTE®?

Today, increasing requirements are made on secure documentation of the results both by customers and Public Health Inspectors. The HY-LiTE® System is well equipped for this and is already prepared to fulfil such requirements.

The documentation encompasses the storage of all relevant data, such as date, time, user, serial number of the instrument, measuring point, result and interpretation of the measured value. More than 2000 complete sets of data can be stored with the HY-LiTE® 2 System by using the measurement modes "Test & Store" or "HACCP Plan".

These data can be retrieved, printed with the built-in thermo-printer or transferred to a PC as required.

With the PC, the data can be processed with the high-performance and advanced TREND 2 software for immediate transformation into graphs and tables. Additional data such as the batch of the applied pen etc. can be stored here, authentically and secure from falsification.

Which are the basic features of the TREND 2 software?

TREND 2 is a powerful data analysis program for your computer. It provides the capability, to easily create "HACCP-Plans" for cleanliness control on the PC and to download them to the HY-LiTE® 2 System. A plan can consist of a number of units each containing a list of test points with specified pass/fail limits. A template function allows convenient use of existing plans as a basis for new ones. For data analysis purposes, you can also merge different plans, enabling you to compare performance between different production areas or even factories. Convenient handling steps allow exciting graphical analysis and presentations of the collected data.

TREND 2 is provided on CD-ROM, including all available language versions.

Which security level does TREND 2 provide?

The TREND 2 software provides extremely high security through 3 different password-protected access levels and auditable documentation of all plan and data records. An effective working environment under the different Windows formats allows comfortable handling as well as export of data in spreadsheet format for import into other programs.

Compared to the former Trend software the on-line help functions are very much extended, eliminating the need for a printed software manual.

CCP

HY-LiTE® 2 System

Luminometer complete with accessories, for portable use in food & drink production, to monitor the hygienic status acc. to HACCP requirements in a rapid and reliable way.

The hygiene monitoring system

HY-LiTE® 2 kit contains a HY-LiTE® 2 luminometer with built-in printer and 1 roll of printer paper, Mains power supply, Batteries, PC-cable, TREND 2 Software, Operator manual (Different language versions available) and Step-by-step guide. All parts are delivered as COMPACT KIT in shoulder bag, or as SUPERVISOR KIT in robust, handy case.

Features and benefits

- Reliable and robust product
- Easy 5-buttons operation
- Half-automatic lid
- Temperature compensation
- Unique self check of calibration
- Advanced data management
- Runs from mains or from standard batteries
- Built-in memory, PC-port, ROM-key port and Printer

Experimental procedure

From Main Menu, select "Test Only" using the up and down arrow keys, and press "OK".

The lid of the measurement chamber will open automatically, and the test pen can be easily inserted. Then close the lid, and the measurement starts automatically.

This is the simplest and fastest means of making measurements with the HY-LiTE® 2. The results are displayed immediately on the screen and can be printed with the internal printer.

More than 2000 complete sets of data can be stored with the HY-LiTE® 2 System by using the measurement modes "Test & Store" or "HACCP Plan".

Data can easily be printed out at a later stage, or downloaded to a PC.

Specification

Dimension	11 x 13 x 28 cm
Application	Primarily for examination of cleaning/ sanitation in food production environments. The HY-LiTE® 2 System may only be used with HY-LiTE® pens.
Display of results	Display of the light intensity (from biolu-minescence) in relative light units.
Working range	Linear: 0 - 99.000 RLU, Logarithmic: 0 - 5.00 log 10 RLU
Self-checks	Automatic self-check against a built-in standard, automatic correction of the background signal, and automatic temperature compensation during each measurement.
Data handling	Optimum usage of the memory capacity by intelligent data handling. More than 2000 sets of data stored. Display information of the free memory capacity.
Test modes	HACCP Plan, Test & Store, Test Only.
Display	Graphic LCD display with 14 lines and adjustable contrast.
Control buttons	1 on/off button and 4 function buttons.
Printer	Built-in thermal printer.
Ambient conditions	Measurements at 5-35°C, 5-95% air humidity. When moved from a cold to a moist, warm environment, the develop-ment of condensation must be prevented.
Connections	Two serial RS232 interfaces for data transfer between system and a computer. One low-voltage socket for the HY-LiTE® power supply unit. One ROM key port.
Mains operation	The HY-LiTE® 2 can be connected to the mains with the power supply unit and 4 mains adapters for worldwide use.
Portable operation	Recommended batteries: 4 normal, sealed 1.5 V alkali batteries size Mignon LR6 AA.
Approvals	CE TÜV GS UL

Literature

DE ZUTTER, L., HELLWIG, K., a. LINDHARDT, C.: ATP method is highly suitable for hygiene monitoring (translated from the Dutch original) - De Keurmeester, 3; 5-10 (1998)

HY-LiTE® 2 System

Ordering Information

Product	Merck Cat. No.	Pack contents	Manual Version
HY-Lite® 2 System	1.30100.0221	HY-LiTE® 2 luminometer complete with accessories in shoulder bag	English
HY-Lite® 2 System	1.30100.0220	HY-LiTE® 2 luminometer complete with accessories in shoulder bag	German
HY-Lite® 2 System	1.30100.0223	HY-LiTE® 2 luminometer complete with accessories in shoulder bag	Spanish
HY-Lite® 2 System	1.30100.0224	HY-LiTE® 2 luminometer complete with accessories in shoulder bag	French
Printer paper	1.30110.0205	5 paper rolls for use with HY-LiTE® 2	

Technical Information about HY-LiTE® System

Power supply

Before disconnecting the HY-LiTE® System from the mains (power supply unit) please take care, that

a. the system has been switched off before and

b. the system has been left adapted to the mains for additional 5 seconds, afeter the last screen ("Testing for empty chamber...") has been disappeared.

Please take care, that the system is always equipped with the Activation card, in case no batteries or mains power supply unit is used. If batteries have to be changed, don't leave the system more than a few minutes without Activation card. Best you change batteries within about half a minute, because then you don't have to set time and date again.

Printer paper

Before using the HY-LiTE® System for the first time, the end of the paper should be pulled out for 1-2 cm of the slit. This should also be repeated, if the built-in printer has not been used for longer (some weeks). Otherwise it could happen, that the first lines of the printout can not be read properly.

In all cases, when you pull out the paper manually, please pull the paper

a. only in pieces of few centimeters, then starting again (to pull out next short piece of paper)

b. by pulling slightly upward (not horizontally or downward).

These measures will reduce the risk of paper jam.

CCP

HY-LiTE® Pens

Ready prepared Pens to test the cleanliness / hygiene of liquids (CIP rinse water) in food & drink industry, or to test biomass in water treatment application, by measuring with the HY-LiTE® 2 luminometer.

Typical composition

Adenosine triphosphate (ATP) is detected specifically by reaction with a luciferin/luciferase reagent in buffered solution.

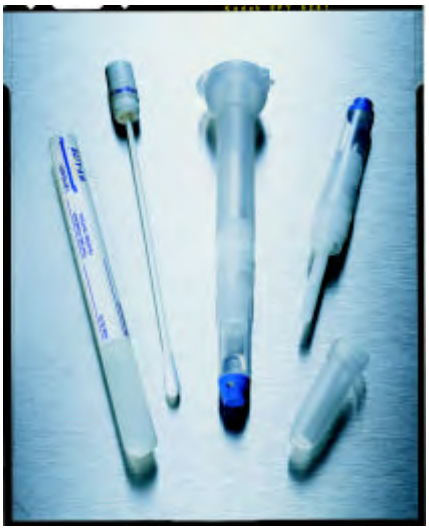
Features and benefits

- Dedicated pen for liquid testing
- Ideal format for CIP rinse water tests
- Also of use for biomass testing in water treatment
- Dilution factor and unique buffer eliminates interference
- Test procedure takes less than 1 minute
- Patented uptake of sample volume
- Long shelf-life

Experimental procedure

Rinse water in food & drink production, filling heads and transport tankers, which bear risk of endangering a complete lot of product, should be controlled by HY-LiTE® routinely. For surface testing a special test format including a standard swab is available (compare item 1.30101).

Dip the white stick of the pen into the liquid sample for 1 second, and press the stick home into the Pen cuvette. Press and twist (screw) the upper part of the Pen until it contacts the lower part. Shake the Pen, then put into luminometer for measurement. Close lid and read the result on the display.



Specification

Application	Primarily examination of cleaning/ sanitation of liquids/CIP rinse water in food & drink production
Format	Ready prepared cuvette test format for use with HY-LiTE® 2 luminometer
Reagent	Contains freeze-dried and stabilized luciferin/luciferase reagent (U.S. patents 5583024, 5674713, 5700673)
Test parameter	Total ATP
Detection limit	1.4×10^{-14} mol ATP
Interference	For normal application within clean production areas no interferences will occur, due to the built-in dilution step and the unique buffering capacity of the HY-LiTE® pen
Ambient conditions	Measurements at 5-35 °C
Storage conditions	The test pens are stable up to the date stated on the pack, when stored closed at +2 to +8°C. The shelf-life includes a period of transport or storage of up to 3 weeks at room temperature
Disposal	HY-LiTE® pens can be disposed off with the normal household waste.

Literature

DE ZUTTER, L., HELLWIG, K., a. LINDHARDT, C.: ATP method is highly suitable for hygiene monitoring (translated from the Dutch original) - De Keurmeester, 3; 5-10 (1998)

Ordering Information

Product	Merck Cat. No.	Pack contents
HY-Lite® Pens	1.30102.0021	50 CIP / liquid test pens

CCP

HY-LiTE® Refill pack

Ready prepared Pens to test the cleanliness / hygiene of surfaces in food & drink industry by measuring with the HY-LiTE® 2 luminometer.

Typical composition

Adenosine triphosphate (ATP) is detected specifically by reaction with a luciferin/luciferase reagent in buffered solution.

Features and benefits

- Reliable and convenient surface testing
- Can also be used for CIP liquid testing
- Separate swab
- No residues left on the tested surface
- Dilution factor and unique buffer eliminates interference
- Test procedure takes less than 1 minute
- Patented uptake of sample volume
- Long shelf-life

Experimental procedure

Surfaces in food production like cutting boards or slicing machines, which bear risk of endangering a complete lot of food product, should be controlled by HY-LiTE® routinely. Rinse water samples from cleaning in place (CIP) procedures in tanks or filling heads should also be checked regularly (compare item 1.30102).

Swab the surface of a defined area (10 x 10 cm). Transfer the sample into the rinse solution. Dip the white stick of the pen into the liquid sample and press the stick into the Pen cuvette. Press and twist (screw) the upper part of the Pen until it contacts the lower part. Shake the Pen, then put into luminometer for measurement. Close lid and read the result on the display.

Specification

Application	Primarily examination of cleaning/ sanitation of surfaces in food & drink production
Format	Ready prepared cuvette test format for use with HY-LiTE® 2 luminometer
Swab	Standfard sterile and ATP free swab for use in microbiology
Reagent	Contains freeze-dried and stabilized luciferin/luciferase reagent (U.S. patents 5583024, 5674713, 5700673)
Test parameter	Total ATP
Detection limit	1.4×10^{-14} mol ATP
Interference	For normal application within clean production areas no interferences will occur, due to the built-in dilution step and the unique buffering capacity of the HY-LiTE® pen
Ambient conditions	Measurements at 5-35 °C
Storage conditions	The test pens are stable up to the date stated on the pack, when stored closed at +2 to +8°C. The shelf-life includes a period of transport or storage of up to 3 weeks at room temperature
Disposal	HY-LiTE® pens can be disposed off with the normal household waste.

Literature

DE ZUTTER, L., HELLWIG, K., a. LINDHARDT, C.: ATP method is highly suitable for hygiene monitoring (translated from the Dutch original) - De Keurmeester, 3; 5-10 (1998)

Ordering Information

Product	Merck Cat. No.	Pack content
HY-Lite® Refill pack	1.30101.0021	100 surface test pens

CCP

HY-LiTE® Free ATP Pens

Ready prepared Pens for applications like biomass monitoring in biocide water treatment by measuring with the HY-LiTE® 2 luminometer.

Typical composition

Adenosine triphosphate (ATP) is detected specifically by reaction with a luciferin/luciferase reagent in buffered solution. Compared to item 1.30102, the white sampling stick of Free ATP pens 1.30194 does not contain a lysis reagent. Therefore bacterial cells are not lysed and only the free ATP in solution is measured.

A comparative measurement using the HY-LiTE® pen 1.30102 can provide information on the proportion of cellularly bound ATP.

Features and benefits

- Dedicated pen for liquid testing
- Main application for biomass testing in water treatment, especially for biocides, which are acting by cell lysis
- Dilution factor and unique buffer eliminates interference
- Test procedure takes less than 1 minute
- Patented uptake of sample volume
- Long shelf-life

Experimental procedure

To assess the effectiveness of the lysing effect of a Biocide, one usually tests the proportion of Free ATP (Read result from measurement with item 1.30194) compared to Total ATP (as measured with item 1.30102).

Dip the white stick of the pen into the liquid sample for 1 second, and press the stick home into the Pen cuvette. Press and twist (screw) the upper part of the Pen until it contacts the lower part. Shake the Pen, then put into luminometer for measurement. Close lid and read the result on the display.

Specification

Application	Primarily examination of biomass in biocide water treatment application
Format	Ready prepared cuvette test format for use with HY-LiTE® 2 luminometer
Reagent	Contains freeze-dried and stabilized luciferin/luciferase reagent (U.S. patents 5583024, 5674713, 5700673)
Test parameter	Free ATP
Detection limit	1.4×10^{-14} mol ATP
Interference	For normal application within clean production areas no interferences will occur, due to the built-in dilution step and the unique buffering capacity of the HY-LiTE® pen
Ambient conditions	Measurements at 5-35 °C
Storage conditions	The test pens are stable up to the date stated on the pack, when stored closed at +2 to +8°C. The shelf-life includes a period of transport or storage of up to 3 weeks at room temperature
Disposal	HY-LiTE® pens can be disposed off with the normal household waste.

Literature

DE ZUTTER, L., HELLWIG, K., a. LINDHARDT, C.: ATP method is highly suitable for hygiene monitoring (translated from the Dutch original) - De Keurmeester, 3; 5-10 (1998)

Ordering Information

Product	Merck Cat. No.	Pack content
HY-LiTE® Free ATP Pens	1.30194.0021	50 CIP / liquid test "free ATP" pens

HY-LiTE® Jet A1 Fuel Test Kit

Ready-to-use Pens for detection of total biological contamination of Jet A1 / Jet A fuel as used in civil aviation.
The HY-LiTE® Jet A1 Fuel Test reagent kit is used in conjunction with the HY-LiTE® 2 luminometer.

Typical Composition

Patented capture solution for sampling from Jet A1 fuel samples. Adenosine triphosphate (ATP) is detected specifically by reaction with a luciferin/luciferase reagent in buffered solution.

Features and Benefits

- Results in minutes compared to days
- De-skilled method
- Field test - no need for laboratory facilities
- Quantitative, Objective results
- Numerical read-out. Easy interpretation
- Same protocol for all jet fuel samples (with / without water)
- Same action limits for all samples (with / without water)
- Detects biological activity directly in the sample. Is not dependant on growth of microorganisms in laboratory media
- Flexible sample volume. 1 litre recommended but lower volumes can be tested. For comparison with guidelines results can be volume adjusted: RLU/litre = RLU x (1000 ml/ ml sample volume)
- Recommended by IATA guideline, 2nd Edition

Experimental Procedure

Fuel contains small amounts of water and bears risk of microbial contamination. The biomass (e.g. bacteria, fungi) may grow and cause blocking of filters or corrosion of tanks. Such damage is a very expensive issue especially in civil aviation, when leading to unplanned maintenance in airport hangars and when waiting three days for microbiology results.

Protocol: Transfer the capture solution into 1 liter fuel sample. Close the bottle tightly, shake and let stand at least 5 minutes. Transfer the blue capture solution back to the pen sample tube. Dip the white stick of the pen into the liquid sample and press the stick into the Pen cuvette. Press and twist (screw) the upper part of the Pen until it contacts the lower part. Shake the Pen, then put into luminometer for measurement. Close lid and read the result on the HY-LiTE® luminometer display.

Specification

Application	Examination of biomass contamination of jet fuel samples
Format	Ready prepared cuvette test format for use with HY-LiTE® 2 luminometer
Pipettes	Each one small and large pipette for sample transfer
Reagent	Contains freeze-dried and stabilized luciferin/luciferase reagent (U.S. patents 5583024, 5674713, 5700673)
Capture solution	Patented solution for capturing of biomass from 1 liter fuel sample
Detection limit	1.4×10^{-14} mol ATP
Interference	Chemical additives and contaminants such as FSII and anticorrosive agents may interfere with the efficiency of the Capture Solution and the HY-LiTE® reaction and cause lower than expected readings. Biocides used for treatment of contaminated fuel may interfere with the reaction chemistry, depending on the concentration and type of biocide in the fuel. The effect of Kathon FP1.5 at 100 ppm w/w and Biobor JF at 270 ppm w/w have been tested in fuel and cause no significant interference on the HY-LiTE® test.
Ambient conditions	Measurements at +5 to +35 °C
Storage conditions	The test pens are stable up to the date stated on the pack, when stored closed at +2 to +8°C. The shelf-life includes a period of transport or storage of up to 3 weeks at room temperature.
Disposal	HY-LiTE pens can be disposed off with the normal waste.

Literature

IATA "Guidance Material on Microbiological Contamination in Aircraft Fuel Tanks" 2nd ed. 2004

Ordering Information

Product	Merck Cat. No.	Pack contents
HY-LiTE® Jet A1 fuel Test Kit	1.30196.0021	20 fuel test pens and 2 x 20 pipettes
HY-LiTE® 2 luminometer	1.30100.0221	Luminometer with accessories in shoulder bag

HY-RiSE® Colour Hygiene Test Strip

An analyser-free rapid test for qualitative testing of cleanliness of surfaces

What is the purpose of test?

HY-RiSE® indicates surface cleanliness by measuring product residues, e.g. from food and beverages, left after inadequate cleaning.

Typical applications are assessments of food and hand contact surfaces, e.g. worktops, slicers, cutting boards, fridge handles, microwaves, ovens, etc.

Measurements made with HY-RiSE® can provide early warning of possible contamination on specific surface areas allowing immediate corrective action, e.g. removing food soils.

What does the test measure?

- Nicotinamide Adenine Dinucleotides (NAD, NADH)
- Nicotinamide Adenine Dinucleotide phosphates (NADP, NADPH)

These are a group of compounds found in all living cells like animal and vegetable matter, including food and food debris, in addition to bacteria, fungi and other microorganisms.

How does the test work?

If NAD(P) and/or NAD(P)H is present in the sample, Gluc-DH® converts ß-D-Glucose into D-Gluconolactone, then Diaphorase converts a Tetrazolium Salt into a coloured Formazan Salt.

Any colour development on the test strip within minutes indicates a positive result:
surface not clean.

Who can use the kit?

- small to medium size food and beverage producers
- cleaning companies, contract cleaners
- caterers, restaurants, canteens, hotels
- super markets, retailers, butchers, bakehouses
- hygiene inspectors, HACCP trainers

What is the test good for?

- Monitoring of surface cleanliness of
  - food and beverage production and storage areas as well as transport equipments
  - cooking areas, food and beverage preparation and storage areas, especially after deep cleaning
  - toilets, bath rooms, rest rooms
- Supervising and training/instructing of food and beverage producers resp.

Which are the benefits?

- Analyser-free hygiene test:
  - no investment cost
  - portable in your pocket
  - applicable at any time and place
  - offers low-end alternative to Merck HY-LiTE®
- Test takes 3 drops of reagents on strip:
  - easy to perform
  - easy to dispose
- Clear test result available within 5 min:
  - dirty surfaces can be re-cleaned immediately
  - simple Yes/No answer
- Test is much more sensitive than visual inspection of cleanliness:
  - can be included into HACCP procedures
  - provides confidence that HACCP regulations are met
  - improves level of hygiene risk management
  - can be used as a tool to identify risk areas for microbiological testing
- Test is more sensitive than protein test
- Test is an essential part of the service package of contract cleaners:
  - enhances cleaning service package
  - assures validated cleaning of HCPs

HY-RiSE® Colour Hygiene Test Strip

An analyser-free rapid test for qualitative testing of cleanliness of surfaces.

Kit components

Package for 50 tests consists of

- 50 foil wrapped test strips
- bottle A with wetting solution
- bottle B with substrate solution
- bottle C with enzyme solution
- colour chart and insert sheets

Preparation and storage

Test strips and solutions are ready-for-use.

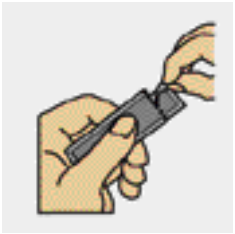
Strips and solutions stable up to the expiry date stated on the package when stored at +2 to +8 °C. Protect from light.

After first opening of each bottle and while in use, stable for 12 weeks when stored at +20 to +25 °C, if immediately re-sealed with the same screw cap after each use.

Alternative: After first opening of each bottle and while in use, stable for 6 months when stored at +2 to +8 °C, if immediately re-sealed with the same screw cap and refrigerated after each use.

Procedure

1. Tear open the foil package at the coloured line, remove the test strip from the foil.



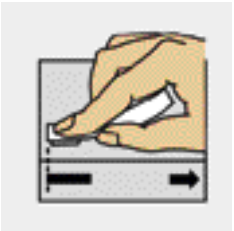
2. Add one drop of Reagent A to the pad on the strip.

Note: If the test surface is wet, do not use Reagent A.

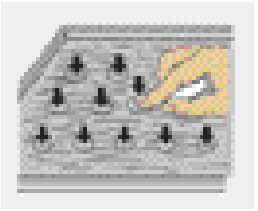
3. **Sampling for testing surfaces:**

   In case of smooth surfaces, place the entire test strip pad onto the test surface and draw back approximately 30 cm in sample area.

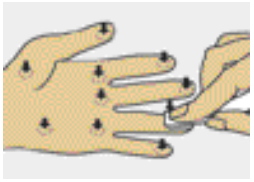
   On rough surfaces, collect sample by pressing the test strip pad onto a minimum of ten different spots on the surface. For hand testing, sample each fingertip and five spots on the palm of the hand.



Smooth surface



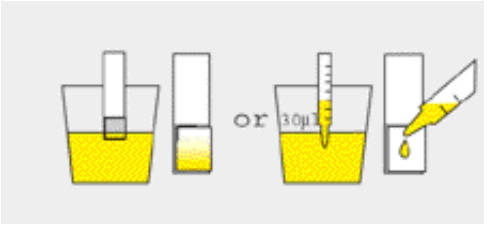
Rough surfaces



Hands

Sampling for testing Clean in Place (C.I.P) rinse waters:

Do not use Reagent A. Dip the test strip pad into the rinse water so that half of the pad is wet. Alternatively, use a pipette and transfer 30 µl sample onto the pad.



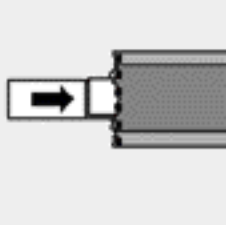
Note: After sampling, the test strips can be left for up to 2 hours at room temperature (up to 25 °C) before further processing, if they are returned to the packaging.

4. Add one drop of **Reagent B** (substrate solution, yellow cap) to the test strip pad.

HY-RiSE® Colour Hygiene Test Strip

5. Add one drop of **Reagent C** (enzyme solution, blue cap) to the test strip pad.

6. Return test strip to foil packaging so that the pad is covered. Leave for **4-5 minutes in the dark at +15 to +30 °C** after addition of Reagent C.

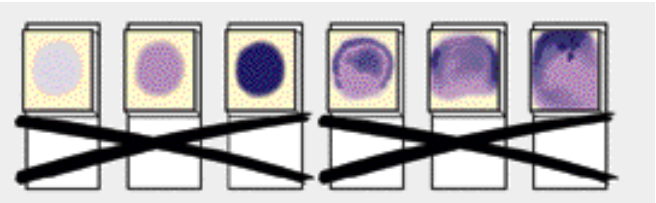


7. **Yellow** colour of the pad indicates a Clean/PASS condition. There are no or undetectable levels of residue on the surface.

   Pink/purple to bluish violet colouring of the pad indicates a Dirty/FAIL condition. There are detectable levels of residue on the surface. The surface should be re-cleaned. PASS/FAIL conditions are marked clean √ and dirty XX.

clean/pass **dirty/fail, re-cleaning is recommended**

8. Document test results on the table to be found in the test kit.



Literature

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Goll M, Kratzheller B, Bülte M, 2003: Kontrolle von Rückständen auf Oberflächen. Evaluierung des HY-RiSE® Colour Hygiene Test Strips zur Überprüfung der Sauberkeit (Evaluation of HY-RiSE® Colour Hygiene Test Strip for checking the cleanliness of surfaces) - Fleischwirtschaft **9**, 152 - 154.

Gierse S, Babel W: Zum Einsatz von Hy-RiSE® im Rahmen der betriebseigenen Kontrolle nach §§3 und 4 LMHV - Bb Bundesverband der beamteten Tierärzte, Kongress am 22./23. April 2002 , Bad Staffelstein.

Ordering Information

Product	Merck Cat. No.	Description
HY-RiSE®	1.31200.0001	Colour Hygiene Test Strip for assessing the general cleanliness of surfaces. Kit for 50 tests.

Introduction to microbial air monitoring

What is microbial air monitoring?

A method, to collect and count the amount of microbes in a defined volume of air.

Why air monitoring?

There is increasing need for effective air sampling in all areas where airborne microorganisms may contaminate or affect industrial products and processes. Air monitoring is therefore highly important in all industries which do have cleanroom areas with filtered air.

How will the microbes be deteced?

Any microbes, spores, bacteria, yeasts or moulds in a distinct volume of air are sampled on a standard Petridish. Colonies are counted after incubation.

Which are the basic features of MAS-100®?

The MAS-100 samples the microbes by using the impaction principle. It is a user-friendly and precise air sampler for standard Petridishes. Housing and sampling head are made of aluminium.

The MAS-100® aspirates 100 liters of air per minute. Up to 2000 liters can be sampled per cycle.

For improved presicion the MAS-100® has a built-in mass anemometer to compensate for any variation in temperature, pressure, agar layer or other factors possibly affecting the sampling.

In which industries should MAS-100® be used?

All industries with high requirements for filtered/sterile/clean air, such as pharmaceutical, food, drink, cosmetic, semiconductor or any other highcare industry.

Other fields of use are environmental, military or research applications, as well as the control of aircondition filters in public buildings and offices etc.

Which official requirements for air monitoring do exist?

For pharma industry: the ISO 14698-1 (2003) defines microbial airmonitoring in pharmaceutical cleanrooms. Different cleanroom classes and the required limits of microbes/1000 liter air are defined in the European GMP (2003), in the USP 26 <1116> (2003) and the FDA draft guide (2003).

For food & Drink industry the HACCP concept is valid, whereby cleanroom classification may be adapted for example from the GMP guidelines.

Which level of microbes in the air is allowed?

According to European GMP in Cleanroom Class A max. 1 count per 1000 liters is allowed. Other areas like Cleanroom Class D allows max. 200 counts.

Compared to this in low-care or packaging areas a level of for example 1000 counts per 1000 liters may be defined, depending on the situation and requirements.

Which arguments do count for MAS-100®?

- Highest precision due to built-in mass sensor
- Aluminium housing and high-end modules for highest reliability
- Easy and reliable calibration
- Use of standard Petridishes for lowest running costs

Which detection principle is used?

The MAS-100® is an Impactor, that is based on the principle of the Andersen air sampler, which aspirates air through a perforated plate. This is the most frequently used airmonitoring method. The impaction velocity (speed at which airborne microorganisms hit the surface of the agar) is corresponding to stage 5 of the Andersen Sampler. This velocity guarantees that all particles >1 µm are collected. The resultant airflow is directed onto a standard agar poured plate. After the collection cycle, the Petridish is incubated and the colonies are counted and corrected statistically by using a Feller table.

Can I trust in the precision of the system?

The MAS-100® is calibrated to 100 liters / minute. In contrary to probably all other airsamplers available, the "mass" of the air is measured by a built-in anemometer. This "standard liters" or "mass" is constant under all environmental conditions and thus independent of physical variables like the Temperature or the Pressure (due to wheather conditions or height above sealevel!). As these variables are compensated by the anemometer during the measurement, a precision of 2.5 % is achieved, probably not reached by any other comparable airsampler.

How can data be stored electronically?

Download to a PC can be done for proper and safe documentation of measurement data with MAS-100®, MAS-100® ISO and MAS-100® CG. The results of the bacterial counts are added at a later stage.

Which microbes are found usually?

In industrial production aeras there can often bacteria be found with human origin from the skin, like Staphylococci, Micrococci or Coryneforme bacteria, from water or aerosoles, like Pseudomonas, or common species from environment or soil, like Bacilli.

Which culture media are recommended?

The most often used medium is of course the CASO Agar, which is equivalent to Tryptic Soy Agar (colony count after 2-3 days incubation).

For yeasts and moulds the RBC (Rose bengal chloramphenicol agar) or DRBC (Dichloran rose bengal chloramphenicol agar) are used as well as the DG 18 Agar (Dichloran glycerol agar) or Maltextract Agar are recommended, especially for environmental or indoor studies (all yeasts and moulds counts after 5-7days of incubation).

For special applications selective agars may be used like the CCA (Chromocult coliform agar, in soft-drink industry). Blood Agar is occasionally used in hospital areas or blood banks etc.

Which evaluation or publication available?

Very interesting results, also evaluating the performance of other available airsamplers, have been published internationally:

MEIER, R. and ZINGRE, H.: Qualification of airsampler systems: The MAS-100®, Swiss Pharma 22 (2000) No. 1-2: page 15-21

The compliance with the requirements of the ISO 14698-1 has been documented in a written report of the CAMR institute.

IQ/OQ?

Especially the pharmaceutical industry has to do special documentation, showing what, why and how methods or instruments are used. For the MAS-100® a file is available on request, with all basic paperwork of use for the documentation.

What is done regarding calibration?

All airsamplers are provided with a detailed factory QC and calibration protocol. The airsamplers have an adjustable built-in calibration reminder, so that the required routine calibration check is not missed during use of the instrument.

How can recalibration be done?

For calibration the special DA-100 calibration unit is a high precision state-of-the-art electronic free-flying wheel anemometer with built-in display for airflow, airvelocity and temperature. This unit is used for the whole range of MAS-100® airsamplers (except CG). The authorized Merck dealers are offering a recalibration service for their customers.

Users can also purchase the DA-100 as separate item and perform recalibrations easily, precise and secure even on their own.

Which customers do trust in MAS-100®?

Among many thousands of satisfied customers worlwide, just a few references shall be mentioned here: Novartis, Roche, Eli Lilly, GSK, Schering, Coca-Cola, etc.

Can the airsampler be used in EX areas?

For the special requirements in EX-areas the MAS-100® Ex has been developed. It is an explosion-proof version, based technically on the MAS-100® airsampler. The housing is black, and fully sealed electrical compounds ensure safe operation of the instrument even in areas, where Ex-proof features are required for electrical and electronic equipment.

What can be done in production or sterility test isolators?

The MAS-100® ISO has been developed according to the special requirements in Isolators. It is a highly precise, modular system for stationary use in sterility test isolators, production isolators or other similar applications.

The MAS-100® ISO allows adaptation to all possible individual needs: depending on the special requirements, the air inlet and outlet can be located nearby eachother, or in completely different rooms. A special, automatically working Valve unit allows complete and convenient hydrogen peroxide sterilization together with the Isolator. The Aspiration unit is based on the well-known MAS-100®, to ensure precise sampling of up to 2000 liters air. The Control unit allows data management from another room, allows to control up to 4 sampling heads, print-out of data, remote control, or even connection to a PC.

What can be used in special producion areas in food or drink industry?

The MAS-100® Eco is an attractive, low-budget version made for cleanrooms in food & drink industry. Since customers are increasingly health-concious and demanding the food manufacturers for low or even no preservatives in food products, the cleanroom technology is coming up in the relevant industries and leading to improved microbial airsampling. Like the other MAS-100® airsamplers the Eco is using standard Petridishes. An airflow of 100 liters/minute is used to sample up to 1000 liters. A lightweight carry bag is available as separate item, for ease of use in all areas of food and drink production.

How can compressed gases be tested acc. to ISO 14698-1

The new MAS-100® CG EX is of use for all compressed gases used in industry, to ensure proper measurement of any microbes. Based on the use of standard Petridishes and the typical airflow of 100 liters/minute, the different gases are recognized automatically (!) and sampled in a way, ensuring that any potentially available microbes in the gas are recovered carefully on the agar without loss of viability due to sudden pressure change. Examples of the long list of special features like Ex-proof, overpressure safety system, standard Tri-clamp, electronic pressure sensor etc. allow convenient and safe microbial monitoring of compressed gases.

MAS-100® airsampler

Precise and reliable microbial airsampler complete with accessories, for portable use in pharmaceutical and other cleanrooms, to count the microbes in a defined volume of air acc. to ISO, GMP, HACCP, FDA, USP etc. requirements.

The microbial airsampler system

The MAS-100® kit contains the MAS-100® microbial airsampler, Mains power supply unit, Built-in rechargeable NiMH battery pack, Operator manual (Different language versions available), Feller table, QC and Calibration-Certificate. All parts are delivered in a robust, handy case.

Features and benefits

- Housing and sampling head made of aluminium
- Convenient 2-buttons operation
- Reliable and robust Swiss-made instrument
- Airflow compensation for highest precision
- Low running costs by using Standard Petridishes
- 100 l/min acc. to ISO 14698
- Only 2.5 % deviation
- Isokinetic: no turbulence in laminar flow
- Sample volume programmable up to 2000 liters
- Runs 7.5 hours with built-in NiMH battery
- Built-in memory, PC-port
- Automatic calibration reminder

Experimental procedure

Open the sampling head and insert a standard Petridish.

The MAS-100® has built-in dialog software. Press "Yes" to switch on the MAS-100® or to accept a parameter or command. To ignore it, press the "No" button. The program works its way automatically through all the required parameters.

Once you have set the aspirated volume and delay, the program goes to the "Start ?" prompt. When you press "yes", the MAS-100® starts to sample the pre-set volume of air, and a green light indicates that the instrument is operating.

After the collection the red LED will illuminate and the total volume collected will be displayed.

Remove the Petridish and count the colonies after incubation.

Specification

Dimensions	26 x 11 cm
Application	Microbial air monitoring in pharma, food & drink, hospital and other cleanrooms.
Display	Alphanumeric liquid crystal display, 32 characters.
Working range	Free selection of volume of 1 - 2000 liters. Predefined Volume selectable.
Sampling head	Perforated aluminium lid with 400 holes, for standard 90/100 mm Petridishes.
Processor	Type 80C552.
Sensor	Hot-wire anemometer, numerical control.
Nominal airflow	100 liters / min. ± 2.5%.
Menu modes	Measurement: Manual mode, PC mode, Selectable mode. Settings: Data mode, Parameter mode, Anemometer mode.
Control buttons	1 YES-button and 1 NO-button for easy operation.
Ambient conditions	Temperature 0 to 40°C; Maximum relative humidity 80 percent for temperatures up to 31°C decreasing linearly to 50 percent relative humidity at 40°C.
Connections	Low-voltage socket for the MAS-100® power supply unit. PC interface for data transfer between system and a computer (cable as separate item). Tripod screw for connection with standard camera tripod.
Mains operation	Can be connected to the mains with the power supply unit: 110-240 Volt, 50-60KHz.
Portable operation	NiMH rechargeable 10 V battery pack, for 7.5 hours operation. Recharge cycle 3.5 hours.
Approvals	CE (EN 55022 Class B, EN 61000-4-2, ENV 50140, ENV 50204, EN 61000-4-4, ENV 50141), UL
Techn. revisions	Technical modifications in compliance with technical progress reserved.

MAS-100® airsampler

Literature

MEIER, R. and ZINGRE, H.: Qualification of airsampler systems: The MAS-100®, Swiss Pharma 22 No. 1-2: page 15-21, 2000

ANDERSEN, A.: New Sampler for the Collection, Sizing, and Enumeration of Viable Airborne Particles, U.S. Army Chemical Corps Proving Ground, Dugway, Utah, 1958

FELLER, W.: An introduction to the probability theory and its application, John Wiley and sons Inc. New York, page 175, 1950

Ordering Information

Product	Merck Cat. No.	Pack contents
MAS-100® System	1.09090.0001	MAS-100® airsampler complete with accessories in carry case
Perforated lid	1.09088.0001	Extra perforated lid/sampling head
Dust cover	1.09084.0001	Extra dust cover for perforated lid
Software pack	1.09226.0001	Special cable for PC-connection
Tube adapter	1.09224.0001	For connection with flexible tube up to 1.5 m
Tripod 100-325 cm	1.09326.0001	Tripod
DA-100 anemometer	1.09228.0001	Digital anemometer for calibration of MAS-100®

MAS-100® EX

Precise and reliable microbial airsampler - in special Ex-proof version - complete with accessories, for portable use in pharmaceutical and other cleanrooms, to count the microbes in a defined volume of air acc. to ISO, GMP, HACCP, FDA, USP etc. requirements.

The microbial airsampler system

The MAS-100® EX kit contains the MAS-100® EX microbial airsampler, Mains power supply unit, Built-in rechargeable NiMH battery pack, Operator manual (Different language versions available), Feller table, QC and Calibration-Certificate, SEV ASE certificate of Swiss electrotechnical association. All parts are delivered in a robust, handy case.

Features and benefits

- Housing and sampling head made of aluminium black colour for electrical isolation
- EX proof version acc. DIN/VDE 0165 and prEN 50021
- Convenient 2-buttons operation
- Airflow compensation for highest precision
- Low running costs by using Standard Petridishes
- 100 l/min acc. to ISO 14698
- Only 2.5 % deviation
- Isokinetic: no turbulence in laminar flow
- Sample volume programmable up to 2000 liters
- Runs 7.5 hours with built-in NiMH battery
- Automatic calibration reminder

Experimental procedure

Open the sampling head and insert a standard Petridish.

The MAS-100® has built-in dialog software. Press "Yes" to switch on the MAS-100® or to accept a parameter or command. To ignore it, press the "No" button. The program works its way automatically through all the required parameters.

Once you have set the aspirated volume and delay, the program goes to the "Start ?" prompt. When you press "yes", the MAS-100® starts to sample the pre-set volume of air, and a green light indicates that the instrument is operating.

After the collection the red LED will illuminate and the total volume collected will be displayed.

Remove the Petridish and count the colonies after incubation.

Specification

Dimensions	26 x 11 cm, Black aluminium housing
Application	Microbial air monitoring in cleanrooms, especially in explosion risk areas.
Display	Alphanumeric liquid crystal display, 32 characters.
Working range	Free selection of volume of 1 - 2000 liters. Predefined Volume selectable.
Sampling head	Perforated aluminium lid with 400 holes, for standard 90/100 mm Petridishes.
Processor	Type 80C552.
Sensor	Hot-wire anemometer, numerical control.
Nominal airflow	100 liters / min. ± 2.5%.
Menu modes	Measurement: Manual mode. Settings: Data mode, Parameter mode, Anemometer mode.
Control buttons	1 YES-button and 1 NO-button for easy operation.
Ambient conditions	Temperature 0 to 40°C; Maximum relative humidity 80 percent for temperatures up to 31°C decreasing linearly to 50 percent relative humidity at 40°C.
Connections	Low-voltage socket for the MAS-100® power supply unit. Tripod screw for connection with standard camera tripod.
Mains operation	Can be connected to the mains with the power supply unit: 110-240 Volt, 50-60 KHz. Important note: Do NOT connect to mains in explosion risk areas!
Portable operation	NiMH rechargeable 10 V battery pack, for 7.5 hours operation. Recharge cycle 3.5 hours.
Approvals	CE (EN 55022 Class B, EN 61000-4-2, ENV 50140, ENV 50204, EN 61000-4-4, ENV 50141, prEN 50021), UL
Techn. revisions	Technical modifications in compliance with technical progress reserved.

MAS-100® EX

Literature

MEIER, R. and ZINGRE, H.: Qualification of airsampler systems: The MAS-100®, Swiss Pharma 22 No. 1-2: page 15-21, 2000

ANDERSEN, A.: New Sampler for the Collection, Sizing, and Enumeration of Viable Airborne Particles, U.S. Army Chemical Corps Proving Ground, Dugway, Utah, 1958

FELLER, W.: An introduction to the probability theory and its application, John Wiley and sons Inc. New York, page 175, 1950

Ordering Information

Product	Merck Cat. No.	Pack contents
MAS-100® EX System	1.09075.0001	MAS-100® EX airsampler complete with accessories in carry case
Perforated lid	1.09124.0001	Extra perforated lid/sampling head, black
Dust cover	1.09123.0001	Extra dust cover for perforated lid, black
Tube adapter	1.09224.0001	For connection with flexible tube up to 1.5 m
Tripod 100-325 cm	1.09326.0001	Tripod
DA-100 anemometer	1.09228.0001	Digital anemometer for calibration of MAS-100® EX

MAS-100 Eco®

Microbial airsampler based on the wellknown MAS-100® principle, for portable use in food & drink production or other cleanrooms, to count the microbes in a defined volume of air acc. to ISO or HACCP requirements.

The microbial airsampler system

The MAS-100 Eco® microbial airsampler is supplied with Mains power supply unit, Standard rechargeable NiMH batteries, Operator manual (Different language versions available), Feller table, QC and Calibration-Certificate.

Features and benefits

- Robust and easy to handle airsampler
- Convenient 2-buttons operation
- Low running costs by using Standard Petridishes
- 100 l/min acc. to ISO 14698
- Isokinetic: no turbulence in laminar flow areas
- Sample volume programmable up to 1000 liters
- Runs 7.5 hours with built-in NiMH battery
- Automatic calibration reminder

Experimental procedure

Open the sampling head and insert a standard Petridish.

The MAS-100 Eco® has built-in dialog software. Press "Yes" to switch on or to accept a parameter or command. To ignore it, press the "No" button. The program works its way automatically through all the required parameters.

Once you have set the aspirated volume and delay, the program goes to the "Start ?" prompt. When you press "yes", the MAS-100 Eco® starts to sample the pre-set volume of air.

After the collection the total volume collected will be displayed.

Remove the Petridish and count the colonies after incubation.

Specification

Dimensions	18 x 11 cm, silver aluminium housing with blue handle.
Application	Microbial air monitoring in food & drink and all other cleanrooms.
Display	Alphanumeric liquid crystal display, 16characters.
Working range	Free selection of volume of 10 - 1000 liters. Predefined Volume selectable.
Sampling head	Perforated aluminium lid with 400 holes, for standard 90/100 mm Petridishes.
Nominal airflow	100 liters / min. ± 4 %.
Menu modes	Measurement: User menu. Settings: Setup menu.
Control buttons	1 YES-button and 1 NO-button for easy operation.
Ambient conditions	Temperature 0 to 40°C; Maximum relative humidity 80 percent for temperatures up to 31°C decreasing linearly to 50 percent relative humidity at 40°C.
Connections	Low-voltage socket for the MAS-100® power supply unit.
Mains operation	Can be connected to the mains with the power supply unit: 110-240 Volt, 47-63 kHz. 4 plug adapters for worldwide use.
Portable operation	2 standard NiMH rechargeable batteries, for 3.5 hours operation. Recharge cycle 9 hours.
Approvals	CE (EN 50081-1:1992 + EN 50082-1:1997, EN 50081-2:1993 + EN 50082-2:1995 + prEN 500082-2:1996)
Techn. revisions	Technical modifications in compliance with technical progress reserved.

MAS-100 Eco®

Literature

MEIER, R. and ZINGRE, H.: Qualification of airsampler systems: The MAS-100®, Swiss Pharma 22 No. 1-2: page 15-21, 2000

ANDERSEN, A.: New Sampler for the Collection, Sizing, and Enumeration of Viable Airborne Particles, U.S. Army Chemical Corps Proving Ground, Dugway, Utah, 1958

FELLER, W.: An introduction to the probability theory and its application, John Wiley and sons Inc. New York, page 175, 1950

Ordering Information

Product	Merck Cat. No.	Pack contents
MAS-100 Eco® System	1.09227.0001	MAS-100 Eco® airsampler
Shoulder bag	1.09126.0001	Blue shoulder bag for MAS-100 Eco®
Tripod screw	1.09127.0001	Special screw adapter, allows usage with standard camera tripod

MAS-100 ISO®

Precise and reliable microbial airsampler with modular concept for stationary use in sterility test or production Isolators, to count the microbes in a defined volume of air acc. to ISO, GMP, HACCP, FDA, USP etc. requirements.

The microbial airsampler system

The MAS-100 ISO® is based on 1 to 4 airsamplers (built-up from moduls SH + VU + AU), controlled by 1 Control unit (CU). This modular concept avoids pumps, filters, vacuum or other inconvenient features, and is in fact a highly secure and precise system, perfectly dedicated for use in Isolators.

Features and benefits

- Based on MAS-100® concept
- Reliable and robust modular system
- Special Valve unit for secure hydrogenperoxide sterilization
- Airflow compensation for highest precision
- Low running costs by using Standard Petridishes
- 100 l/min acc. to ISO 14698, only 2.5 % deviation
- Isokinetic: no turbulence in laminar flow
- Sample volume programmable up to 2000 liters
- Built-in Memory, PC-port, Printer, Calibration reminder

Experimental procedure

The MAS-100® has a built-in dialog software. The Sampling head is within the Isolator, all other parts outside. Automatic driven security valves ensure convenient and proper hydrogen-peroxide sterilization of the modules SH and VU together with the Isolator.

Specification

Dimensions	CU and VU: 23.5 x 17 x 12 cm, AU: 23.5 x 25.4 cm, SH: 8.6 x 11 cm
Application	Microbial air monitoring in Isolators and other cleanrooms.
Display	Alphanumeric liquid crystal display, 32 characters.
Working range	Free selection of volume of 1 - 2000 liters. Predefined Volume selectable.
Sampling head	Perforated aluminium (SH-SA) or stainless stell (SH-SS) lid with 400 holes, for standard 90/100 mm Petridishes.
Tube connection	Standard ¾" Tri-clamp.
Sensor	Hot-wire anemometer, numerical control.
Nominal airflow	100 liters / min. ± 2.5%.
Menu modes	User menu, Setup menu, Service menu
Remote start	At the rear of the aspiration unit is a 4-pole connector for remote control of the unit.
Valves	Rigid PVC / Viton / SS.
Ambient conditions	Temperature 0 to 40°C; Maximum relative humidity 80 percent for temperatures up to 31°C decreasing linearly to 50 percent relative humidity at 40°C.
Power consumption	AU + VU = 0.25A (58V) when active, or 0.06A (12V) on standby
Mains operation	Connected to the mains with the power supply unit: 110-240 Volt.
Printer	Integrated matrix printer Citizen MD-911.
Approvals	CE (EN 61326-1: 1997 + A1:1998, EN 55022, Class A, EN 61000-4-2, EN 61000-4-3, EN 61000-4-4, EN 61000-4-5, EN 61000-4-6, EN 61000-4-11)
Techn. revisions	Technical modifications in compliance with technical progress reserved.

MAS-100 ISO®

Literature

MEIER, R. and ZINGRE, H.: Qualification of airsampler systems: The MAS-100®, Swiss Pharma 22 No. 1-2: page 15-21, 2000

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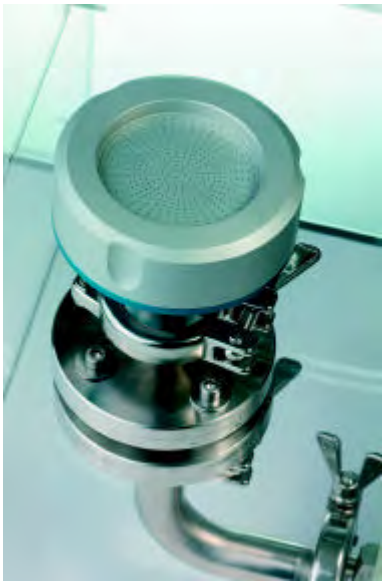

Ordering Information

Product	Merck Cat. No.	Pack contents
MAS-100 ISO® CU	1.09812.0001	Control unit with built-in printer
MAS-100 ISO® SH-SA	1.09816.0001	Sampling head, aluminium
MAS-100 ISO® SH-SS	1.09817.0001	Sampling head, stainless steel
MAS-100 ISO® VU	1.09814.0001	Valve unit
MAS-100 ISO® AU	1.09813.0001	Aspiration unit
Software pack	1.09819.0001	Special cable for PC-connection
Pressure test kit	1.09326.0001	Test kit, to ensure presseure tight connections
DA-100 anemometer	1.09228.0001	Digital anemometer for calibration of MAS-100®

MAS-100 CG Ex®

According to ISO norm 14698-1 not only the environment, but also compressed gases must be analyzed for the presence of microorganisms. Therefore a special MAS-100 CG Ex® airsampler had been developed. Parameters such as impaction speed, mass flow, the use of standard Petridishes and the handling of the firmware are based on the wellknown MAS-100® concept.

The MAS-100 CG Ex®

The MAS-100 CG Ex® is a high-performance instrument that is based on the principle of the Andersen air sampler, uses Standard Petridishes and collects the microorganisms fully automatically. The instrument operates with two sensors, one measuring the mass flow and the other one measuring the pressure. These two sensors calculate a constant flow of 100L/min over a range of 1.5 to 10 bar.

Features and benefits

- 4 compressed gas types pre-programmed: Air, nitrogen, carbon dioxide and argon
- Up to 10 gas types programmable
- Automatic adjustment of environmental pressure
- Automatic decompress cycle after sample collection
- Automatic calibration reminder selectable from 1 up to 12 months
- Sampling volumes individually programmable from 0 up to 2000 liters
- Password access for calibration parameters
- Fully automated sampling cycle
- Flashing LED if instrument is under pressure
- PC mode for programming test schedules
- Integrated mass flow and pressure sensors
- Uses standard 90 mm Petridishes
- Real time clock and calendar
- Easy handling of instrument
- Movable sampling head
- Security valve > 10 bar
- Battery operated
- Ex-proof version

Experimental procedure

The MAS-100 CG Ex® has an integrated dialog software. To accept a parameter or command, press the "Yes" button. To ignore it, press the "No" button. The program works in its way automatically through all the required parameters.

Once you have set the aspirated volume and delay time, the program goes to the "Start ?" prompt. When you press "Yes", the airsampler automatically starts to sample the pre-set volume of compressed air, automatically compensated for the gas type and possible pressure variation. A green light indicates that the instrument is operating.

Specification

General: Nominal flow rate:	100 liter / min. ± 5.0%, over the pressure range of 1.5 bar to 10 bar (absolute)
Sampling volume:	1 to 2000 liter, volumes individually selectable.
Pre-programmed gas types:	Air, nitrogen, carbon dioxide, argon
Sampling head: Head without clamps, height:	16.0 cm
Diameter:	10.0 cm
Weight:	1.5 kg
Material:	Anodized aluminum, clamps of stainless steel
Autoclavable:	20 minutes at 121°C
Tubing:	Length, 1.5 m ID=10 mm OD=19 mm, sterilize for 20 min at 121°C
Rapid connectors:	Chromium-plated brass
MAS-100 CG Ex® with handle: Height:	32.5 cm
Length:	37.0 cm
Width:	11.0 cm
Weight without sampling head:	10 kg
Trolley case:	Coated aluminum
Additional information: Battery pack:	20 cells NiMH, 3800 mAh, voltage 24 V
Battery charger:	110-240 Volt, 50-60 Hz
Charger input:	36 V DC, 1.5 A
Display:	Alphanumeric liquid crystal display, 32 characters
Lifetime RTC Battery:	Approx. 10 years
Flow valve:	Proportional, 24 Volt
Processor:	Type : 80C552
Gas regulation:	Mass flowmeter and pressure sensor 0-10 bar and proportional valve
CE approval:	EN 61000-6-1; 2001, EN 61000-6-3; 2001, EN 61000-6-2; 2001, EN 61000-6-4; 2001, EN 61326-1 + A1, 1998
Ex-proof:	SNCH 02 ATEX 3418, EN 1127; 1997, EN 50021; 1999

MAS-100 CG Ex®

Literature

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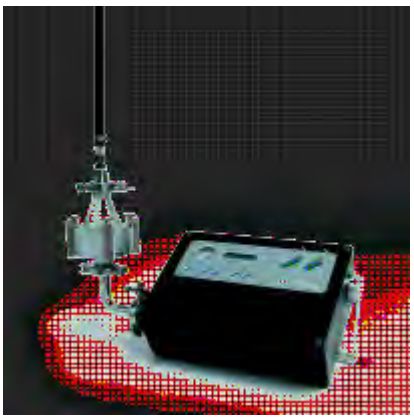


Ordering Information

Product	Merck Cat. No.	Pack contents
MAS-100 CG Ex®	1.09327.0001	Airsampler complete with accessories in trolley case
Sampling head	1.09231.0001	Additional sampling head for MAS-100 CG®

DA-100®

DA-100® calibration unit is a high precision state-of-the-art electronic free-flying wheel anemometer with built-in display for airflow, airvelocity and temperature.

The Digital calibration unit

The DA-100® is used for checking and recalibration of MAS-100® airsamplers like MAS-100®, MAS-100 Ex®, MAS-100 Eco® and MAS-100 ISO® (but not for MAS-100 CG® and instruments with firmware version < V2.62).

Features and benefits

- Highly precise and reliable
- Air flow indicated in liters/minute
- Air velocity indicated in meters/second
- Results within only about 30 seconds
- Handy and easy-to-use
- Battery operation
- Including recognized calibration certificate

Experimental procedure

The DA-100® digital anemometer is fixed onto an MAS-100® air sampler. The vane, which is positioned above the air inlet, rotates at a speed in response to the volume of the aspirated air. The values obtained are transmitted to an optical sensor. From the impulses, the sensor calculates the airflow in liters per minute and the air velocity in meters per second. The temperature of the air is measured by an integrated temperature sensor. The digital anemometer works on the principle of volume displacement and therefore displays volumes independently of pressure and temperature.

For highest precision the MAS-100®, MAS-100 Ex® and MAS-100 ISO® are calibrated by including the pressure into calculation, and by performing a 2-point calibration.

Specification

Accuracy at 100 l/min	± 1.0%
Height	8.5 cm
Diameter	11 cm
Weight	0.9 kg
Vane bearing	Magnetic (patent pending)
Material	Anodized aluminum
Battery pack	9V battery
Display	Alphanumerical LCD display, 2 x 8 characters
Temperature sensor	± 2.0°C
Temperature resolution	± 0.5°C
Ambient conditions	Temperature 0 to 40°C, Humidity 0 to 80% r.h.
CE tests	EN 50081-1:1992 + EN 50082-1:1997, EN 50081-2:1993 + EN 50082-2:1995 + prEN 500082-2:1996
Contents of kit	1 Robust, handy carry case 1 DA-100® calibration unit 1 Calibration certificate 1 Silicone ring for 2-point calibration of MAS-100 / Ex / ISO 1 User manual for DA-100

Literature

MEIER, R. and ZINGRE, H.: Qualification of airsampler systems: The MAS-100, Swiss Pharma 22 No. 1-2: page 15-21, 2000

Ordering Information

Product	Merck Cat. No.	Pack contents
DA-100® anemometer	1.09228.0001	Digital anemometer for calibration of MAS-100®

Envirocheck® Contact Slides (with flexible paddle) for surface- and liquid-testing

Envirocheck® Contact Slides are widely used to check critical control points (CCP's) within the HACCP (**h**azardous **a**nalytical **c**ritical **c**ontrol **p**oints) for the microbiological hygiene control of surfaces in production lines and equipment. Additionally these slides can be used to check liquids for the microbiological hygiene status.
Envirocheck® Contact Slides are packed in boxes of 10 slides, in polycarbonate container and sealed with a blew screw cap.

1. Unscrew cap on the tube and remove the Envirocheck® slide from the tube **taking care not to touch the agar surfaces**. Check for any dehydration or contamination before use.
2. Inoculation procedure
3. Labelling

   Enter the sample, source, date and time. **Indicate whether the sample has been taken before or after cleaning.**
4. Incubation

   Place the container in an upright position into an incubator, e.g. CULTURA Mini-Incubator 1.13311.0001 (230 V version) or 1.15533.0001 (110 V version) for 24-48 h at 35-37 °C (for bacteria) and/or for 2-7 days at 27-30 °C (for yeasts and moulds). However, alternative times and temperature for incubation may be used according to individual requirements.
5. Reading of results (colony counting)

   liquid testing:

   Remove the slide from the tube and compare with the density chart on the front page of the insert-sheet (upper chart for bacteria/yeasts and lower chart for moulds).

   surface testing:

   The recovery rate of viable organisms from surfaces with contact slides is about 50 %. The calculation for the colony count per cm2 has to be done as follows:

$$\frac{\text{number of colonies x 2}}{9}$$

   (number of colonies counted on one side; correction factor 2 to have 100 %; division by 9: surface area of the slide has 9 cm^2)
6. Disposal

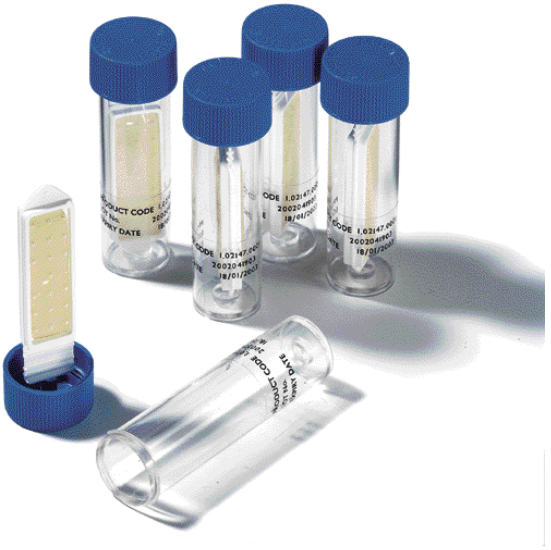
   Infected slides should be handled with care! The slides should be autoclaved, incinerated or soaked with disinfectant (e.g. 1.07551.2000 Extran® MA 04).

Surface testing

- With two fingers hold the terminal end of the paddle against the surface to be tested. Press down on the spike to bend the paddle still holding the slide by the cap.
- With a firm and even pressure press one medium against the surface to be tested. Take care not to smear the agar over the area.
- Repeat the procedure with the other side of the paddle on an area adjacent to the initial test site.
- Replace the slide back into the tube and close tightly.

Liquid testing

- Dip the Envirocheck® slide for about 5-10 seconds into the test fluid. Both agar surfaces should become totally covered. In case of insufficient liquid, pour over both surfaces of the slide.
- Tip slide on clean absorbant paper to drain off excess fluid.
- Replace the slide back into the tube and close tightly.



Envirocheck® Contact TVC (Total Viable Counts)

Envirocheck® Contact TVC is used to check for Total Colony Counts. One side has Nutrient Agar with 0.05 % TTC (Triphenyl Tetra-zolium Chloride) indicating bacterial growth in form of red colonies due to the formation of red formazan dye. The other side is coated with Nutrient Agar, a medium that is recommended by APHA (American Public Health Association) for testing of foodstuffs.

Typical Composition (g/litre)

side 1:	Nutrient Agar Meat extract 3.0; meat peptone 5.0; Agar Agar 12.0
side 2:	Nutrient Agar w/ TTC Meat extract 3.0; meat peptone 5.0; Agar Agar 12.0; TTC 0.05 (only in Nutrient Agar w/TTC)

pH: 7.0 ± 0.2 (at 25 °C)

Ordering Information

Product	Merck Cat. No.	Pack size
Envirocheck® Contact TVC (Total Viable Counts)	1.02149.0001	1 x 10 slides

Quality Control

Test strains	Nutrient Agar w/TTC	Nutrient Agar
Escherichia coli ATCC 8739	good growth	good growth
Staphylococcus aureus ATCC 6538	fair / good growth	good growth
Candida albicans ATCC 10231	no / poor growth	good growth
Pseudomonas aeruginosa ATCC 9027	good growth	good growth
Bacillus subtilis ATCC 6633	good growth	good growth
Aspergillus niger ATCC 16404	good growth	good growth

Envirocheck® Contact DC

Envirocheck® Contact DC is used to control the disinfection efficiency. For neutralizing a broad spectrum of antiseptic and disinfectants one side is coated with CASO Agar containing neutralizers: Tween® 80, lecithin, histidine and sodium thiosulfate.

Typical Composition (g/litre)

side 1:	CASO Agar 40.0
side 2:	CASO Agar 40.0; Tween® 80 5.0; lecitihin 0.7; sodium thiosulfate 0.5; histidine 1.0

pH: 7.3 ± 0.2 (at 25 °C)

Ordering Information

Product	Merck Cat. No.	Pack size
Envirocheck® Contact DC	1.02147.0001	1 x 10 slides

Quality Control

Test strains	CASO Agar	CASO Agar w/ neutralizers
Escherichia coli ATCC 8739	good growth	good growth
Staphylococcus aureus ATCC 6538	good growth	good growth
Candida albicans ATCC 10231	good growth	good growth
Pseudomonas aeruginosa ATCC 9027	good growth	good growth
Bacillus subtilis ATCC 6633	good growth	good growth
Aspergillus niger ATCC 16404	good growth	good growth

Envirocheck® Contact C

Envirocheck® Contact C is used to detect total coliforms / E. coli. For total counts coated with Plate Count Agar and for the specific differentiation of total coliforms from E. coli with Chromocult® Coliform Agar.

Typical Composition (g/litre)

side 1:	Plate Count Agar 22.5
	pH: 7.0 ± 0.2 (at 25 °C)
side 2:	Chromocult® Coliform Agar 26.5
	pH: 6.8 ± 0.2 (at 25 °C)

Ordering Information

Product	Merck Cat. No.	Pack size
Envirocheck® Contact C	1.02136.0001	1 x 10 slides

Quality Control

Test strains	Plate Count Agar	Chromocult® Coliform Agar
Escherichia coli ATCC 11775	good growth	good growth; colonies dark blue to violet; indole positive
Citrobacter freundii ATCC 8090	good growth	good growth; colonies salmon to red
E. coli 0157:H7 ATCC 35150	good growth	fair/good growth; colonies salmon to red; indole positive
Salmonella enteritidis ATCC 13076	good growth	good growth; colourless colonies

Envirocheck® Contact E

Envirocheck® Contact E is used to detect Enterobacteriaceae. For total counts coated with Plate Count Agar and on the other side VRBD Agar (Violet Red Bile Dextrose): the degradation of dextrose is accompanied by acid production which is indicated by a colour change to red and by zones of precipitated bile acids surrounding the colonies.

Typical Composition (g/litre)

side 1:	Plate Count Agar 22.5
	pH: 7.0 ± 0.2 (at 25 °C)
side 2:	VRBD Agar 39.5
	pH: 7.3 ± 0.2 (at 25 °C)

Ordering Information

Product	Merck Cat. No.	Pack size
Envirocheck® Contact E	1.02137.0001	1 x 10 slides

Quality Control

Test strains	Plate Count Agar	VRBD Agar
Escherichia coli ATCC 11775	good growth	fair / good growth
Staphylococcus aureus ATCC 6538	good growth	no growth
Salmonella typhimurium ATCC 14028	good growth	fair / good growth
Shigella sonnei ATCC 25931	good growth	fair / good growth

Envirocheck® Contact YM (R)

Envirocheck® Contact YM(R) is used for detecting yeasts and moulds. Rose Bengal Chloramphenicol Agar is recommended for the selective isolation and enumeration of yeasts and moulds from environmental materials and foodstuffs. Chloramphenicol inhibits the growth of bacteria additionally to Rose Bengal, which restricts the height and size of mould colonies so that slow-growing fungi are not overgrown by more rapidly growing species.

Typical Composition (g/litre)

side 1:	CASO Agar 40.0; TTC 0.05
	pH: 7.3 ± 0.2 (at 25 °C)
side 2:	Rose Bengal Chloramphenicol Agar 32.0
	pH: 7.2 ± 0.2 (at 25 °C)

Ordering Information

Product	Merck Cat. No.	Pack size
Envirocheck® Contact YM (R)	1.02139.0001	1 x 10 slides

Quality Control

Test strains	CASO Agar	Rose Bengal Chloramphenicol Agar
Escherichia coli ATCC 8739	good growth	no growth
Staphylococcus aureus ATCC 6538	fair / good growth	no growth
Candida albicans ATCC 10231	no / poor growth	good growth; pink colonies
Pseudomonas aeruginosa ATCC 9027	good growth	no / poor growth
S. cerevisiae ATCC 9763	no / poor growth	good growth
Aspergillus niger ATCC 16404	good growth	good growth; black after 5 days

Envirocheck® Contact plates (Ø 56 mm) for surface-testing

Envirocheck® Contact plates (Replicate Organism Detection and Counting) plates are widely used to check critical control points (CCP's) within the HACCP (hazardous analytical critical control points) for the microbiological hygiene of surfaces in production premises (GMP) or protected areas e.g. intensive care units.
Envirocheck® Contact plates are packed in boxes of 2 x 10 plates, triple cellophane wrapped and γ-irradiated at £12.5 Kgray to fulfill the requirement also for protected areas.

Experimental Procedure

1. Open the outer wrapping one before and one in the airlock entrance of the protected area - in the protected area remove the third wrapping.
2. Take off the lid and press the convex agar for 10 seconds onto the surface to be investigated ensuring an even pressure (25 g/cm^2) over the whole plate. Replace the lid and mark the plate with appropriate data.
3. Clean the sample area on the surface in order to remove any remainings of the agar.

Envirocheck® Contact TVC

mesophilic bacteria	68 ± 4 h	at 25-30 °C ± 1 °C
yeasts and moulds; (micromycetes)	minimum 7 days from 2nd day on controls for growth	at 25 °C ± 1 °C
environmental bacteria	68 ± 4 h in the dark afterward for 3 days	at 30 °C ± 1 °C at room temperature

Envirocheck® Contact Y + M

yeasts and moulds (micromycetes)	minimum 7 days from 2nd day on controls for growth	at 25 °C ± 1 °C

Results

Count the number of typical colonies on the contact plates directly after the specified incubation period and confirm these, if necessary, according to the microorganisms sought. Divide the number of characteristic colonies by the surface area of the plate. Report the count as a number of colony-forming units (CFU) per square centimetre of surface (acc. to ISO18593).

Storage / shelf life

see expiry date shown on the package; storage at room-temperature: +15 to +25 °C; avoid draught and temperature fluctuations.

Safe Disposal

Envirocheck® Contact plates should be burnt in an incinerator or sterilized in an autoclave. If this is not possible, put the plates in a disinfectant solution (e.g. EXTRAN® MA 04) overnight. It is recommended to use disinfectants that are recognized by official authorities in your country!

Ordering Information

Product	Merck Cat. No.	Pack size
EXTRAN® MA disinfectant	1.07551.2000	2 liter
CULTURA® Mini-Incubator (220-235 V)	1.13311.0001	1 ea
CULTURA® Mini-Incubator (100-110 V)	1.15533.0001	1 ea

Envirocheck® Contact plates TVC (Total Colony Count)

Envirocheck® Contact plates TVC is used to check for Total Colony Counts. In order to inactivate any residual disinfectants on the surface to be tested, the base medium (CASO Agar, Merck Cat. No. 1.05458.) additionally contains neutralizing agents.

Typical Composition (g/litre)

Peptone from casein 15.0; Peptone from soymeal 5.0; sodium chloride 5.0; Tween® 80 5.0; lecithin 0.7; sodium thiosulfate 0.5; L-histidine 1.0; Agar Agar 20.5.

pH: 7.3 ± 0.2 (at 25 °C)

Ordering Information

Product	Merck Cat. No.	Pack size
Envirocheck® Contact plates TVC	1.07084.0001	2 x 10 plates

Quality Control

Test strains	recovery rate
Staphylococcus aureus ATCC 6538	≥ 50 %
Bacillus subtilis ATCC 6633	≥ 50 %
Pseudomonas aeruginosa ATCC 9027	≥ 50 %
Candida albicans ATCC 10231	≥ 50 %
Asperigillus niger ATCC 16404	≥ 50 %

Envirocheck® Contact plates Y + M (Yeasts and moulds)

Envirocheck® Contact plates Y + M is used to check for yeasts and moulds. For the inhibition of accompanying bacterial flora the base medium (Malt Extract Agar, Merck Cat. No. 1.05398.) contains additionally chloramphenicol.

Typical Composition (g/litre)

Malt Extract Agar 45.0; Agar Agar 5.5; chloramphenicol 0.1

pH: 5.4 ± 0.2 (at 25 °C)

Ordering Information

Product	Merck Cat. No.	Pack size
Envirocheck® Contact plates Y + M	1.07088.0001	2 x 10 plates

Quality Control

Test strains	recovery rate
Candida albicans ATCC 10231	+
Saccharomyces cerevisiae ATCC 9763	+
Escherichia coli ATCC 25922	-
Staphylococcus aureus ATCC 25923	-
Enterococcus faecalis ATCC 19433	-

Envirocheck® Contact plates Blister

ENVIROCHECK® Contact plates in blister (snap pack) format

economical	**snap pack format enables the use of each single plate, while the other plates remain sterile !**
convenient	**storage at room-temperature (20 - 25 °C) !**
multifunctional	**double-wrapping and sterilization by irradiation allows usage also in sterile areas !**

Experimental Procedure

1. Open the outer wrapping in the airlock entrance of the protected area - in the protected area press the plate(s) out of the snap pack.
2. Take off the lid and press the convex agar for 10 seconds onto the surface to be tested ensuring an even pressure (25 g/cm^2) over the whole plate. Replace the lid and mark the plate with appropriate data.
3. Clean the sample area on the surface in order to remove any remainings of the agar.
4. Incubate the plate(s).

Storage / shelf life

see expiry date shown on the package; storage at room-temperature: +15 to +25 °C; avoid draught and temperature fluctuations.

Safe disposal

Envirocheck® Contact plates should be burnt in an incinerator or sterilized in an autoclave. If this is not possible, put the plates in a disinfectant solution (e.g. EXTRAN® MA 04) overnight. It is recommended to use disinfectants that are recognized by official authorities in your country!

Ordering Information

Product	Merck Cat. No.	Pack size
EXTRAN® MA 04 disinfectant	1.07551.2000	2 liter
CULTURA® Mini-Incubator (220-235 V)	1.13311.0001	1 ea
CULTURA® Mini-Incubator (100-110 V)	1.15533.0001	1 ea

Envirocheck® Contact plates Blister TVC w/neutralizers for Total Colony Count

The media composition is the same as Envircheck® Contact plates TVC (product code 1.07084.0001).

Ordering Information

Product	Merck Cat. No.	Pack size
Envirocheck® Contact plates Blister TVC w/neuralizers for Total Colony Count	1.07042.0001	6 x 5 plates

Envirocheck® Contact plates Blister Y + M w/chloramphenicol for yeasts and moulds

The media composition is the same as Envirocheck® Contact plates Y + M (product code 1.07088.0001).

Ordering Information

Product	Merck Cat. No.	Pack size
Envirocheck® Contact plates Blister Y + M w/chloramphenicol for yeasts and moulds	1.07044.0001	6 x 5 plates


Duopath®
VEROTOXINS
O
VT2
VT1
Be there
first!
New technologies will prevail.

Lateral Flow Tests

Singlepath® and Duopath® lateral flow tests reliable results in less time

Rapid testing?

World-wide food - and water borne infections of microbial origin are an important health challenge. The increasing occurrence in food borne illness calls for careful, efficient, rapid and reliable baseline monitoring for the presence of food borne pathogens. On the other hand significant increase in production costs or production delays as a consequence of testing would be commercially unacceptable.

Merck developed a new generation of assays for detecting of pathogens: Lateralflow tests which are essentially mobile mini-laboratories. Unequivocal results are available after as little as 20 minutes for *Salmonella, Campylobacter*, *E.coli* O157, its verotoxins 1 and 2 in one assay (Duopath®), and Listeria giving a customer upside of up to two days. Singlepath and Duopath form the second generation of lateral flow assays. They offer improved detection limits and easier reading of test results due to a more intensive colouring of the test line.

The attractive "rapid" pathogen test kits use the culture protocols of the standard methods and have the same standard of reliability. The unique combination of Merck's specially adapted enrichment media helps to guarantee precise, fast and reliable test results.

All Merck's Singlepath® and Duopath® lateral flow tests are **AOAC-RI** approved.

Automated or manual?

For rapid testing on the major food borne pathogens there is a choice between a manual test kit or an automated system. Normally automated systems are used in central laboratories where large numbers of pathogen test (> 50) are done per day. Choosing an automated system implies choosing for a capital investment in equipment of 10.000 to 50.000 Euro. Unfortunately automated systems do generally not offer great flexibility in the number of tests per run. When the number of test samples is markedly less than the capacity of a "walk away" processing run, there is an inefficient use of the run. Also for each "walk away" processing run, space must be allocated, and test kits used for calibration and validation. This leads to extra "hidden" costs. After the classical culture enrichment step the time to a result of automated systems ranges from up to 90 to 120 min. The detection levels may vary from 10^{3-5} cfu per ml for a PCR® based automated system to 10^{5-7} per ml for an immuno-assay based system.

When on a daily basis a variable number of pathogen test are conducted and the number of tests is less than 30 per day, Merck's Singlepath® and Duopath® are the methods of choice.

Figure 1:
Singlepath® Salmonella
Negative test - No red line at T with positive internal control
(red line at C)

Figure 2:
Singlepath® Salmonella
Positive test - Red line at T with positive internal control
(red line at C)

Lateral flow tests are ideal rapid test assays for pathogen and toxin detection. There is no instrument required, and there is no need for sample preparation after the classical culture. Just load a test kit by pipeting 0.16 ml culture from the appropriate standard enrichment medium. Walk away and read the test within 20 min. There is no need for calibration or validation of the test kit, hence no "hidden costs". Each lateral flow test device has an internal standard. A control red line (the internal control) demonstrates that the test kit functioned properly. If there is no red control line appearing within 20 min. the test kit is malfunctioning. When the test result is negative there is only the red control line. The occurrence of a clear red line within 20 min. at position T (Figure 1) indicates the presence of the pathogen sought. A positive proper functioning test gives two red lines; one at T position and one at C position (Figure2).

Using the lateral flow test gives a head start of up to two days in releasing products to the market place.

If you have already an automated pathogen detecting system?

The efficiency of your automated system is optimised when you use it in combination with Singlepath® and Duopath® lateral flow assays. Their incorporation in the routine direct pathogen testing compensates the disadvantages of using an automated system. Singlepath® and Duopath® offer what an automated system lacks: flexibility, ease of handling, and speed. If the number of samples is greater than a run, and/or if the sample arrive late: there is no need to complete a run, there is no need to set up controls, there is no need to wait 90-120 min to your test result when using Singlepath® and Duopath®. Using Singlepath® and Duopath® lateral flow tests you read the test result, for example, of up to 20 samples within 30 min. This is about the time it takes to bring the enrichments to the bench, to inoculate Singlepath® and Duopath®, to walk away for 15-20 min, and to start reading the test result.

Dedicated enrichment media

Using the many years of experience in the field of classical enrichment media Merck has precisely adjusted its various enrichment broths to suit the technology behind the lateral flow tests. For the detection of verotoxins a modified CAYE broth and a supplement is developed. The special selection of peptones in CAYE broth enhances the production of verotoxins. The new supplement of the CAYE broth further enhances the production and the release of verotoxins from toxin producing bacteria. As a result of this, the red detection line of the lateral flow test is clear and easy to read, the detection of verotoxins is more sensitive and less false negative results occur.

Singlepath® and Duopath® lateral flow tests

= Same safety standard as the classical detection method

> Wider product range
Lateral flow tests for the detection of Salmonella, Campylobacter, E.coli O157, Listeria and in one test verotoxin 1 and 2

+ Additional plus
Especially adapted media for precise and reliable results

The principle of lateral flow assay

Singlepath® and Duopath® lateral flow test is an immunodiagnostic assay in which the principles of immunity are applied to detect specifically a pathogen or toxin. Immunity is the ability of a host to resist intruders and infection. An essential element of immunity is that the host specifically recognises foreign molecules that are termed antigens. The host immune system reacts on the intrusion of such foreign molecules e.g. by production of immunoglobulins or antibodies. These react specifically with the antigen. The normal response of a host is that many B cells are stimulated to produce antibodies to a complex antigen. The antiserum that is produced consists of a mixture of antibodies and is polyclonal. For immuno-diagnostic procedures these types of antibodies are very potent but difficult to standardise. Monoclonal antibodies are products of single cells. A monoclonal antibody is generally highly specific for a single antigenic determinant.

Singlepath® and Duopath® is a sandwich ELISA (Enzyme Linked Immuno Sorbent Assay). In this method the antigen e.g. a pathogen or a toxin is trapped between layers of antibodies. The antigen can be a toxin molecule, or a marker molecule of a pathogenic subspecies, or a group of subspecies or serovars, for example *Salmonella* spp. In food, environmental and water samples the antigen *Salmonella* spp. occurs in low numbers. For immunodiagnostic detection a culture procedure step is required that enriches the antigen *Salmonella* spp. to a detectable level. In case of Singlepath® and Duopath® a 0.16ml of the enrichment culture is transferred to the sample window. This window gives access to a conjugate pad impregnated with an excess of gold labelled antibodies specific for *Salmonella* spp. The *Salmonella* spp. cells in the sample bind to gold-labelled antibody. The solution with gold-labelled antibody *Salmonella* spp. complexes migrate from the conjugate pad via a connecting pad towards the absorbent pad on the opposite end of the test device. The connecting pad of a Singlepath® contains two lines of catcher antibodies; one is the test or sample line (T), and the other is a internal control line (C). A Duopath® contains two test lines and a control line. The gold-labelled antibody- *Salmonella* spp. complexes migrate from the conjugate pad to the T line containing catcher antibodies. These react with gold-labelled antibody-*Salmonella* spp complexes, stopping the migration, and when sufficient gold-labelled antibody-*Salmonella* spp. complexes are bound to catcher antibodies a clear and sharp red line becomes visual within 20 min. If there is no sufficient gold-labelled antibody-*Salmonella* spp. bound there is no red line produced. The excess of gold-labelled antibody that is not bound to Salmonella spp. migrates past the test line (T) and reaches the control catcher antibody line (C). There the gold-labelled antibodies bind to the catcher antibodies producing a clear red line at C. A red line indicates that the test device is working properly.

The important features of an immunodiagnostic assay is the **specificity** and the sensitivity. Specificity is the ability of an antibody to recognise an antigen e.g. *Salmonella* spp. A high specificity implies that the antibody is specific for *Salmonella* spp. and will not cross react with any other antigen producing a **false positive** reaction for example with bacteria close to *Salmonella* spp. e.g. Hafnia alvei. Sensitivity defines the lowest amount of an antigen (*Salmonella* spp.) that can be detected. High sensitivity prevents **false negative** reactions.

The sensitivity of Singlepath® assays is 10^4 to 10^6 cfu of a target organism per ml.

The culture procedure is a crucial part of each immuno-diagnostic assay for the detection of pathogens, or bacterial toxin. False negative immuno-diagnostic test results are mostly due to the sue of a not optimal performing culture medium or mistakes in culture conditions. The best results are obtained when immuno-diagnostic assays are used in combination with dedicated culture media. These specifically designed enrichment media repair quickly sub-lethal injured target organisms, shorten the lag-phase, promote an optimal growth of the target organism in the log-phase, enhance the expression of antigenic molecules, and inhibit the growth of interfering microorganisms (antigens). The inoculation of enrichment medium direct from the refrigerated should be avoided. The manufacturers' instructions regarding time and temperature of incubation shoud be implemented to get a reliable detection of the target.

Your Benefits

Reliable	As sensitive as the official culture media method. Provides accurate test results.
Fast	Result in just 20 minutes.
Ease of Use	One step format avoids working errors during handling.
Convenient	Simmply add sample and read off the result.
Safe	Clear and distinctive positive or negative test results with buildt-in positive control.
Economical	Rapid results save labour and inventory costs and reduce labour intensive plating methods. No capital investment for example for instru-mentation.
Flexible	No need to complete a run.

Singlepath® E. coli O157

GLISA-Rapid Test (Gold Labelled ImmunoSorbent Assay) for the qualitative detection of E.coli O157 in food.

Intended Use

Singlepath® E. coli O157 is intended to be used in food-analysing laboratories for the presumptive qualitative detection of E. coli O157 (including H7) from a variety of foods. The test has been validated and received AOAC approval for use in raw ground beef and pasteurised whole milk from which levels as low as one E. coli O157 per 25 grams or ml of sample could be detected after 18 h enrichment.

Introduction

Amongst the E.coli human pathogens, Verotoxin (a Shiga like toxin) - forming strains (VTEC) have gained in importance in recent years. The group of enterohaemorrhagic E.coli (EHEC) with its highly pathogenic serovar O157:H7 strain is particularly interesting in this respect. EHEC are capable of initiating life-threatening illnesses, particularly in those with immune deficiency, young children and the elderly. The main sources of infection are contaminated, raw or insufficiently heated foods of animal origin, e.g. meat and dairy products. The reservoir for EHEC is the faeces of the cattle, sheep and goats. These microorganisms can enter food during the processing of meat and dairy products if hygienic conditions are inadequate.

The drastic increase in the incidence of food infection caused by E.coli O157 demands reliable and rapid methods of detection. Apart from traditional culture methods, immunological techniques are becoming more and more popular with users due to their better specificity and sensitivity.

Singlepath® E.coli O157 is an immunological screening test based on the immune flow principle and is designed for testing food and environmental samples in such a way that time-consuming and personnel intensive working steps are avoided.

Mode of Action

Singlepath® E.coli O157 (1.04141) is an immunochromatographic rapid test based on gold-labelled antibodies. The test device has a circular sample port, and an oval shaped test (T) and control (C) window.

1. The sample is applied to the chromatography paper via the circular sample port.
2. The sample is absorbed through the pad to the reaction zone containing colloidal, gold-labelled antibodies specific to E.coli O157.
3. Any E.coli O157 antigen present complexes with the gold-labelled antibody and migrates through the port until it encounters a binding zone in the test (T) area.
4. The binding zone (T) contains another anti-E.coli O157 antibody, which immobilises any E.coli O157-antibody complex present. Due to the gold-labelling, a distinct red line is then formed.
5. The rest of the sample continues to migrate to a second binding reagent zone within the control (C) zone, and also forms a second distinct red line (positive control). Regardless of whether any E.coli O157 is present or not, this distinct red line is always formed in the control (C) zone, thus ensuring the test is working correctly.

Storage / Stability

Singlepath® E.coli O157 is stable until the expiry date printed on the box, when stored at +2 to +8°C

Sample Material / Sample Enrichment

- Mix 25 g solid sample or 25 ml liquid sample with 225 ml enrichment medium 1 and homogenise with a Stomacher if necessary.
- Incubate for 18 - 24 h at +35 °C to +37°C.
- Allow to cool to room temperature

For dairy products, mTSB + Novobiocin selective enrichment broth (MERCK 1.09205.) is recommended.

For meat and meat products, mEC + Novobiocin selective enrichment broth (MERCK 1.14582.) should be used.

Test Procedure

Sample Preparation (optional boiling step)

The following boiling step is not mandatory for the test performance but can be done in order to reduce the potential risk of contamination when handling live bacteria.

1. Transfer approx. 1-2 ml of enrichment culture to an appropriate (polypropylene) tube. Cover with a loose-fitting cap.
2. Place tubes in boiling water bath for 15 min.
3. Remove and allow cooling to room temperature (18 - 26 °C), prior to use.

Allow test devices to warm to room temperature if stored at +2 to +8°C.

Procedure

1. Remove the foil pouches from the required number of Singlepath® E.coli O157 devices. Place the test device(s) on a flat surface and label with appropriate sample identification. (Note: Perform the tests within a period of 2 hours after opening!).
2. Using a micro pipette and disposable pipette tip, draw up 150 µl from the boiled or non-boiled and cooled enrichment. Dispense 150 µl of the sample into the circular sample port on the test device.
   Alternatively using a disposable transfer pipette, squeeze the pipette bulb, insert the stem into the boiled sample and release pressure on bulb. This will draw sample up into the pipette. Dispense five (5) free falling drops (about 150-160µl) into the circular sample port on the test device.

Singlepath® E. coli O157

3. Incubate the test device at room temperature and observe the test result immediately 20 minutes after applying the sample to the device.

Interpretation of Results

The test can be regarded as working correctly if a distinct red line appears in the control zone (C) within 20 minutes.

A sample can be considered POSITIVE if at or prior to 20minutes, red lines appear on both test (T) and control (C) zones.

A sample can be considered NEGATIVE if no red line appears in the test (T) zone but does appear distinctly in the control (C) zone 20 minutes after application of sample to the device.

Technical Specifications

Detection limit

1 colony-forming unit of E. coli O157 (including H7) in a 25gram food sample can be regarded as being the lowest detection limit. These levels meet the minimum detectable limits as defined by the AOAC Research Institute. These data were verified by an independent testing laboratory.

Interferences

Singlepath® E. coli O157 has been validated and received AOAC approval for use in raw ground beef and pasteurised whole milk. Results obtained to date on numerous food samples indicate that there is no interference of Singlepath® E.coli O157 with food ingredients.

The test has been developed based on using mEC + N and mTSB + N selective enrichment broths from MERCK and EHEC enrichment broth + cefixime, cefsulodin and vancomycin. Interference from other types of selective enrichment broths and other brands cannot be excluded. In particular use of broth of red-brown colour could potentially mask weak signals due to background coloration of the test zone.

Sensitivity (according to AOAC trials)	**>99 %**
Specificity (according to AOAC trials)	**>99 %**
False-negative rate	**<1 %**
False-positive rate	**<1 %**
Efficiency	**>99 %**

Trouble-shooting

Problem	Measures
No line appears in either zone after 20 minutes test period	Re-run sample

Precautions

E. coli O157 (including H7) isolates have been shown to be infective at very low dosage (<50 bacteria). Users of Singlepath® E. coli O157 must be familiar with the appropriate aseptic techniques for the isolation and identification of E. coli O157 (including H7). Extreme care must be kept in handling samples, enriched culture media and devices.

Disposal

Decontaminate Singlepath® devices, tubes, pipettes, and culture media by autoclave, bleach, etc. in accordance with local, state, and federal regulations.

Technical Assistance

For technical assistance, please contact your local Merck representative or Merck KGaA 64271 Darmstadt, Germany.
Tel : +49-6151-720, Fax : +49-6151-72 20 00,
Email: service@merck.de.

Ordering Information

Product	Merck Cat. No.	Pack size
Singlepath® E. coli O157	1.04141.0001	25 tests
mEC Novobiocin selective enrichment broth	1.14582.0500	500 g
mTSB Novobiocin selective enrichment broth	1.09205.0500	500 g

Additionally required materials and instrumentation

- Stomacher/Stomacher bags
- Incubators +35 °C and +42 °C
- Distilled or deionized water
- Autoclave
- Water bath for boiling of samples (optional)
- Disposable Polypropylene tubes for boiling of samples (optional)
- Disposable plastic transfer pipettes and/or appropriate micro pipettes and disposable tips for dispensing 1-2 ml (sample for boiling) and 150 µl (application of boiled sample onto tests)

Singlepath® E. coli 0157
Test result negative

Singlepath® E. coli 0157
Test result positive

Singlepath® Listeria

GLISA-Rapid Test (Gold Labelled ImmunoSorbent Assay) for the qualitative detection of Listeria spp. in food and environmental samples.

AOAC approval pending.

Introduction

Listeria are gram-positive, non-spore-forming, rod-shaped bacteria. Of the six known species of the Listeria genus, Listeria monocytogenes deserves particular mention as a human and animal pathogen, while L. ivanovii is pathogenic only in animals and L. innocua, L. seeligeri, L. grayi, and L. welshimeri are considered harmless environmental bacteria [1].

Listeriosis, the disease caused by L. monocytogenes, manifests itself not only as sepsis, but also and primarily as meningitis or even as encephalitis. Since L. monocytogenes is capable of crossing the placenta barrier, an infection of the pregnant mother with listeria constitutes a special risk for the fetus or result in the infection of the newborn child. As a result of the ubiquitous distribution of listeria and their capability to grow at refrigerator temperatures (+4° C to + 8° C), foods constitute one of the main sources of infection [1].

In the conduct of risk-related quality controls in foods and in the context of state-of-the-art hygiene-status monitoring procedures, tests should be run not only for L. monocytogenes, but also for the Listeria genus in general. The presence of listeria - in particular of L. innocua - is an indicator for critical hygienic conditions in the production process.

The drastic increase in the incidence of food infection caused by Listeria demands reliable and rapid methods of detection. Apart from traditional culture methods, immunological techniques are becoming more and more popular with users due to their better specificity and sensitivity.

Singlepath® Listeria is an immunological screening test based on the immune flow principle and is designed in such a way that time-consuming and personnel intensive working steps are avoided.

Mode of Action

Singlepath® Listeria (1.04142) is an immunochromatographic rapid test based on gold-labelled antibodies. The test device has a circular sample port, and an oval shaped test (T) and control (C) window.

1. The sample is applied to the chromatography paper via the circular sample port
2. The sample is absorbed through the pad to the reaction zone containing colloidal, gold-labelled antibodies specific to Listeria.
3. Any Listeria antigen present complexes with the gold-labelled antibody and migrates through the port until it encounters a binding zone in the test (T) area.
4. The binding zone (T) contains another anti-Listeria antibody, which immobilises any Listeria-antibody complex present. Due to the gold-labelling, a distinct red line is then formed.
5. The rest of the sample continues to migrate to a second binding reagent zone within the control (C) zone, and also forms a second distinct red line (positive control). Regardless of whether any Listeria is present or not, this distinct red line is always formed in the control (C) zone, thus ensuring the test is working correctly.

Storage / Stability

Singlepath® Listeria is stable until the expiry date printed on the box, when stored at +2 to +8°C.

Sample Material / Sample Enrichment

- Mix 25 g solid sample or 25 ml liquid sample with 225 ml of half-concentrated FRASER broth or bLEB or UVM-I broth and homogenise with a Stomacher if necessary.
- Incubate for 18 - 24 h at +28 - 30°C.
- Transfer 0,1 ml in 9.9 ml buffered LEB or Fraser broth or UVM-II broth.
- Incubate for 18-24 h at +28 - 30°C.

Experimental Procedure and Evaluation

Sample Preparation

1. Transfer approx. 1 - 2 ml enrichment culture to an appropriate (polypropylene) tube. Cover with a loose-fitting cap.
2. Place tubes in an incubator adjusted at 95 °C or boiling water bath for 15 min.
3. Remove and allow cooling to room temperature (18 - 26 °C), prior to use.

Allow test devices to warm to room temperature.

Procedure

1. Remove the foil pouches from the required number of Singlepath® Listeria devices. Place the test device(s) on a flat surface and label with appropriate sample identification. (Note: Perform the tests within a period of 2 hours after opening!).
2. Using a micro pipette and disposable pipette tip, draw up 150 µl from the boiled, cooled enrichment. Dispense 150 µl of the sample into the circular sample port on the test device.
   Alternatively using a disposable transfer pipette, squeeze the pipette bulb, insert the stem into the boiled sample and release pressure on bulb. This will draw sample up into the pipette. Dispense five (5) free falling drops (about 150-160µl) into the circular sample port on the test device.
3. Observe the test result within 25 minutes after applying the sample to the device.

Singlepath® Listeria

Interpretation of Results

The test can be regarded as working correctly if a distinct red line appears in the control zone (C) within 25 minutes.

A sample can be considered POSITIVE if at or prior to 25minutes, red lines appear on both test (T) **and** control (C) zones.

A sample can be considered NEGATIVE if no red line appears in the test (T) zone but does appear distinctly in the control (C) zone 25 minutes after application of sample to the device.

Technical Specifications

Detection limit

Depending on serogroup, approx. 1 x 10^6 bacteria/ml can be regarded as being the lower detection limit. Negative results may occur if the amount of antigen extracted is below the minimum sensitivity of the tests or if an incubation temperature above 30°C are used during enrichment or if the boiling step was omitted.

Interferences

Results obtained to date on numerous food samples indicate that there is no interference of Singlepath® Listeria with food ingredients.

The test has been developed based on using LEB and Fraser selective enrichment broth from MERCK. Interference from other types of selective enrichment broths and other brands cannot be excluded. In particular use of broth of red-brown colour could potentially mask weak signals due to background coloration of the test zone.

Singlepath® Listeria has demonstrated not to detect Listeria at lower concentration in raw beef sample/ground beef.

Sensitivity	**98 %**
Specificity	**100 %**

Trouble-shooting

Problem	Measures
No line appears in either zone after within 25 minutes test period	Re-run sample.

Ordering Information

Product	Merck Cat. No.	Pack size
Singlepath® Listeria	1.04142.0001	25 tests
FRASER Listeria selective enrichment broth (base)	1.10398.0500	500 g
FRASER Listeria Supplement	1.10399.0001	16 vials
Listeria enrichment broth, buffered (base) acc. to FDA/BAM 1995 (bLEB)	1.09628.0500	500 g
Listeria selective enrichment supplement acc. to FDA/BAM 1995	1.11781.0001	16 vials
UVM-Listeria selective enrichment broth modified	1.10824.0500	500 g
UVM-II Supplement	1.04039.0001	1 vial

Additionally required materials and instrumentation

- Stomacher / Stomacher bags
- Incubators +28 - 30°C
- Distilled or deionized water
- Autoclave
- Water bath or incubator for boiling of samples
- Disposable Polypropylene tubes for boiling of samples
- Disposable plastic transfer pipettes and/or appropriate micro pipettes and disposable tips
- Disposable inoculation loops

Singlepath® Listeria
Test result negative

Singlepath® Listeria
Test result positive

Singlepath® Campylobacter

GLISA-Rapid Test (Gold Labelled ImmunoSorbent Assay) for the qualitative detection of Campylobacter spp in food.

Introduction

Campylobacter spp. are now the leading cause of human enteritis in both the western world and developing countries. Recent infection with certain *C.jejuni* strains has also been associated with the debilitating neurological disorder, Guillain-Barre Syndrome (GBS) and reactive arthritis.

Campylobacter spp. are components of the intestinal flora of a wide range of wild animals and birds, farm animals and domestic pets. Human infection is mainly acquired from consumption of contaminated undercooked food, essentially, meats, poultry, shellfish and unpasteurised milk. Also, less commonly, some fruit and vegetables. However, infection can also be acquired from the environment. Water can become contaminated with animal and avian faeces, agricultural run-off and sewage effluent.

Campylobacter spp. are highly infective: as few as 500 bacteria are required to cause illness. *C.jejuni* and *C.coli* are the most common causative agents of human enteritis. C.lari and the emerging human pathogen, *C.upsaliensis*, have also been reported in a small percentage of cases. *C.fetus* infection is more rare, mainly systemic, especially in immunocompromised patients, and has been associated with abortion in humans.

The majority of Campylobacter spp. have low biochemical activity, therefore, identification is difficult on phenotypic characteristics. The standard detection method is enrichment for 48 hours in a microaerophilic environment, followed by isolation on selective agars for 48 hours in a microaerophilic environment. Results are therefore only available after 4- 5 days.

The Singlepath® Campylobacter test, however, greatly reduces the time-to-result. Following 48 hour enrichment, a result is obtained on the heat-killed sample within 20 minutes, thereby eliminating the isolation step. The need for microaerophilic enrichment can also be eliminated if the Sample Enrichment Protocol below is followed.

Singlepath® Listeria is an immunological screening test based on the immune flow principle and is designed in such a way that time-consuming and personnel intensive working steps are avoided.

Mode of Action

Singlepath® Campylobacter is an immunochromatographic rapid test based on gold-labelled antibodies. The test device has a circular sample port, and an oval shaped test (T) and control (C) window.

1. The sample is applied to the chromatography paper via the circular sample port.
2. The sample is absorbed through the pad to the reaction zone containing colloidal, gold-labelled antibodies specific to Campylobacter spp.
3. Any Campylobacter antigen present complexes with the gold-labelled antibody and migrates through the port until it encounters a binding zone in the test (T) area.
4. The binding zone (T) contains another anti-Campylobacter antibody, which immobilises any Campylobacter-antibody complex present. Due to the gold-labelling, a distinct red line is then formed.
5. The rest of the sample continues to migrate to a second binding reagent zone within the control (C) zone, and also forms a second distinct red line (positive control). Regardless of whether any Campylobacter is present or not, this distinct red line is always formed in the control (C) zone, thus ensuring the test is working correctly.
6. The rest of the sample continues to migrate to a second binding reagent zone within the control (C) zone, and also forms a second distinct red line (positive control). Regardless of whether any Listeria is present or not, this distinct red line is always formed in the control (C) zone, thus ensuring the test is working correctly.

Storage / Stability

Singlepath® Campylobacter is stable until the expiry date printed on the box, when stored at +2 to +8 °C.

Sample Material / Sample Enrichment

- Add 25 g solid sample to 225 ml Bolton enrichment broth in a 250ml Polystyrene Bottle or 25 ml liquid sample to 200ml Bolton enrichment broth in a 250ml Polystyrene Bottle.
- If necessary, transfer to the filter unit of a Stomacher bag and homogenise in Stomacher for 1 minute.
- Transfer homogenate back to 250ml Polystyrene Bottle, ensuring a headspace of 10 - 15 % is provided (see Note below).
- Discard the Stomacher bag and filter unit.
- Incubate for 4 h at 37°C.
- Transfer to 41.5°C and incubate for a further 44 h.

Note: If headspace is more than 15%, incubate enrichment culture microaerophilically, using a controlled atmosphere chamber, "Anaerocult" C gas packs (1.16275. or 1.13682.) can be used to generate this.

Experimental Procedure and Evaluation

Sample Preparation

1. Transfer approx. 1-2 ml enrichment culture to an appropriate (polypropylene) tube. Cover with a loose-fitting cap.
2. Place tubes in boiling water bath for 15 min.
3. Remove and allow cooling to room temperature (18- 26 °C), prior to use.

Allow test devices to warm to room temperature if stored at +2 to +8°C.

Singlepath® Campylobacter

Procedure

1. Remove the foil pouches from the required number of Singlepath Campylobacter devices. Place the test device(s) on a flat surface and label with appropriate sample identification. (Note: Perform the tests within a period of 2 hours after opening!
2. Using a disposable transfer pipette, draw up a sample from the boiled, cooled enrichment. If the broth contains sediment, such as horse blood, RESUSPEND the sediment before taking out the sample.
3. Dispense five (5) free falling drops (about 150 - 160µl) into the circular sample port on the test device. Alternatively using a micro pipette and disposable pipette tip, dispense 160 µl sample into the circular sample port on the test device.
4. Observe the test result 20 minutes after applying the sample to the device.

Interpretation of Results

The test can be regarded as working correctly if a distinct red line appears in the control zone (C) within 20 minutes.

A sample can be considered POSITIVE if at or prior to 20minutes, distinct red lines appear on both test (T) **and** control (C) zones.

A sample can be considered NEGATIVE if no red line appears in the test (T) zone but does appear distinctly in the control (C) zone 20 minutes after application of sample to the device.

Technical Specifications

Detection limit

Depending on serogroup, a range of approx. 10^4 - 10^7 bacteria/ml can be regarded as being the lower detection limit.

Interferences

Results obtained to date on numerous food samples indicate that there is no interference of Singlepath® Campylobacter with food ingredients.

The test has been developed based on using Bolton enrichments. Interference from other types of enrichment broth and other brands of Bolton broth cannot be excluded.

Sensitivity **98 %**
Specificity **100 %**

Trouble-shooting

Problem	Measures
No line appears in either zone after 20 minutes test period	If sediment has accidentally been deposited in the sampling well, try carefully scraping this off using a disposable inoculation loop **If unsuccessful, re-run sample avoiding sediment when sampling.**
Delay in sample reaching Nitrocellulose	Touch sample pad with pipette tip

Ordering Information

Product	Merck Cat. No.	Pack size
Singlepath® Campylobacter	1.04143.0001	25 tests
Anaerocult® C	1.16275.0001	1 x 25
Anaerocult® C mini *	1.13682.0001	1 x 25
Bolton Broth	1.00068.0500	500 g
Bolton Broth Selective Supplement	1.00079.0001	16 vials
Campylobacter blood free Selective agar Base (modified CCDA-Preston)	1.00070.0500	500 g
CCDA Selective Supplement	1.00071.0001	16 vials
Lysed Horse Blood		

* Optional

Additionally required materials and instrumentation

- Disposable 250ml Sterile Polystyrene Bottles with flow-seal cap for enrichment
- Stomacher / Stomacher bags with net - lined inserts
- 5 ml disposable tubes; 2 ml and 200 ml disposable pipettes
- Incubators +37 °C and +41.5°C
- Destilled or deoinized water
- Waterbath for boiling of samples
- Disposable Polypropylene tubes for boiling of samples
- Disposable plastic transfer pipettes and/or appropriate micro pipettes and disposable tips for dispensing 1-2 ml (sample for boiling) and 160 µl (application of oiled sample onto tests)
- Autoclave

Singlepath® Campylobacter Test result negative

Singlepath® Campylobacter Test result positive

Singlepath® Salmonella

GLISA-Rapid Test (Gold Labelled ImmunoSorbent Assay) for the presumptive qualitative detection of *Salmonella spp* in food.

Intended Purpose

Singlepath® Salmonella GLISA test is an immunochromatographic rapid test intended for use in microbiology laboratories analyzing food and animal feeds for the presumptive qualitative detection of *Salmonella spp.* from food matrices such as meats (raw ground beef and raw ground turkey), spices (black pepper), dairy (dried skimmed milk), dried foods (coconut) and seafood (cooked, peeled frozen prawns).

Introduction

Salmonella is one of the most common causes of food poisoning world-wide. Salmonella have been isolated from most types of raw food (meats, eggs, as well as plant products), and their high resistance to drying combined with a very high heat resistance once dried, makes Salmonella a potential problem in most foods and particular in dried products.

Screening for the presence of Salmonella in foods by conventional methods involve a 3-step technique: non-selective pre-enrichment (18- 24h), selective enrichment in (at least) two different selective broth media (24- 48h) followed by plating on (at least) two different selective/indicative agars (24 - 48h).This leads to a total time for Yes/No screening result of up to 5 days. For products where a positive release system is enforced, this means a delay of 5 days before release of the product.

The Singlepath® Salmonella test is an immunological screening test performed from only one selective enrichment culture, and gives a Yes/No answer in 20 minutes, meaning that products can be released 2 days earlier than by conventional microbiology.

Mode of Action

Singlepath® Salmonella is an immunochromatographic rapid test based on gold-labelled antibodies. The test device has a circular sample port, and an oval shaped test (T) and control (C) window.

1. The sample is applied to the chromatography paper via the circular sample port.
2. The sample is absorbed through the pad to the reaction zone containing colloidal, gold-labelled antibodies specific to Salmonella spp.
3. Any Salmonella antigen present complexes with the gold-labelled antibody and migrates through the port until it encounters a binding zone in the test (T) area.
4. The binding zone (T) contains another anti-Salmonella antibody, which immobilises any Salmonella-antibody complex present. Due to the gold-labelling, a distinct red line is then formed.
5. The rest of the sample continues to migrate to a second binding reagent zone within the control (C) zone, and also forms a second distinct red line (positive control). Regardless of whether any Salmonella is present or not, this distinct red line is always formed in the control (C) zone, thus ensuring the test is working correctly.

Storage / Stability

Singlepath® Salmonella is stable until the expiry date printed on the box, when stored at +2 to +8°C.

Sample Material / Sample Enrichment

- Mix 25 g solid sample or 25 ml liquid sample with 225 ml pre-enrichment broth (BPW) and homogenise for approximately 2 minutes in stomacher if necessary.
- Incubate for 18 ± 2 h at 37°C.
- Inoculate 10 ml RVS selective enrichment broth with 0.1 ml of pre-enrichment culture.
  Incubate for 24 ± 3 h at 41.5°C. Test procedure.

Experimental Procedure and Evaluation

Sample Preparation

1. Transfer approx. 1 - 2 ml of selective enrichment culture to an appropriate (polypropylene) tube.
2. Place tubes in boiling water bath for 15 min.
3. Remove and allow cooling to room temperature (18 - 26 °C), prior to use.

Allow test devices to warm to room temperature if stored at +2 to +8°C.

Procedure

1. Remove the foil pouches from the required number of Singlepath® Salmonella devices. Place the test device(s) on a flat surface and label with appropriate sample identification.
2. Using a micropipette and disposable pipette tip, dispense 160 µl into the circular sample port on the test device.
3. Observe the test result 20 minutes after applying the sample to the device.

Note: It is recommended to read results no later than 25 minutes after sample application, before the device starts to dry.

Interpretation of Results

The test can be regarded as working correctly if a distinct red line appears in the control zone (C) within 20 minutes.

A sample can be considered POSITIVE if at or prior to 20minutes, red lines appear on both test (T) **and** control (C) zones.

A sample can be considered NEGATIVE if no red line appears in the test (T) zone but does appear distinctly in the control (C) zone 20 minutes after application of sample to the device.

As with all rapid immunoassays, this method is presumptive. All positive results should be confirmed by plating the selective enrichments to the selective agars indicated in the ISO 6579:2002 or equivalent analysis methods such as USDA-FSIS MLG 4.02 method, and by analyzing typical isolated colonies using the biochemical and serological confirmatory techniques also recommended in this method.

Singlepath® Salmonella

Technical Specifications

Detection limit

Depending on serogroup, less than 1 colony forming unit in a 25g food sample can be regarded as being the detection limit.

Interferences

Results obtained to date on food samples, such as dried skimmed milk powder, black pepper, dried pet food (dog food), desiccated coconut and cooked peeled frozen prawns, indicate that there is no interference of Singlepath® Salmonella with these food ingredients.

The test has been developed based on using Merck media. Interference from components of other brands of media cannot be excluded.

Limitations of the Procedure

- The strength of signal is dependent on serogroup and the concentration of Salmonella cells.
- A positive or negative result does not preclude the presence of other infectious organisms.

Trouble-shooting

Problem	Measures
No line appears in either zone after 20 minutes test period	Re-run sample
Delay in sample reaching Nitrocellulose membrane	Touch sample pad with pipette tip
Blue-green colour appearing on membrane	In the rare event that dye from RVS medium reaches the test zone within 20 minutes, the colour does not interfere with the test signal

Precautions

Users of Singlepath® Salmonella must be familiar with the appropriate aseptic techniques for the isolation and identification of *Salmonella spp.* Care must be taken when handling samples, enrichments and devices.

Disposal

Decontaminate Singlepath® devices, enrichments, tubes and pipettes by autoclave, bleach etc in accordance with local, state, and federal regulations.

Technical Assistance

For technical assistance, please contact your local Merck representative or Merck KGaA 64271 Darmstadt, Germany.
Tel : +49-6151-720, Fax : +49-6151-72 20 00,
Email: service@merck.de.

Ordering Information

Product	Merck Cat. No.	Pack size
Singlepath® Salmonella	1.04140.0001	25 tests
Peptone Water (Buffered)	1.07228.0500	500 g
Salmonella Enrichment Broth acc. to Rappaport-Vassiliadis (RVS broth)	1.07700.0500	500 g

Additionally required materials and instrumentation

- Stomacher / Stomacher bags
- Incubators +37 °C and +41.5°C
- Distilled or deionized water
- Autoclave
- Waterbath for boiling of samples
- Disposable heat-stable Polypropylene tubes for boiling of samples
- Disposable plastic transfer pipettes and/or appropriate micro pipettes and disposable tips for dispensing 1 - 2 ml (sample for boiling) and 160 µl (application of boiled sample onto tests)

Singlepath® Salmonella
Test result negative

Singlepath® Salmonella
Test result positive

Singlepath® Salmonella

Singlepath® Salmonella

Strains that were tested and detected positive:

Salm. paratyphi A ATCC 9150	Salm. kentucky ATCC 9263	Salm. unnamed ssp.II Serotype: 11;-;1,5	Salm. karamoja
Salm. derby ATCC 6960	Salm. gallinarum ATCC 9184	Salm. friedenau	Salm. sheffield
Salm. abortus-equi ATCC 9842	Salm. pullorum ATCC 19945	Salm. luanshya ssp.II	Salm. wandsworth
Salm. typhimurium ATCC 6994	Salm. panama ATCC 7378	Salm. warragul	Salm. waycross
Salm. paratyphi B ATCC 8759	Salm. dublin ATCC 15480	Salm. zwickau ATCC 15805	Salm. unnamed ssp. III Serotype :42;z41,z24;
Salm. typhimurium ATCC 14028	Salm. enteritidis ATCC 13076	Salm. kirkee ATCC 8822	Salm. irigney
Salm. bredeney ATCC 10728	Salm. javiana ATCC 10721	Salm. fluntern	Salm. lohbruegge
Salm. chester ATCC 11997	Salm. maarssen ATCC 15793	Salm. infantis ATCC 51741	Salm. deversoir
Salm. infantis ATCC 51741	Salm. anatum ATCC 9270	Salm. london ATCC 9389	Salm. quinhon
Salm. bareilly ATCC 9115	Salm. matroosfontein	Salm. eschersheim	Salm. ngozi ssp. II
Salm. choleraesuis ATCC 12011	Salm. vejle	Salm. schalkwijk ATCC 15785	Salm. bonaire ssp. IV
Salm. choleraesuis ATCC 13312	Salm. butantan	Salm. minnesota ATCC 9700	Salm. arizonae NCTC 8297
Salm. newport ATCC 6962	Salm. illinois ATCC 11646	Salm. pomona ATCC 10729	Salm. uccle
Salm. breukelen ATCC 15782	Salm. westerstede	Salm. kitenge ATCC 19126	
Salm. düsseldorf	Salm. chittagong	Salm. morningside	
Salm. münchen ATCC 8388	Salm. yehuda	Salm. arizonae ssp. III	

This list represents Salmonella strains from most food-relevant serogroups. However, it cannot be ruled out that Salmonella strains from other serogroups may not be detectable.

Duopath® Verotoxins

GLISA-Rapid Test (Gold Labelled ImmunoSorbent Assay) for the qualitative detection of Verotoxins from Verotoxinogenic E. coli isolated from food enrichments.

IVD *in vitro diagnosticum –*
For professional use only

See also General Instruction of Use
Warnings and precautions see ChemDAT®
(www.chemdat.info)

Intended Purpose

The Duopath® Verotoxins GLISA test is an immunochromatographic rapid test intended to be used in food-analysing laboratories for the qualitative detection of Verotoxins (Shiga-like toxins) 1 and 2 from Verotoxinogenic E. coli (including E.coli O157:H7) isolated from food enrichments using FDA, USDA or other food enrichment methods. This test has been validated and received AOAC approval for detection of Verotoxins 1 and 2 from isolated Verotoxin-producing E.coli (including E. coli O157:H7).

Duopath® Verotoxin is also intended to be used in clinical laboratories for the qualitative identification of Verotoxins 1 and 2 (Shiga-like toxins 1 and 2) produced by E. coli isolated in cultures derived from clinical stool specimens. The identification aids in the diagnosis of diseases caused by enterohemorrhagic E.coli infections.

Introduction

Among the E. coli human pathogens, Verotoxin (Shiga-like toxin) forming strains (VTEC) have gained in importance in recent years. The group of enterohaemorrhagic E. coli (EHEC) with its highly pathogenic serovars 0157:H7, 026, 0103, 0111, 0145, and other strains are of particular concern. Production of Verotoxins is the most common criteria for the detection of this group of bacteria. Verotoxins can be classified into two main categories Verotoxin 1 (VT1, SLT1, Stx1) and Verotoxin 2 (VT2, SLT2, Stx2). EHEC strains may produce either VT1 or VT2 only or both VT1 and VT2 simultaneously. EHEC are capable of initiating life threatening illnesses, particularly in those with immune deficiency, young children and the elderly. The main sources of infection are contaminated, raw or insufficiently heated foods of animal origin, e.g. meat and dairy products. The reservoir for EHEC is the feces of cattle, sheep and goats. These microorganisms can enter food during the processing of meat and dairy products if hygienic conditions are inadequate. The drastic increase in the incidence of food infection caused by E.coli 0157 demands reliable and rapid methods of detection. In addition to traditional culture methods, immunological techniques are becoming more useful due to their improved specificity and sensitivity. Duopath® Verotoxins is an immunological screening test based on the immune flow principle.

Typical Composition

Package contents:

25 test devices individually packaged in aluminum foil. Each device consists of a plastic housing with 2 ports which encloses and protects the pads containing test reagents.

Reagent components of the test device:

1. Membrane associated Mouse monoclonal anti-VT1 antibody
2. Membrane-associated mouse monoclonal anti-VT2 antibody
3. Membrane-associated goat polyclonal anti-mouse antibody
4. Gold-labeled mouse monoclonal anti-VT1 and anti-VT2 antibodies
5. Buffer
6. Blocking agents

Storage and shelf-life after first opening

Duopath© Verotoxins is stable until the expiry date printed on the box when stored at +2 to +8°C. The test device should be used with 2 hr after removal from the sealed foil pouch. Tests should not be used if the foil pouch is torn or has been previously opened. If no red line appears in the poitive control zone C during a test the test device is not working properly and should be discarded.

Specimen Collection and Handling

Appropriate stool specimens may be held at controlled room temperature for up to 4 h prior to preparing cultures. Stool specimens that cannot be cultured within 4 hours should be placed at 2 - 8°C and cultured within 24 hours. If the specimens cannot be cultured within 24 h they should be frozen at -70°C as soon as possible after receipt.

Sample Preparation

Stool

Using a swab inoculate stool samples onto Sorbitol-MacConkey agar plates containing no tellurite or cefixime. Incubate for 18-24 h at 35°C. Using a swab, sweep a few times across the confluent growth area of the plate avoiding mucoid colonies. Mucoid colonies may interfere with migration of the sample. Dacron swabs are preferred to cotton swabs since less liquid is retained by the Dacron swabs for subsequent testing. Suspend the swab in 0.5 ml distilled water containing 50 µg/ml polymyxin B to enhance the release of Verotoxins from VTEC. Incubate the mixture for 30 minutes at 35°C.

Duopath® Verotoxins

Isolated bacteria from food

Pick 1 - 5 colonies from SMAC, CT-SMAC or Brain Heart Infusion (BHI) agar and dispense it in 1 ml CAYE broth containing CAYE broth supplement C.

Incubate for 6 h at +37 °C.

Pipette 180 µl CAYE-culture into a Eppendorf Cup.

Dissolve powder in Polymyxin B vial with 1 ml sterile distilled H_2O, the add 20 µl of Polymyxin B solution (concentration: 5 mg/ml) to the 180 µl CAYE culture and mix.

Incubate the Eppendorf Cup for 10 min. at 35 - 37°C

Allow to cool to room temperature.

Isolation of E. coli O157 from foods

Mix 25 g solid sample or 25 ml liquid sample with 225 ml enrichment medium 1 and homogenise with a Stomacher if necessary.

Incubate for 18 - 24 h at +42°C (mEC + N) or at +35 - 37°C (mTSB + N).

Inoculate CT-SMAC Agar with an aliquot from the enrichment broth.

Incubate for 18 - 24 h at +35 to +37°C.

Pick up 1 - 5 typical colonies from the CT-SMAC Agar and dispense it in 1 ml of the CAYE broth containing CAYE broth supplement C.

Incubate for 6 h at +37 °C.

Pipette 180 µl CAYE-culture into a Eppendorf Cup.

Add 20 µl Polymyxin B solution (preparation see above) and mix.

Incubate the Eppendorf Cup for 10 min. at 35-37°C.

Allow to cool to room temperature.

For dairy products, mTSB + Novobiocin selective enrichment broth (MERCK 1.09205.) is recommended.

For meat and meat products, mEC + Novobiocin selective enrichment broth (MERCK 1.14582.) should be used.

Only E.coli O157 serovars are able to grow on CT-SMAC Agar. To detect Verotoxins of other serovars, CT-SMAC agar must be replaced by SMAC or BHI agar.

Test Procedure

Stool

Prior to use allow the enriched sample and the required number of foil sealed test devices to reach room temperature (+15 to +25 °C).

Remove the foil pouches from the required number of Duopath® Verotoxins devices.

Place the test device(s) on a flat surface and label with appropriate sample identification. Note: Perform the test within 2 h of opening.

Gently swirl the polymyxin B sample to mix.

Using a micropipetter and disposable pipette tip, draw up 200 µl and dispense it into the circular sample port on the test device. Alternatively using a disposable transfer pipette, squeeze the pipette bulb, insert the stem into the sample tube and release pressure on the bulb. This will draw sample up into the pipette. Dispense six (6) free falling drops into the circular sample port on the test device (about 190 µl). Incubate at room temperature for 10 min and read immediately.

Food

Remove the foil pouches from the required number of Duopath® Verotoxins devices. Place the test device(s) on a flat surface and label with appropriate sample identification. (Note: Perform the tests within a period of 2 hours after opening!).

Briefly stirr the Eppendorf Cup with a Vortex mixer.

Using a micro pipette and disposable pipette tip, draw up 150 µl and pipette it into the circular sample port on the test device.

Alternatively using a disposable transfer pipette, squeeze the pipette bulb, insert the stem into the Eppendorf Cup and release pressure on bulb. This will draw sample up into the pipette. Dispense five (5) free falling drops (about 150 µl) into the circular sample port on the test device.

Incubate the test at room temperature (+15 to 25°C) and observe the test result immediately 20 minutes after applying the sample to the device.

Methodology

Principle of the method

Duopath® Verotoxins (1.04144.) is an immunochromatographic rapid test utilizing monoclonal antibodies which are labeled by red-colored gold particles. The test device has a circular sample port and an oval shaped test (VT1, VT2) and control (C) window.

1. The sample is applied to the chromatography paper via the circular sample port.
2. The sample is absorbed through the pad to the reaction zone containing colloidal, gold labeled antibodies specific to Verotoxins.
3. Any Verotoxin (VT1 and VT2) antigen present complexes with the gold-labeled antibody and migrates through the pad until it encounters the binding zones in the test (VT1, VT2) area.
4. The binding zones (VT1 and VT2) contain another anti VT1 or VT2 antibody, which immobilizes any Verotoxin antibody complex present. Due to the gold labeling, a distinct red line is then formed.
5. The remainder of the sample continues to migrate to another binding reagent zone within the control (C) zone, and also forms a further distinct red line (positive control). Regardless of whether any Verotoxin is present or not, a distinct red line is always formed in the control (C) zone and confirms that the test is working correctly.

Duopath® Verotoxins

Interpretation of Results

The test is working correctly if a distinct red line appears in the control zone (C) within the read time of 10 - 20 min. A sample can be considered POSITIVE if at or prior to the read time, red lines appear on test (VT1 and/or VT2) and control (C) zones. A sample can be considered NEGATIVE if no red line appears in the test (VT1and VT2) zones but does appear distinctly in the control (C) zone within 10 - 20 min after application of sample to the device. The result line must be red to be considered Positive. A dark line which is not red should be considered Negative. A positive result indicates that Verotoxin 1 and/or Verotoxin 2 (Shiga-like toxins) from E. coli were detected in the sample. A negative result indicates that neither Verotoxin 1 nor Verotoxin 2 was detected in the sample.

Detection Limit

One colony is the lower detection limit. The lower limits of detection are 25 ng/mL for VT1 and 62.5 ng/mL for VT2.

Interferences

Detection of Verotoxins from E. coli O157 isolated from foods has been successfully tested in different laboratories with food samples such as raw ground beef and pasteurised whole milk when the above described protocol was used. Results obtained to date with numerous E. coli isolates indicate that there is no interference of Duopath® Verotoxins with non-Verotoxinogenic E. coli or food ingredients. Duopath Verotoxin has been validated and AOAC approved for use of bacteria isolated from food enrichments using FDA, USDA or other food enrichment methods.

The test has been developed based on using CAYE medium from MERCK. Interference from other types of CAYE medium and other brands cannot be excluded. In particular use of broth of red-brown colour could potentially mask weak signals due to background coloration of the test zone.

Limitations of the Procedure

- The Duopath® Verotoxins assay detects the presence of Shiga-like toxin from culture isolates. The level of toxin has not been shown to be correlated with either the presence or severity of disease.
- The performance of this assay has not been evaluated with direct stools and enrichment broth testing.
- A positive result does not preclude the presence of other infectious organisms.
- Overgrowth of normal fecal flora could mask sorbitol negative colonies.
- Toxin expression may be lost upon serial passage. Colony sweeps may increase the likelihood of detecting Shiga toxin producing organisms.
- Enteric media other than Sorbitol/MacConkey (SMAC) has not been tested for use with this assay.

Trouble-shooting

Problem	Action
No line appears in Control zone after 10 minutes test period.	Re-run sample

Performance Characteristics

Stool specimen

REPRODUCIBILITY:

Three independent laboratories tested three samples in triplicate, on each of three different times in one day (intra-assay variability) and on three different days (inter-assay variability). Samples consisted of three negative, three low positive and three strong positive. The Duopath® Verotoxins produced 100% reproducibility including control lines.

SPECIFICITY:

The specifictiy of Duopath® Verotoxins was tested with the following clincial isolates which were inoculated onto SMAC plates and followed by polymyxin B extractions.

Microorganisms (number of strains tested)

Pseudomonas aeruginosa (10)

Klebsiella pneumoniae (10)

Enterobacter species (10)

Proteus species (10)

Non-Stx-producing E. coli (10)

Aeromonas species (3)

Serratia marcescens (5)

Shigella species (3)

None of the above isolates cross-react with the Duopath© Verotoxins

ASSAY SENSITIVITY (WITH STOCK CULTURES):

The following 40 STEC stock cultures were cultivated on SMAC plates and followed by the polymyxin extraction.

Number of Strains Tested	Serotype
32	O157:H7
1	O96:H9
1	O111:NM
2	O26:H11
1	O103:H2
1	O145:NM
1	O45:H2
1	O45:NM

All of the above isolates produced positive reactions on Duopath® Verotoxins Test Devices.

Duopath® Verotoxins

Performance Data

The Duopath® Verotoxin test was evaluated in the United States and the tested specimens included 249 fresh stools and 41 Shiga-toxin positive frozen stools.

DUOPATH® VEROTOXIN
Fresh Stool Specimen

Reference Method*	Positive	Negative	Totals
Positive	5	0	5
Negative	1**	243	244
Totals	6	243	249
% agreement +	100 % 41/41		
% agreement -	99.6% 243/244		

DUOPATH® VEROTOXIN
Frozen Stool Specimen

Reference Method*	Positive	Negative	Totals
Positive	41	0	41
Negative	0	0	0
Totals	41	0	41
% agreement +	100 % 41/41		
% agreement -	No negativ results		

* Premier EHEC (Meridian Bioscience, Inc.)

** E. coli O157:H was recovered from the culture but was not detected by the reference method.

Foods

Sensitivity	VT1	>99 %	VT2	>99 %
Specificity	VT1	>99 %	VT2	>99 %

Precautions

E. coli O157 (including H7) isolates have been shown to be infective at very low dosage (<50 bacteria). Users of Duopath® Verotoxin must be familiar with the appropriate aseptic techniques for the isolation and identification of E. coli O157 (including H7). Extreme care must be kept in handling samples, enriched culture media and devices.

Disposal

Decontaminate Duopath® devices, tubes, pipettes, and culture media by autoclave, bleach, etc. in accordance with local, state, and federal regulations.

Technical Assistance

For technical assistance, please contact your local Merck representative or Merck KGaA 64271 Darmstadt, Germany.
Tel : +49-6151-720, Fax : +49-6151-72 20 00,
Email: service@merck.de.

Literature

C.H. Park, H.J. Kim, D.L. Hixon, and A. Bubert; Evaluation of the Duopath® Verotoxin Test for Detection of Shiga Toxins in Cultures of Human Stools; Journal of Clinical Microbiology 41, June 2003, p. 2650-2653

Ordering Information

Product	Merck Cat. No.	Pack size
Duopath® Verotoxins	1.04144.0001	25 tests
CAYE Broth mod. acc.to Evans	1.00060.0100	100 g
CAYE Broth Supplement C	1.00051.0001	16 vials
mEC Selective Enrichment Broth w/ Novobiocin	1.14582.0500	500 g
mTSB Selective Enrich-ment Broth w/ Novobiocin	1.09205.0500	500 g
Sorbitol-MacConkey (SMAC) Agar	1.09207.0500	500 g
CT-Supplement	1.09202.0001	16 vials

Additionally required materials and instrumentation

- Polymyxin Solution: 5 mg Polymyxin B sulfate (1.09875.0001 Bacillus Cereus Selective Supplement) in 1 ml of deionized water
- Stomacher/Stomacher bags
- Incubators +35 °C and +42 °C
- Distilled or deionized water
- Autoclave
- Disposable plastic transfer pipettes and/or appropriate micro pipettes and disposable tips for dispensing 200 µl
- Disposable inoculation loops

Staining of Microorganisms

Fixing

In most cases, staining with appropriate dyes is necessary to make microorganisms and their details visible under the microscope. Bacteria staining, apart from supravital staining (e.g. fluorescense stains), is carried out on fixed cells. Particulary heat sensitive bacteria are merely air dried.

1. Heat fixing

Reagent

Merck Cat. No.	Product
1.06404	Sodium chloride for analysis ACS, ISO

Solution

Physiological sodium chloride solution: Dissolve 9 g sodium chloride in 1 litre of demineralized water.

Experimental procedure and application

Using a loop, place the specimen on a fat free slide and streak either directly (e.g. viscous exsudate, puss or liquid cultures) or after dilution with physiological sodium chloride solution (e.g. centrifuge sediment, pure cultures from solid culture media). After complete air drying, or for the sake of speed after careful heat drying, fix by slowly moving the slide in a circle of about 2.5 cm three times through the dark flame of a Bunsen burner (the specime side of the slide should be at the top).

2. Chemical fixing

In contrast to heat fixing, chemical fixing permits a better contrasted visualisation of bacteria details e.g. the cilia or the relationship between bacteria and body cells.

Reagents

Merck Cat. No.	Product
1.06009	Methanol GR for analysis ACS, ISO, Reag. Ph Eur
1.00983	Ethanol absolute GR for analysis ACS, ISO, Reag. Ph Eur
1.00921	Diethylether GR for analysis ACS, ISO, Reag. Ph Eur
1.04419	Mercury(II) chloride GR for analysis ACS, ISO, Reag. Ph Eur
1.09266	Osmic acid solution 2%
1.00062	Acetic acid 96% for analysis

Solutions

1. Ethanol ether: Ethanol abs. 50 ml; diethyl ether 50 ml.
2. Sublimate alcohol: Mercury(II) chloride 3 g; demin. water to 60 ml; ethanol abs. 30 ml.
3. Osmic acid solution: Osmic acid solution 2% 5 ml; conc. acetic acid 5 drops. Store in a bottle with a wide neck.

Experimental procedure and evaluation

Cover specimens with the following fluids or lay specimens in the baths:

a) methanol 2–3 min
b) or ethanol ether (1) 10–15 min
c) or sublimate alcohol (2) 3–5 min
d) It is also possible to hold the slide above hot steaming water and then place the wet slide on the opening of the wide neck bottle (3) to impregnate it with osmic acid fumes.

Methylene blue staining

The methylene blue stain is an appropriate staining method to obtain a general picture e.g. of Gonococci, lactobacilli and to visualize polar bodies of Pasteurella.

Reagents

Merck Cat. No.	Product
1.01287	Löffler's Methylene blue solution
1.00062	Acetic acid 96% GR for analysis

Experimental Procedure and Application

Stain fixed, air dried specimes for about 15 sec (thin smears) to 45 sec (thick specimens) with Löffler's methylene blue solution. Only stain Gonococci very briefly. If necessary differentiate with 0.5 to 1% acetic acid solution. Rinse with water and dry.

Gram-staining

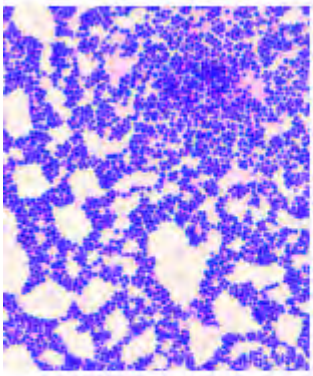
In the staining procedure aniline dyes are coupled with iodine to the bacterial cell wall to form a dye-iodine complex. All bacteria can be classified as Gram-positive or Gram-negative on the basis of this staining technique. In the case of Gram-positive organisms the dye-iodine complex cannot subsequently be dissolved from the cells with decolorizing agents such as alcohol or acetone; the cell remains blue-violet. In Gram-negative bacteria the dye-iodine complex is dissolved by these agents. The decolorated cells are then stained pink to red as a result of counterstaining with carbol fuchsin, or orange with safranine.

Staining

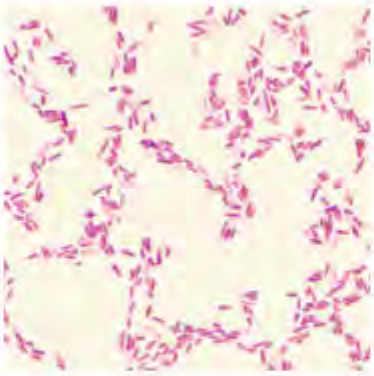
Merck Cat. No.	Product
1.11885	Gram-color staining set:
	Solution 1
	Crystal violet solution 500 ml
	Solution 2
	Lugols's solution stab. 500 ml
	Solution 3/4
	Decolorization solution 2 x 500 ml
	Solution 5
	Safranine solution 500 ml

Individual reagents

Merck Cat.No.	Product
1.09218	Gram's crystal violet solution
1.09217	Gram's safranine solution
1.10218	Gram's decolorization solution
1.09215	Ziehl-Neelsen's carbol fuchsin solution
1.09261	Lugol's solution
1.00567	Lugol's solution stabilized
1.06009	Methanol GR for analysis ACS, ISO, Reag. Ph Eur (decolorizer)
1.00972	Ethanol absolute for analysis
1.00014	Acetone GR for analysis ACS, ISO, Reag. Ph Eur



Culture, Gram-positive, Gram-color



Culture, Gram-negative, Gram-color

Gram-staining (original method)

1) Cover the slide completely with crystal violet solution, stain for 3 min, pour off, do not rinse.
2) Cover the slide completely with Lugol's solution, leave for 2 min, pour off, do not rinse.
3) Immerse the slide completely in decolorization solution (acetone, ethanol or methanol) and move for about 20–60 sec until no more clouds of stain are released and the smear is blue-grey.
4) Rinse carefully with distilled water for about 5 sec.
5) Cover the slide completely with Ziehl-Neelsen's carbol fuchsin solution diluted 1:10, stain for 1 min, pour off.
6) Rinse carefully with distilled water for about 5 sec.
7) Allow to dry.

Results

Gram-positive bacteria: dark violet
Gram-negative bacteria: red

Gram-staining acc. to Hucker with Gram-color staining set (Staining rack)

1) Cover the fixed smear with crystal violet and stain for 1 min.
2) Pour off the crystal violet solution and rinse with Lugol's solution.
3) Cover with Lugol's solution and leave for 1 min.
4) Rinse with water.
5) Swirl in the decolorizing solution for 1 min.
6) Rinse with water.
7) Stain for 1 min in safranine solution.
8) Rinse with water and dry.

Results

Gram-positive bacteria: dark violet
Gram-negative bacteria: orange

Gram-staining acc. to Hucker with Gram-color staining set (automated/coplin jar staining)

1) Immerse the fixed smear for 3 min in crystal violet solution (dilute 1:5 with water).
2) 1 min in Lugol's solution.
3) 3 min in a second cuvette containing Lugol's solution.
4) 1 min in water.
5) 1 min in decolorization solution.
6) 1 min in water.
7) 1 min in safranine solution.
8) 1 min in water (not necessary if the smear is immediately rinsed under running water).

Results

Gram-positive bacteria: dark violet
Gram-negative bacteria: orange

Gram-color modified, phenol-free

Principle

Aniline dyes in the cell tissue of microorganisms form a red dye-iodine complex when exposed to iodine. Sodium hydrogen carbonate enhances the formation of this complex further still. In Gram-positive microorganisms the dye-iodine complex cannot be subsequently dissolved from the cells by decolorizing agents such as alcohol or acetone. The cell remains dark blue in color. In Gram-negative microorganisms the dye-iodine complex is dissolved and the cell turns red as a result of counterstaining.

Reagents

Merck Cat. No.	Product
1.01603	Gram-color modified, phenol-free:
	Solution 1a
	Crystal-violet solution, phenol-free 100 ml
	Solution 1b
	Sodium hydrogen carbonate solution 100 ml
	Solution 1c
	Bottle for working solution of 1a und 1b
	Solution 2
	Iodine solution, stabilized with PVP 200 ml
	Solution 3
	Decolorization solution 200 ml
	Solution 4
	Fuchsin solution, phenol-free 200 ml

Storage

The staining kit must be stored at room temperature. Storage at temperatures below 15 °C may result in a coloured precipitate settling out of the staining solutions; in such a case the bottles should be conditioned by placing them in a water bath set at 60 °C for 2-3 hours. This will redissolve the greater part of the dye precipitates. Subsequently filter the staining solutions through a paper filter.

Preparing the smears

Apply the specimen material – for example body liquids, exsudates, puss, or a liquid or solid culture – to a degreased microscopic slide using an annealed loop. Subsequently distribute the specimen, either directly or after adding 1 to 2 drops of physiological saline solution, and smear out. Allow to airdry and heatfix the smear by slowly drawing the slide (with the smear side facing upwards) through the upper part of a Bunsen flame three times; subsequently allow to cool and then perform the staining procedure.

Preparing the staining solution

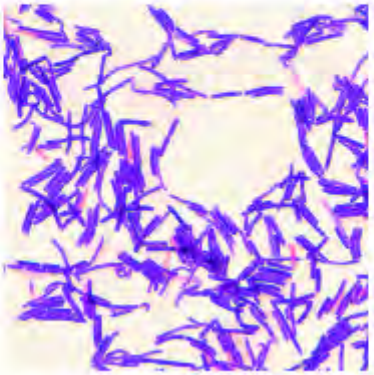
Mix the reagents Crystal-violet solution (solution 1a) and Sodium hydrogen carbonate solution (solution 1b) 1:1 in the bottle provided (1c). This mixture is sufficient for approximately 65-70 specimens and can be stored at room temperature for 10 days and refrigerated 14 days, respectively. If this amount appears to be too large for this period of time, it is advisable to prepare a smaller quantity (approx. 3 ml per microscopic slide).

Procedure on the staining bench

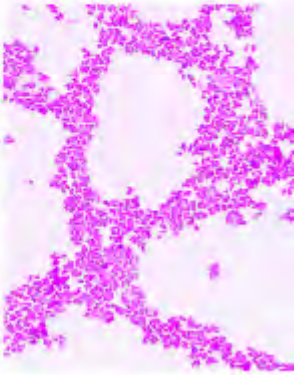
1) Cover the microscopic slide completely with working solution 1c (mixture of crystal-violet solution (1a) and sodium hydrogen carbonate solution (1b) 1:1) and stain.	1 min
2) Carefully rinse with distilled water.	5 sec
3) Cover the microscopic slide completely with solution 2, stabilized iodine solution.	1 min
4) Carefully rinse with distilled water.	5 sec
5) Decolor by covering completely with solution 3, decolorization solution.	5-10 sec
6) Carefully rinse with distilled water.	5 sec
7) Counterstain by covering completely with solution 4, fuchsin solution.	15-30 sec
8) Carefully rinse with distilled water.	5 sec
9) Dry.	

Results

Gram-positive microorganisms	dark blue
Gram-negative microorganisms	red



Culture, Gram-positive, Gram-color modified, phenol-free



Culture, Gram-negative, Gram-color modified, phenol-free

Checking the staining kit

The staining kit can be checked using Gram-positive bacteria (staphylococci) and Gram-negative bacteria (Escherichia coli). Cultures taken from a culture medium incubated for 18-24 hours must be used for this purpose.

Auxiliary reagents

Merck Cat.No.	Product
1.04699	Immersion oil for microscopy
1.15577	Immersion oil acc. to DIN ISO 8036-1, modified
1.07961	Entellan® new
1.15525	Ringer tablets

Diphtheria bacteria staining acc. to Neisser

At the correct pH, methylene blue and crystal violet are bound in the polar body structure but not in the bacteria body. Counterstaining with chrysoidine shows particularly the bacteria body and only apart of the polar body.

Reagents

Merck Cat. No.	Product
1.09238	Neisser's solution Ia (methylene blue solution)
1.09239	Neisser's solution Ib (crystal violet solution)
1.09240	Neisser's solution II (chrysoidine solution)
1.09261	Lugol's solution (diluted iodine-potassium iodide solution)
1.00567	Lugol's solution stabilized with PVP
1.00366	Lactic acid about 90% purified

Solutions

1. Methylene blue crystal violet solution: Solution Ia 2 parts; solution Ib 1 part; prepare fresh each time.
2. Lugol's solution with lactic acid: Lactic acid 0.9 ml; Lugol's solution to 100 ml.

Experimental procedure and application

After fixing, stain specimen for 2 sec with methylene blue crystal violet solution (1), if at all rinse very shortly with water and stain immediately for 10 sec with Neisser's solution II, again rinse only shortly with water and dry between blotting paper.
Gin's modification: Stain specimen for 1-2 min with methylene blue crystal violet solution (1), rinse for 3 to 5 sec with Lugol's solution with lactic acid (2), rinse well with water and restain with Neisser's solution II.

Results

Polar bodies: dark blue to black
Bacteria body: brownish

Diphtheria bacteria staining acc. to Albert and Laybourn

Reagents

Merck Cat. No.	Product
1.15930	Toluidine blue O (C.I. 52040) Certistain®
1.15942	Malachite green-oxalate (C.I. 42000) Certistain®
1.00972	Ethanol absolute for analysis
1.00063	Acetic acid (glacial) 100% unhydrous GR for analsyis ACS, ISO, Reag. Ph Eur
1.09261	Lugol's solution (diluted iodine-potassium iodide solution)
1.00567	Lugol's solution stabilized with PVP

Solutions

1. Toluidine blue malachite green solution: Toluidine blue 1.5 g; malachite green 2.0 g; dissolve in ethanol 20 ml; demineralised water 1l; glacial acetic acid 10 ml; leave to stand for 1 to 2 days; filter.
2. Lugol's solution.

Experimental procedure and application

After heat fixing, stain the smear for 4-6 min with toluidine blue malachite green solution (1), rinse with water, cover with Lugol's solution for 2 min, rinse with water and dry between blotting paper.

Results

Polar bodies: blue-black
Bacteria body: green

Gonococci staining acc. to Pappenheim-Unna

Reagents

Merck Cat. No	Product
1.15944	Methyl green (C.I. 42590) Certistain®
1.07518	Pyronine G (C.I. 45005) Certistain®
1.04094	Glycerol about 87% GR for analysis
1.00983	Ethanol abs. GR for analysis ACS, ISO, Reag. Ph Eur
1.00206	Phenol GR for analysis ACS

Solution

Carbol methyl green pyronine solution: Methyl green 0.1 g; pyronine 0.9 g; ethanol abs. 9 ml; glycerol 10 ml; phenol water 0.5% 100 ml; dissolve at about 50 °C while shaking and leave to stand for 14 days.

Experimental procedure and application

Fix the air dried smear with methanol or ethanol ether and then stain for 2-5 min with the staining solution, rinse with demineralized water and dry.

Results

Nuclei:	blue-green
Bacteria (Gonococci), fungi hyphae:	ruby red
Plasma:	light red

The color contrast makes it easier to find the Gonococci. *The colouring, however, is not specific for Gonococci!*

Literature

Unna, P. jr: Dermat. Wschr., 8; 314 (1929)

Gonococci staining acc. to Schlirf

Reagents

Merck Cat. No	Product
1.15940	Crystal violet (C.I. 42555) Certistain®
1.15943	Methylene blue (C.I. 52015) Certistain®
1.09261	Lugol's solution (diluted iodine-potassium iodide solution)
1.00567	Lugol's solution stabilized with PVP
1.00206	Phenol GR for analysis ACS
1.00983	Ethanol abs. GR for analysis ACS, ISO, Reag. Ph Feur

Solutions

1. Crystal violet solution: Crystal violet 4 g; ethanol abs. 100 ml; dissolve at 40 to 50 °C, filter after cooling.
2. Methylene blue solution: Methylene blue 2 g; ethanol abs. 100 ml; dissolve at 40 to 50 °C, filter after cooling.
3. Carbol crystal violet methylene blue solution: solution (1) 15 ml; solution (2) 10 ml, phenol water 2% 100 ml; demineralized water 50 ml. Filter before use.
4. Carbol methyl green pyronine solution: (Manufacture: see Gonococci staining acc. to Pappenheim-Unna). Before use, dilute 1+5.

Experimental procedure and application

Stain fixed smears for 1 min in staining solution (3), rinse with water, dry between blotting papier and rinse gently for 1 min with Lugol's solution. Differentiate with ethanol abs. for about 30 sec, restain with solution (4) for 2 min, rinse and dry.

Results

Gram-positive bacteria:	black
Gonococci (Gram-negative):	red

Enrichment of the Tb examination material

Reagent

Merck Cat. No.	Product
1.08000	Sputofluol®

For the oxidative dissolution of organic material (cell material, mucus, etc.) in order to liberate tubercle bacilli from sputum and other material.

Mode of action

If tubercle bacilli are to be cultivated, they must first be freed from the surrounding cells and mucus. This is achieved by dissolving the material in Sputofluol®. Sputofluol® contains alkaline hypochlorite which dissolves the organic material by oxidation without damaging the acid-alcohol-resistant tubercle bacilli. The undesired accompanying bacterial flora is destroyed.

Composition

Water; sodium hydroxide; sodium hypochlorite

continued on next page

Experimental procedure and evaluation

Place at least 4 ml sputum, urine, punctate, sediment etc. in a sterile centrifuge tube together with approximately 12 ml (ratio 1:3) of a 10–15% Sputofluol® solution prepared with sterile, distilled water (the strength of the solution depends on the degree of contamination), mix with a sterile glas rod. Allow to react for 10 min then centrifuge at 3000 to 4800 rpm for 20 min. The supernatant is decanted. For microscopical detection of bacilli, smear the sediment on an unused, fat-free slide, allow to dry, fix careful over a flame and then stain in the customary way. When identifying tubercle bacilli in culture, a 5–10% Sputofluol® solution is used. In order to prevent irreversible damage to the tubercle bacilli due to the prolonged action of Sputofluol®, after 10 min of activity the solution is immediatly neutralized. 1N HCl is added dropwise until an added pH indicator (e.g. neutral red) changes. Then centifugate. The sediment is inoculated onto appropriate culture media. In the examination of milk or tissue, sediments are obtained from 30 ml milk or 10–50 g tissue comminuted in sterile, physiological saline or sterile Ringer solution.

Mycobacteria staining acc. to Ziehl-Neelsen

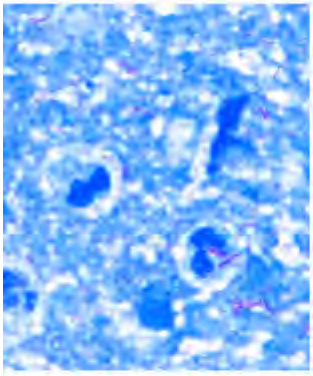
The acid "fastness" of certain bacteria, e.g. Tb bacteria, results from a wax-like shell which, due to an acid reaction, prevents the germs releasing dyes which they have absorbed.

Reagents

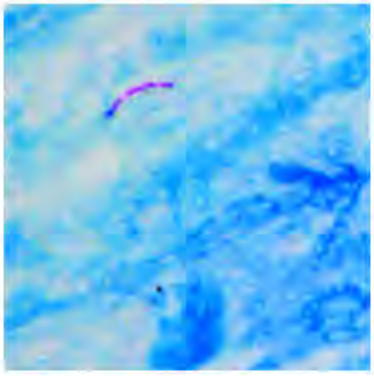
Merck Cat. No.	Product
1.09215	Ziehl-Neelsen's carbol-fuchsin solution
1.15943	Methylene blue (C.I. 52015) Certistain®
1.00327	Hydrochloric acid in ethanol
1.00983	Ethanol abs. GR for analysis ACS, ISO, Reag. Ph Eur
1.01287	Löffler's methylene blue solution

Solution

Methylene blue solution: Methylene blue 2 g; ethanol abs. 100 ml; dissolve at 40 to 50 °C; filter after cooling; dilute 1+9 before use.



Sputum, Ziehl-Neelsen stained



Sputum, Ziehl-Neelsen stained

Experimental procedure and application

1) On the staining rack, cover fixed smears completely with Ziehl-Neelsen's carbol-fuchsin solution.
2) Heat three times carefully with a Bunsen burner from below until fumes form. Do not boil! Take your time and let the solution cool during the process. The dye should be allowed to work for 5 min in all.
3) Pour off the staining solution and rinse with a water jet.
4) Bleach with hydrochloric acid in ethanol until no further red clouds leave the normally thick parts of the preparation.
5) Rinse with water.
6) Restain for 1 min with diluted methylene blue solution.
7) Wash with water and dry in air.

Results

Acid-fast mycobacteria: red
Background: light blue

Diagnosis

A positive result means "acid-fast rods detected" and a negative result "acid-fast rods not detected". Is is impossible to say whether these rods are tubercle bacilli or other "atypical" mycobacteria or whether they are still capable of reproduction or are already dead. In sputum, positive in open lung Tb. Also Tb bacteria can be detected in urine, liquor and gastic juice with the Ziehl-Neelsen method.
Apart from Mycobacterium tuberculosis, other acid-fast rod bacteria are the leprosy pathogen and many harmless saprophytes (e.g. smegma bacteria, Nocardia species).

Tb-color modified, hot staining

Staining kit for the detection of mycobacteria (AFB) by hot staining method. Mycobacteria are difficult to stain because of the high proportion of lipid and wax in their cell walls. Up to now, in order to carry out the classical Ziehl-Neelsen staining, the test material to be heated with carbol fuchsin solution to produce the mycolic acid fuchsin compound. Once stained, acid fast mycobacteria keep their coloring even after treatment with strong decolorizing solutions as HCl-ethanol. They remain red after counterstaining with methylene blue, whereas the micro-organisms susceptible to acid take on the blue.

Reagents

Merck Cat. No.	Product
1.00497	Tb-color modified staining set: Solution 1 Ziehl-Neelsen's carbol-fuchsin solution 500 ml, Solution 2/3 Hydrochloric acid in ethanol 2 x 500 ml, Solution 4 Löffler's methylene blue solution 500 ml

Application

The microscopic investigation of mycobacteria. Tb-color modified staining set uses the classical Ziehl-Neelsen hot staining procedure with methylene blue counterstraining.

Sample material

Heat-fixed smears of sputum, FNAB, lavages, imprints, body fluids, exsudates, puss, liquid, solid cultures and histological sections

Fixation

Fixation is carried out over the flame of a Bunsen burner (2-3 times, avoiding excessive heating). It is also possible to fix the smears in an oven at 100-110 °C for 20 min. Impairment of staining must be expected if a higher temperature or longer heating is employed.

Pretreatment

Sputum

Sputum should be pretreated with Sputofluol® in order to free the mycobacteria from surrounding mucus. One ingredient in Sputofluol® is hypochlorite, which oxidatively dissolves the organic material while sparing the mycobacteria for the greater part. In a centrifuge tube mix 1 part of the sample (at least 2 ml) with 3 parts of a 15% Sputofluol® solution prepared with distilled water, and leave to react for 10 min shaking vigorously from time to time. Centrifuge at 3000 to 4800 rpm for 20 min, decant the supernatant, smear out the sediment allow to dry.

Punction and lavage material, sediments

After carrying out the appropriate enrichment measures smear out samples on the microscopic slides and allow to air dry.

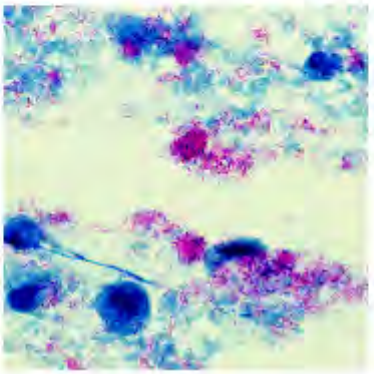
Histological sections

Deparaffinize the sections in the typical manner and rehydrate in a descending alcohol series.

Staining with a staining rack:

1) Cover specimens completely with Ziehl-Neelsen's carbol fuchsin solution. Carefully heat 3 times from below with Bunsen burner to steaming and keep hot for 5 min. Do not allow the stain to boil.
2) Wash with tap water until no further color is given off.
3) Cover completely with hydrochloric acid in ethanol solution and, depending on the thickness of the specimen, allow to stand for 15-30 sec.
4) Wash immediately with tap water.
5) Counterstain for 30 sec in Löffler's methylene blue solution or 1 min in diluted Löffler's methylene blue solution (dilution: 1+9 with dist. water).
6) Wash well with tap water.
7) Dry.

Dehydrate histological specimens (ascending alcohol series), clear in xylene or Neo-Clear® and mount with Entellan® new or Neo-Mount®.



Sputum, Tb-color modified stained

Results

Acid-fast bacteria:	red
Background:	light blue

Assessment

A positive finding is reported as "acid fast bacteria detected" and a negative finding is reported as "acid fast bacteria not detected". It is not possible to state whether there are tuberculosis bacteria or other "atypical" bacteria. It is also impossible to state whether theses mycobacteria are still capable of reproduction or are already dead. When acid-fast bacteria are found in the material examined, further investigations in a special laboratory are indicated.

Capacity

The kit is sufficient for 250 specimen, stained on a staining rack.

Cold staining of Mycobacteria with Tb-color

Staining set for the microscopic examination of Mycobacteria. In the present modification of the Ziehl-Neelsen staining method, consistently good staining results are obtained without to heating the carbol fuchsin solution (phenol vapours) during staining (cold staining).

Reagent

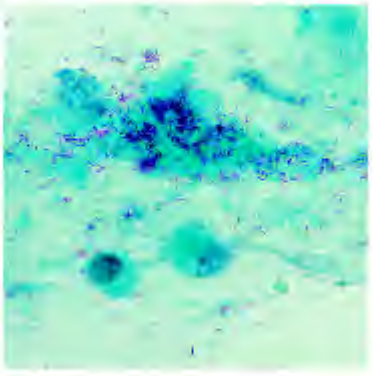
Merck Cat.No.	Product
1.16450	Tb-color staining kit: Solution 1 Sputofluol® solution (contains sodium hydroxide solution and sodium hypochlorite solution) 500 ml, Solution 2 Carbol-fuchsin solution (contains phenol and ethanol) 500 ml, Solution 3 Decolorization solution (contains ethanol with 0.75 % hydrochloric acid) 500 ml, Solution 4 Malachite green-oxalate solution (contains 0.2 % malachite green oxalate) 500 ml

Preparation with solution 1 (Sputofluol® solution)

Add 3 parts of a 15% Sputofluol® solution prepared with distilled water to 1 part of the specimen (at least 4 ml) in a centrifuge tube. Allow to act for 10 min while shaking vigorously, centrifuge for 20 min at 3000 to 4800 rpm. Decant the supernatant and use the sediment to prepare a smear.

Fixation

Fix over a Bunsen burner flame (2-3 times taking care not to heat too strongly). Alternatively the smears can be fixed for 20 min at 100-110 °C in a drying cabinet. Intense heat affects stainability.



Sputum,
Tb-color stained

Staining with a staining rack

1) Cover the air-dried, heat-fixed preparation completely with solution 2 (carbol-fuchsin solution) and stain for 5 min.
2) Wash off with tap water until clouds of stain cease to be formed.
3) Cover completely with solution 3 (decolorization solution) and wash off immediately with tap water (maximum staining period 30 sec).
4) Completely cover the preparations with solution 4 (malachite green solution) and counterstain for 1 min.
5) Wash off for about 10 sec with tap water and dry in air.

Staining in the coplin jar

1) Solution 2 (carbol-fuchsin solution)	5 min
2) Tap water	15 sec
3) Solution 3 (decolorization solution)	45 sec
4) Tap water	15 sec
5) Solution 4 (malachite green solution)	1 min
6) Tap water	10 sec
7) Dry	3 min

Examine the stained preparation under the microscope for at least 5 min using bright-field illumination and a 90-100x objective with immersion oil (Merck Cat. No. 1.15577).

Staining results

Acid-fast mycobacteria: red
Background: light green

Diagnosis

A positive result means "acid-fast rods detected" and a negative result "acid-fast rods not detected".
Is is impossible to say whether these rods are tubercle bacilli or other "atypical" mycobacteria or whether they are still capable of reproduction or are already dead.

Storage/shelf life

Do not store below +15 °C as the stain then precipitates out. If stain precipitate is formed, place the bottle for 2 until 3 hours in water at a temperature of about 60 °C. Filter prior to use. If stored as directed, the set is stable for at least 24 months. If the staining set is used in conjunction with a staining rack the contents are sufficient for at least 250 preparations.

Capacity

The kit is sufficient for 250 specimen, stained on a staining rack.

Literature

Brett, U.: Modifizierte Ziehl-Neelsen-Färbung.
mta, 6; 415–416 (1986).

Auramine staining of Mycobacteria acc. to Hagemann-Herrmann

Reagents

Merck Cat. No.	Product
1.00327	Hydrochloric acid in ethanol
1.05082	Potassium permaganate GR for analysis ACS
1.00206	Phenol GR for analysis ACS
1.01301	Auramine O (C.I. 41000)
1.01287	Löffler's methylene blue solution

Solutions

1. Phenol auramine solution: Auramine 1 g; demineralized water 1 l; liquified phenol 50 ml.
2. Liquified phenol: Melt 10 parts of phenol in a slightly warmed water bath and add 1 part of water.
3. Potassium permanganate solution 0.1 %: Potassium permanganate 1.0 g; demineralized water to 1 litre.

Experimental procedure and application

1) After heat fixing, bring the smears covered with phenol auramine solution (1) to boil and stain for 5 min, shake off superfluous solution and repeat staining.
2) Rinse with water.
3) Differentiate in hydrochloric acid in ethanol until bleached (15–20 sec).
4) Rinse with water if necessary counterstain.
5) Dip for 5 sec into potassium permanganate solution (2).
6) Rinse with water.
7) Dip for 1 sec into Löffler's methylene blue solution.
8) Rinse with water.

Results

Tb bacteria: gold-yellow fluorescence
cells and mucus : dark violet fluorescence

Literature

Hermann, W.: Dtsch. Med. Wschr., 64; 1354 (1938)

Fluorescence staining of Mycobacteria with Tb-fluor

The reason for the fact that the mycobacteria are acid-fast is that a wax-like coating (mycolic acid) in the membrane of these microorganisms prevents the release, on acid treatments, of the dye, once it has been absorbed. Both staining methods – Ziehl-Neelsen (with heated carbolfuchsin) or Tb-color (cold method) for optical microscopy and Tb-fluor (Auramine-Rhodamine) for fluorescence microscopy – are based on this principle.

Reagent

Merck Cat.No.	Product
1.09093	Tb-fluor staining kit: Solution 1 Staining solution (Auramine-Rhodamine solution) 500 ml, Solution 2 Decolorization solution (HCl-isopropanol) 3 x 500 ml, Solution 3 Counterstaining solution (buffered $KMnO_4$) 2 x 500 ml

Specimen for investigation

Sputum, pleural fluid, bronchio-alveolar washing (BAL), urine. The staining can also be performed in histology for acid-fast examination in lymphnodes and any other kind of biopsies.

Pretreatment of the specimen

Sputum

Before the staining, it is recommended to treat the sputum with Sputofluol® in order to free the mycobacteria of the coating of cells and mucus. One ingredient of Sputofluol® is alkaline hypochlorite. This agent dissolves the organic material by oxydation, while essentially leaving the acid-fast bacilli unaffected.
Three parts of a 15% solution of Sputofluol® are added to one part of specimen (4 ml) in a centrifuge tube. The mixture is shaken vigorously for 10 min. It is then centrifuged at 3000 to 4800 rpm for 20 min. The supernatant is decanted and the sediment is smeared thinly on degreased slides and dried in air *(see also page 7/8)*.

Pleural fluids, bronchio-alveolar washing, urine

The specimens have to be smeared thinly on glass slides and dried in air after appropriate enrichment.

Histological specimens

The histological specimens will be treated to remove paraffin and rehydrate the section.

Fixation of smears

Fixation is carried out over the flame of a Bunsen burner (2 or 3 times rapidly, avoiding excessive heating).
The smears can also be fixed for 20 min in an oven (100–110 °C) or on a warming plate.

continued on next page

Staining in the coplin jar

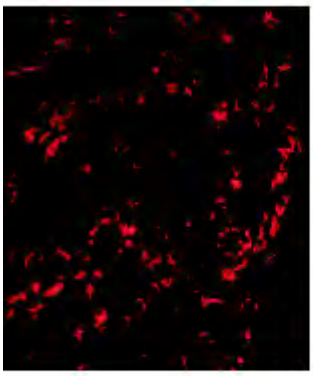
1) Solution 1 (Auramine-Rhodamine solution)	15 min
2) Rinse with tap water	10 min
3) Solution 2 (Decolorization)	1 min
4) Rinse with tap water	5 min
5) Solution 3 (Counterstaining)	5 min
6) Rinse with tap water	5 min

Dry the smears in air and mount them with Entellan® new; the histologigal sections should be treated with increased concentrations of alcohol and with xylene/Neo-Clear® before mounting with Entellan® new/Neo-Mount®.

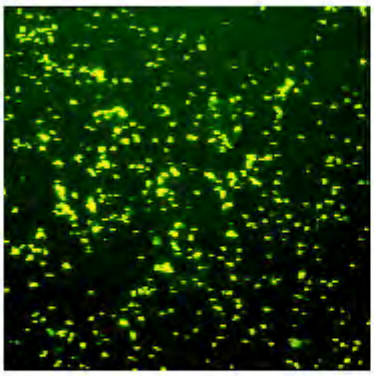
Application for the MIRASTAINER®

Stains	Station	Time	Dip status
Solution 1 (Auramine-Rhodamine solution)	4	15 min	off
Rinse with tap water	5	10 min	on
Solution 2 (Decolorization)	3	1 min	on
Rinse with tap water	5	5 min	on
Solution 3 (Counterstaining)	2	5 min	off
Rinse with tap water	5	5 min	on
Dry	6	5 min	–

Mount the dried smears with Entellan® new; histological sections should be treated with increased concentrations of alcohol and with xylene/Neo-Clear® before mounting with Entellan® new/Neo-Mount®.



Lung section, acid fast bacteria, Tb-fluor stained



Lung section, acid fast bacteria, Tb-fluor stained

Staining result and assessment

The acid-fast bacilli are clearly distinguished by being red-orange or green (depending of the filter combination used for the fluorescence microscope) on a dark background (25x or 40x objective).

Recommended filter combination

Exciation filter:	490-570 nm
Dichromatic mirror:	525 and 635 nm
Suppression filter:	505-600 nm

Diagnosis

A positive result means "acid-fast rods detected" and a negative result "acid-fast rods not detected".
Is is impossible to say whether these rods are tubercle bacilli or other "atypical" mycobacteria or whether they are still capable of reproduction or are already dead. Confirmation tests (culture, PCR, or similar methods) are requested in order to establish the diagnosis of tuberculosis.

Double staining

Any doubtful or suspicious result can be confirmed by performing a double staining Tb-fluor – Tb-color (or Tb-fluor – Ziehl-Neelsen).
The non-mounted slides stained with Tb-fluor are first examined using immersion oil (Merck Cat. No. 1.15577).
Then, they are rinsed with toluene, dried and immediately restained with the Tb-color staining set (or with the Ziehl-Neelsen's carbolfuchsin solution). The mycobacteria are clearly distinguished by being red on a pale green (Tb-color) or blue (Ziehl-Neelsen) more or less amorphous background.

Storage

Storage temperature between 15°C and 25°C. Unopened, the staining kit has a shelf life of 24 months.

Stability

In case of manual staining in coplin jar or with the MIRASTAINER®, the staining kit will allow to stain 300 up to 400 specimens, depending of the number of slides placed in the coplin jar for each staining procedure. It is recommended to replace the solution 1 (Auramine-Rhodamine) after 10 to 15 staining series and 3 (Counterstaining solution) after 5 to 10 staining series; solution 2 (Decolorisation solution) should be replaced after 5 staining series.
If stored below +15°C, a precipitate of dye may form in solution 1 and 3. If this occurs, place the bottle in water at about 60°C for 2-3 hours. Filter before use.

Tb-fluor, phenol-free

Fluorescence staining kit for the microscopic investigation of mycobacteria. The acid-fastness of mycobacteria is based on the fact that a wax-like sheath of the membrane of these bacteria prevents the release of already incorporated dyes by treatment with acid. Here the use of a modified staining solution makes the inclusion of phenol in the staining solution unnecessary. The sensitivity and specifity of the staining results are identical with those obtained using the classical (phenol-employing) staining method.

Reagent

Merck Cat.No.	Product
1.01597	Tb-fluor, phenol-free staining kit: Reagent 1 Auramine-Rhodamine staining solution 200 ml, Reagent 2/3 Decolorization solution 200 ml, Reagent 4 Counterstaining solution (buffered $KMnO_4$) 200 ml

Examination material

Specimen materials offering themselves for examination with this method include e.g. sputum, specimens gained by pleural punctation or bronchial lavage (BAL), urine sediments, FNAB, imprints, culture specimens, and histologic sections.

Sample preparation

The sample material is applied to clean, degreased microscopic slides.

Sputum

Sputum should be pretreated with Sputofluol® in order to free the mycobacteria from the enveloping mucus. One ingredient in Sputofluol® is hypochlorite, which oxidatively dissolves the organic material while sparing the mycobacteria for the greater part.
In a centrifuge tube mix 1 part of the sample (at least 2 ml) with 3 parts of a 15% Sputofluol® solution prepared with distilled water, and leave to react for 10 min shaking vigorously from time to time. Centrifuge at 3000 to 4800 rpm for 20 min, dec ant the supernatant, smear out the sediment, and allow to dry.

Punctation and lavage material, sediments

After carrying out the appropriate enrichment measures smear out samples on the microscopic slides and allow to air-dry.

Histologic sections

Deparaffinize the sections in the typical manner and rehydrate in a descending alcohol series.

Fixation

Fixate samples over the bunsen burner flame (2-3 times, taking care to avoid excessive heating).
The material may also be fixated at 100-110 °C for 20 min in a drying cabinet or on a hotplate.

Staining on the staining bench

1) Cover the air-dried, heat-fixated specimens completely with auramine-rhodamine staining solution (Sol. 1) and stain — 15 min
2) Carefully rinse under running tap water — 30 sec
3) Cover the specimens completely with decolorization solution (Sol. 2) and leave to stand — 1 min
4) Carefully rinse under running tap water — 30 sec
5) Cover the specimens completely with $KMnO_4$ counterstaining solution (Sol. 3) and stain — 5 min
6) Carefully rinse under running tap water — 30 sec

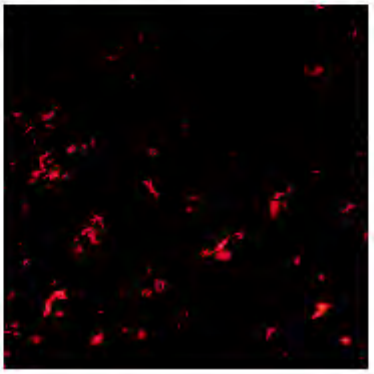
Allow the specimens to dry and, if necessary, mount with Entellan® new or Neo-Mount®. Dehydrate histologic specimens (ascending alcohol series) and mount with Entellan® new or Neo-Mount®. *Note: Carefully shake the Auramine-Rhodamine solution prior to use.*

Staining results

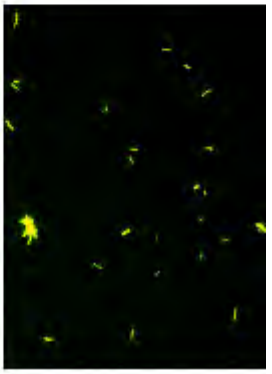
Acid-fast bacteria:	red-orange or yellow-green fluorescence
Background:	dark

Recommended filter combination

Excitation filter:	490-570 nm
Color split:	525 and 635 nm
Suppression filter:	505-600 nm



Lung, paraffin section, acid fast bacteria, Tb-fluor, phenol-free



Lung, paraffin section, acid fast bacteria, Tb-fluor, phenol-free

Double staining

Any doubtful or suspicious result can be confirmed by conducting the double staining method "Tb-fluor – Tb-color" or "Tb-fluor – Tb-color modified". In the case of the unmounted specimens stained with Tb-fluor, first only immersion oil for diagnostic purposes is used. Subsequently the immersion oil is carefully removed and the dried specimens are stained with Tb-color or Tb-color modified. The mycobacteria show up red against a light green (Tb-color) or light blue (Tb-color mod.) background.

Capacity

The kit is sufficient for 60-65 specimen.

Brucella staining acc. to Koslowskij-Treffenstädt

Reagents

Merck Cat. No.	Product
1.15948	Safranine O (C.I. 50240) Certistain®
1.15943	Methylene blue (C.I. 52015) Certistain®
1.06009	Methanol GR for analysis ACS, ISO, Reag. Ph Eur
1.00063	Acetic acid (glacial) 100% unhydrous GR for analysis ACS, ISO, Reag. Ph Eur

Solutions

1. Safranine solution 3%: Safranine 3 g; demineralized water to 100 ml; dissolve under heat and filter. Acetic acid 1%: Glacial acetic acid 1 ml; demineralized water to 100ml.
2. Methylene blue solution: Methylene blue 1 g; demineralized water to 100 ml.

Experimental procedure and application

1) After heat fixing (or methanol fixing), cover smear with safranine solution (1), heat for 3 min until it bubbles.
2) Rinse thoroughly with water. With thick smears, differentiate with acetic acid (2).
3) Counterstain with methylene blue solution (3).
4) Rinse.

Results

Brucella: red
Cells, other bacteria: blue

Capsule staining in Pneumococci

Reagents

Merck Cat. No.	Product
1.05226	New fuchsin (C.I. 42520) Certistain®
1.00983	Ethanol abs. GR for analysis ACS, ISO, Reag. Ph Eur
1.00063	Acetic acid (glacial) 100% unhydrous GR for analysis ACS, ISO, Reag. Ph Eur

Solutions

1. Fuchsin solution: New fuchsin 2.0 g; ethanol abs. 100 ml.
2. Acetic acid 3%: Glacial acetic acid 3 ml; demineralized water to 100 ml.

Experimental procedure and application

Place exsudate or sputum on the slide with a loop and spread. Dry quickly by waving in air and on the staining rack pour 1 drop of ethanol on the smear. Ignite this immediately and extinguish after 1 sec. The re-ignite and re-extinguish until no alcohol remains. After cooling, briefly stain with acetic acid (2) and restain with alcoholic fuchsin solution (1).

Result

Pneumococci: dark red in a pale pink zone

Capsule staining acc. to Olt in anthrax pathogens

Reagent

Merck Cat. No.	Product
1.15948	Safranine O (C.I. 50240) Certistain®

Solution

Safranine solution 3%: Safranine 3.0 g; demineralized water to 100 ml; dissolve under heat and filter.

Experimental procedure and application

After heat fixing, stain with safranine solution for 1-2 min, rinse with water and dry. Prior to the examination cover the smear with water and a cover glass.

Result

Capsules of anthrax pathogens: orange

Negative visualisation of capsules

Reagents

Merck Cat. No.	Product
1.15924	Nigrosin (C.I. 50420) Certistain®
1.03999	Formaldehyde solution min. 37%

Solution

Nigrosine solution: Nigrosine 5g; demineralized water 100ml; boil for 10 min and allow to cool; add formaldehyde solution 0.5ml; filter.

Experimental procedure and application

Rub 1 loop of nigrosine solution on a pre-cleaned slide together with some bacteria material. Press a clean cover glass over the slide.

Results

Capsules: bright
Background: dark

Spore staining acc. to Rakette

Reagents

Merck Cat. No.	Product
1.15942	Malachite green-oxalate (C.I. 42000) Certistain®
1.15935	Eosin Y (yellowish) (C.I. 45380) Certistain®
1.15948	Safranine O (C.I. 50240) Certistain®

Solutions

1. Malachite green solution: Malachite green 5.0g; demineralized water to 100ml.
2. Eosin solution: Eosin Y 2.5g; demineralized water to 100ml.
3. Safranine solution: Safranine 0.5g; demineralized water to 100ml.

Experimental procedure and application

1) Fix the air dried smear: draw 6 to 8 times through the flame.
2) Staining: cover the slide completely with malachite green solution (1), bring to boil for 20 sec and allow to act for 30 sec, if necessary somewhat longer.
3) Rinse for 30 sec in running water.
4) Restaining: 1 min with eosin solution (2) or, acc. to Wirtz, 30 sec with safranine solution (3).
5) Rinse and dry.

Results

Spores: Emerald green
Other cell parts : red

Cilium staining acc. to Lembach and Sous

Reagents

Merck Cat. No.	Product
1.00773	Tannic acid powdered pure DAB 7, Ph Eur, USP
1.00229	Chromium (VI) oxide GR for analysis ACS, ISO
1.01512	Silver nitrate GR for analysis ACS, ISO
1.06648	Sodium sulfate decahydrate GR for analysis ACS, Reag. Ph Eur
1.05432	Ammonia solution 25% GR for analysis
1.09218	Gram's crystal violet solution

Solutions

1. Tannin solution: Tannic acid 20.0g; demineralized water to 100ml.
2. Chromic acid solution: Chromium (VI) oxide 2.5g; demineralized water to 100ml.
3. Silver nitrate solution: Silver nitrate 25g; demineralized water to 100ml.
4. Sodium sulphate solution: Sodium sulphate decahydrate 60g; demineralized water to 100ml.
5. Ammonia solution 1%: Ammonia solution 25% 4.4ml; demineralized water to 100ml.
6. Mordant: Tannin solution (1) 100ml; chromic acid solution (2) 15ml; keep for two days at room temperature; filter before use.
7. Standard solution: Silver nitrate solution (3) 20ml; sodium sulphate solution (4) 100ml; pour off supernatant, wash the precipitate out thoroughly with demineralized water and take-up in 500ml of demineralized water. In a dark bottle it can be kept for long periods.
8. Working solution: Drop by drop add ammonia solution (5) to approx. 25ml of standard solution until the brown precipitate disappears. Make up the solution fresh each time before use.

continued on next page

Experimental procedure and application

1) Put three drops of tap water onto a clean, fat-free slide. Place the culture carefully in the first drop, after 1 min transfer it to the second drop and after a further minute to the third drop. From this, take a loopful and place it on a slide or cover glass. Dry in air and fix (once through the flame).
2) Cover the slide or cover glass with mordant (6) and allow to act for 30 min.
3) Then pour on the working solution (8) and heat gently until a pale brown sheen appears on the specimen.
4) Rinse with water.
5) Restain with Gram's crystal violet solution for 3 min.
6) Rinse with water and dry high above the flame.

Results

Cilia:	grey-black
Bacteria bodies:	blue-violet

Note

The coloured visualisation of cilia is most successful with material from swarm plates.

Literature

Lembach, K. u. Sous, H.: Zbl. Bakt.-Abt. I Orig., 152; 445 (1948).

Spirochaeta staining with Giemsa solution

Reagents

Merck Cat. No.	Product
1.09204	Giemsa's azur eosin methylene-blue solution
1.06009	Methanol GR for analysis ACS, ISO, Reag. Ph Eur
1.04928	Potassium carbonate GR for analysis ACS, ISO, Reag. Ph Eur

Solutions

1. Diluted Giemsa solution: Giemsa's azur eosin methylene-blue solution 10 drops; demineralized water 20 ml.
2. Potassium carbonate solution: Potassium carbonate 0.1 g; demineralized water to 100 ml.

Experimental procedure and application

1) Fixing with methanol.
2) Allow the diluted Giemsa solution (1) to work for 12-24 hours or, after adding a few drops of potassium carbonate solution (2), for 6-8 hours. To avoid the formation of precipitate, lay the specimen on the lower side of the glass rod in the staining solution.
3) Rinse with water.

Results

Spirochaeta pallida:	pink
Other Spirochaeta:	reddish to bluish-violet

Trichomonads staining with Cytocolor®

The Cytocolor® staining set is for the differential coloring of smear preparations in cytodiagnosis using the standard stain acc. to Szczepanik (modified Papanicolaou staining). With this method Trichomonas vaginalis can be readily recognized.

Reagents

Merck Cat. No.	Product
1.15355	Cytocolor® staining set cytological standard staining acc. to Szczepanik: Modified hematoxylin solution 500 ml, Modified polychrom solution 500 ml, Propanol-(2) 3 x 500 ml, Xylene 500 ml
1.03981	Merckofix® spray fixative
1.07961	Entellan® new
1.03973	Merckoglas® liquid cover glass

Experimental procedure and application

The smears, fixed with Merckofix®, are placed one after the other in the following solutions:

1)	Distilled water	10 x 1 sec
2)	Mod. hematoxylin solution	1 x 1 min
3)	Wash under running water	1 x 5 sec
4)	2-Propanol for analysis	2 x 1 sec
5)	Mod. polychrom solution	1 x 1 min
6)	80% 2-Propanol for analysis	5 x 1 sec
7)	2-Propanol for analysis	5 x 1 sec
8)	2-Propanol for analysis	5 x 1 sec
9)	Xylene for analysis	5 x 1 sec
10)	Xylene for analysis	5 x 1 sec

The stained smears can be covered with Merckoglas® or with cover glass and Entellan® new.

Result

Trichomonads:	grey-blue to grey-green

Fungi visualisation in the original preparation

Mode of action

Strong alkalines cause the examination material to well which makes the refractive fungi elements more clearly visible. This process can be enhanced by gentle heating.

Reagents

Merck Cat. No.	Product
1.05588	Sodium hydroxide solution min. 10% GR for analysis or
1.06498	Sodium hydroxide pellets GR for analysis or
1.05033	Potassium hydroxide pellets GR for analysis or
1.08123	Tetramethylammonium hydroxide solution 10%

Experimental procedure and evaluation

Place 1 drop of alkaline (10-30%) and examination material (from the edges of the skin changes) on the slide. Warm carefully. Allow the alkaline to work for 5 to 15 min until a gelatinous consistency is obtained. Squash the examination material with a cover glass, and with a switched down drying system, observe the material under the microscope.

Note

It is only necessary to use oil immersion to observe actinomycetes and streptomycetes. If heating is too intensive the alkaline crystallizes out.

Fungi staining with Lactophenol blue

Reagents

Merck Cat. No.	Product
1.13741	Lactophenol blue solution
1.06498	Sodium hydroxide pellets GR for analysis
1.05033	Potassium hydroxide pellets GR for analysis

Experimental procedure and application

1) Clear the specimen for 1 to 15 min, depending on its thickness, with 1 to 2 drops of alkaline.
2) Apply several changes of water and soak up with filter paper.
3) Stain with 1 to 2 drops of lactophenol blue solution and cover about 2 min.

Note

For untreated culture specimens, apply 1 to 2 drops of lactophenol blue solution and cover with a cover slip. Examine under the microscope after about 2 min.

Result

Fungal elements: dark blue

PAS-Fungal staining

If fungal material is kept for a long time in alkalines, the walls of the fungi are damaged. Therefore fine scales should not be treated with potassium or sodium hydroxide. For softening skin scrapings, nails or hair, use the preparation method acc. to Taschdjian (1955) and Muskat-Blit (1953). Any appropriate staining method can then follow.

Mode of action

Polysaccharides in the cell wall are converted by periodic acid into polyaldehyde which reacts with colorless Schiff's reagent (fuchsine sulphurous acid) forming a red-blue dye (PAS-reaction = Periodic-Acid-Schiff-reaction). The coloring shows the fungal elements clearly.

Reagents

Merck Cat. No.	Product
1.05588	Sodium hydroxide solution min. 10%
1.06498	Sodium hydroxide pellets GR for analysis
1.05033	Potassium hydroxide pellets GR for analysis
1.08123	Tetramethylammonium hydroxide solution 10%
1.09531	pH indicator strips non-bleeding Acilit-Indicator pH 0-6
1.00366	Lactic acid about 90% purified Ph Eur, BP, E 270
1.00972	Ethanol 96% extra pure Ph Eur, BP
1.00524	Periodic acid GR for analysis
1.09033	Schiff's reagent
1.15929	Thionine (acetate) (C.I. 52000) Certistain®
1.12018	Albumin fraction V (from bovine blood)
1.09242	Kaiser's glycerol gelatine

Solutions

1. Lactic acid solution 10%: Lactic acid 90% 11 ml; demineralized water 88 ml.
2. Periodic acid solution: Periodic acid 1.0 g; demineralized water 20 ml; always make up fresh before use.
3. Thionine solution: Thionine 1.0 g; demineralized water to 50 ml.

Preparing the specimen

1) Depending on the thickness of the examination material, allow a 10% alkaline solution to act for 5 to 15 min until decoloration and softening occurs. Do not let the material become too "soupy".
2) Soak up the superfluous alkaline with filter paper.
3) Cover the specimen with lactic acid solution (1) and let it act for about 3 min; test with pH indicator if necessary. The pH should be between 3 and 5.
4) Soak up superfluous acid.
5) Add a few drops of 96% ethanol to the material on the slide and allow it to dry for several hours. If the material does not stick to the slide, place it on a slide covered with albumin or on a piece of adhesive tape. After this staining can take place.

Staining

1) Allow 5% periodic acid solution (2) to act for 10 min.
2) Rinse for 2 min under running tap water.
3) Stain for 15 min with Schiff's reagent.
4) Removes superfluous Schiff's reagent by applying 2% thionine solution (3) for 10 min.
5) Rinse under running tap water.

Notes

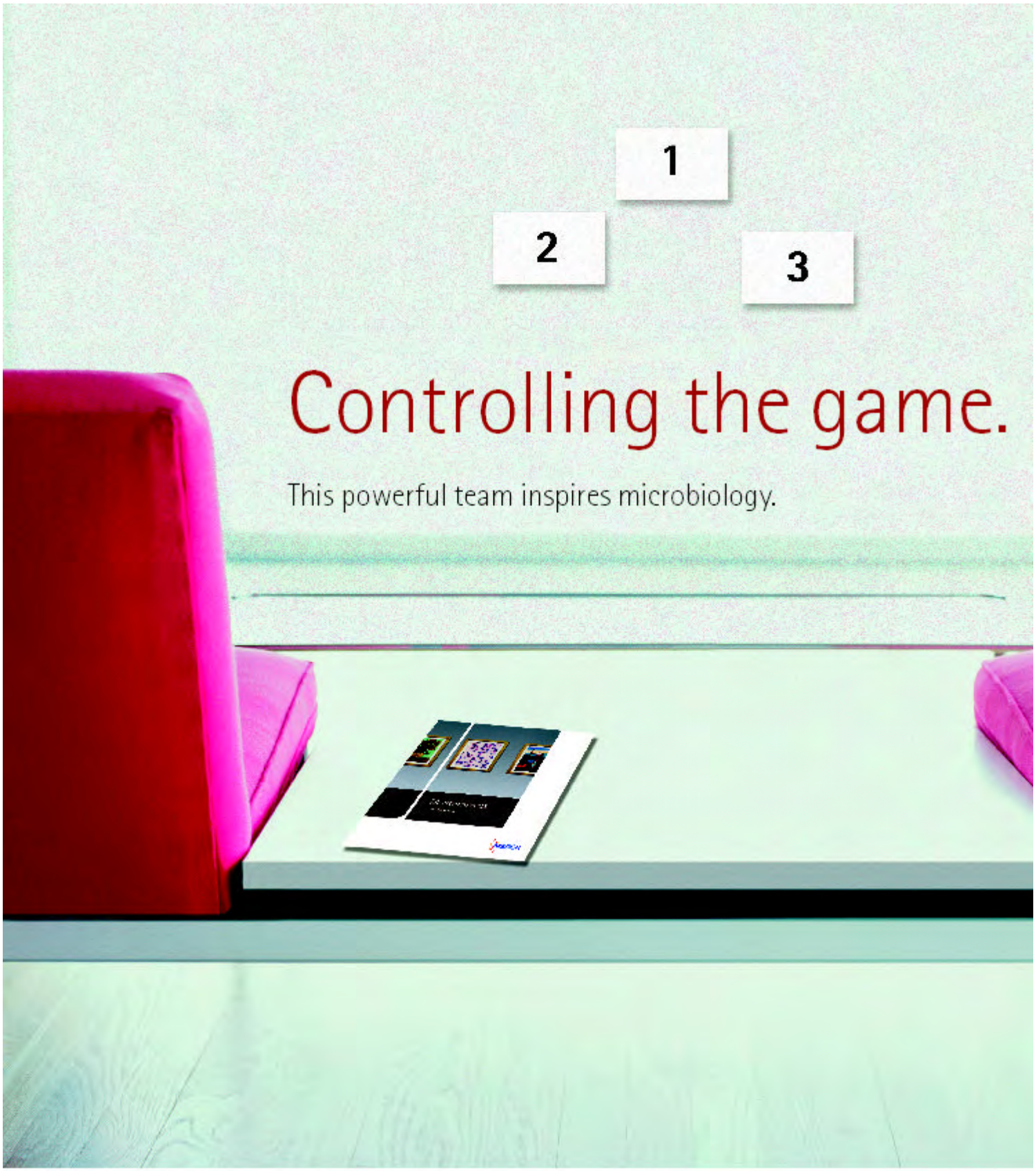
1) With skin scrapings, thionine application is not necessary. This shortens the staining time.
2) Small skin particles can be stuck to adhesive film and placed in the staining dishes. For preparations which are to kept, cut out part of the film strip and embed this in Kaiser's glycerol gelatin. Because the adhesive film is attacked by xylene and alcohol, canada balsam and dehydration in an increasing alcohol series can not be use.
3) Coarse tissue particles on the adhesive film are softened before PAS staining to a semi-gelatinous consistency.

Result

Fungal elements: red-blue

Literature

Hotchkiss, G.: Arch. Biochem., 16; 131 (1948)
McManus, J.F.A.: Stain.Technol., 23; 99 (1948)
Muskat-Blit, E.: Arch. Dermat., 68; 579 (1953)
Taschdjian, C.L.: J. Investigat. Dermat., 24; 77 (1955)


1
2
3
Controlling the game.
This powerful team inspires microbiology.

Designation Comparison

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MICROBIOLOGY CROSS-REFERENCE LIST 2005: Merck - BD/Difco

Merck Cat.No.	BD/Difco Cat.No.	BD/Difco Product Description	Size	UOM
1.00415.0500	218231	A-1 Medium	500	g
1.02245.0500	211843	Acidicase Peptone	500	g
1.01614.0500	212304	Agar, Grade A	454	g
1.11925.1000	281230	Agar, Technical	500	g
1.05452.0500	210926	Anaerobic Agar	500	g
1.05272.0500	210937	Antibiotic Medium #1 (Penicillin Assay Seed Agar)	500	g
1.05272.0500	210938	Antibiotic Medium #1 (Penicillin Assay Seed Agar)	5	lb
1.05272.0500	210939	Antibiotic Medium #1 (Penicillin Assay Seed Agar)	25	lb
1.05269.0500	210977	Antibiotic Medium #11 (Neomycin Assay Agar)	500	g
1.05269.0500	210980	Antibiotic Medium #11 (Neomycin Assay Agar)	5	lb
1.05270.0500	210943	Antibiotic Medium #2 (Base Agar, Penicillin Assay)	500	g
1.05273.0500	210932	Antibiotic Medium #3 (Antibiotic Assay Broth)	500	g
1.05271.0500	210953	Antibiotic Medium #5 (Streptomycin Assay Agar with Yeast Extract)	500	g
1.09877.0001	232671	Antimicrobic Vial Oxytetracycline	10	ml
1.09875.0001	232681	Antimicrobic Vial P	6 x 10	ml
1.10453.0500	210916	APT Agar All Purpose Agar	500	g
1.05406.0500	211023	Baird Parker Agar Base	500	g
1.05406.0500	212276	Baird Parker Agar Base	5	lb
1.01590.0500	276830	Baird-Parker Agar Base	100	g
1.01590.0500	276840	Baird-Parker Agar Base	500	g
1.10493.0500	212424	Brain Heart Infusion	100	g
1.10493.0500	211059	Brain Heart Infusion	500	g
1.10493.0500	211060	Brain Heart Infusion	5	lb
1.13825.0500	212425	Brain Heart Infusion Agar	100	g
1.13825.0500	211065	Brain Heart Infusion Agar	500	g
1.13825.0500	212166	Brain Heart Infusion Agar	5	lb
1.05454.0500	211079	Brilliant Green Bile Broth, 2%	100	g
1.05454.0500	211080	Brilliant Green Bile Broth, 2%	500	g
1.10490.0500	211086	Brucella Agar for Microbiology	500	g
1.07228.0500	212367	Buffered Peptone Water	500	g
1.02249.0001	232801	Campylobacter Agar Kit Skirrow	6 x 1	L
1.13678.0001	271045	CampyPak Plus Disposable Hydrogen+Carbon Dioxide Generator Envelope	10	ea
1.13699.0001	260656	CampyPak Pouch, CO2 enriched microaerophilic generating system	25	ea
1.02245.0500	223050	Casamino Acids	500	g
1.07324.0500	255320	Casein Soy Peptone Agar w/Polysorbate 80 and Lecithin	500	g
1.07324.0500	255310	Casein Soy Peptone Agar w/Polysorbate 80 and Lecithin	2	kg
1.16434.0500	218172	CIN Agar Base, Modified	500	g
1.01638.0500	212218	CLED Agar	500	g
1.13306.0001	240827	Coagulase Plasma, Rabbit, with EDTA	10	ea
1.10455.0500	211124	Columbia Agar Base	500	g
1.10455.5000	211125	Columbia Agar Base	5	lb

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Merck Cat.No.	BD/Difco Cat.No.	BD/Difco Product Description	Size	UOM
1.10270.0500	211144	DCLS Agar	500	g
1.10398.0500	265320	Demi-Fraser Broth Base	500	g
1.10398.0500	265310	Demi-Fraser Broth Base	10	kg
1.02896.0500	211154	Desoxycholate Citrate Agar	500	g
1.02894.0500	211160	Desoxycholate Lactose Agar	500	g
1.08342.1000	211863	Dextrose	1	lb
1.08342.2500	211864	Dextrose	5	lb
1.08342.1000	215530	Dextrose (Glucose, D(+), anhydrous)	500	g
1.08342.2500	215510	Dextrose (Glucose, D(+), anhydrous)	2	kg
1.08342.2500	215520	Dextrose (Glucose, D(+), anhydrous)	10	kg
1.10860.0500	211175	Dextrose Tryptone Agar	500	g
1.00466.0500	258710	Dichloran Rose Bengal Chloramphenicol Agar	500	g
1.10449.0500	211179	DNAse Test Agar	500	g
1.10765.0500	211187	EC Broth	500	g
1.10765.0500	231430	EC Medium	500	g
1.10765.0500	231410	EC Medium	10	kg
1.14582.0500	234020	EC Medium, Modified	500	g
1.03785.0001	212357	Egg Yolk Tellurite Solution	600	ml
1.04044.0500	212434	Endo Agar	100	g
1.04044.0500	211199	Endo Agar	500	g
1.01347.0500	211215	Eosin Methylene Blue Agar	500	g
1.01342.0500	212439	Eosin Methylene Blue Agar, Levine	100	g
1.01342.0500	211222	Eosin Methylene Blue Agar, Levine	5	lb
1.01342.0500	212256	Eosin Methylene Blue Agar, Levine	25	lb
1.05285.0500	210420	Fluid Tetrathionate Medium	2	kg
1.10398.0500	211767	Fraser Broth Base	500	g
1.10398.0500	211766	Fraser Broth Base	2	kg
1.10399.0001	211742	Fraser Broth Supplement	6x10	ml
1.10398.0500	265320	Fraser Broth, Demi	500	g
1.10398.0500	265310	Fraser Broth, Demi	10	kg
9.57008.0000	260411	GasPak 100 Lid	1	ea
1.13681.0001	260463	GasPak 100 Polycarbonate Jar	1	ea
1.13674.0001	260619	GasPak 100 Rack	1	ea
1.13675.0001	270504	GasPak Disposable Anaerobic Indicator	100	ea
1.13677.0001	271040	GasPak Plus Disposable Hydrogen+Carbon Dioxide Generator Envelope	10	ea
1.14255.0001	260652	GasPak Pouch Sealing Bars	10	ea
1.04070.0500	211868	Gelatin	500	g
1.07284.1000	211870	Gelysate Peptone	454	g
1.07284.1000	211870	Gelysate Peptone	1	lb
1.07284.1000	294627	Gelysate Peptone	5	lb
1.10756.0500	211279	GN Broth	500	g
1.10886.0500	244400	Heart Infusion Agar	100	g

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MICROBIOLOGY CROSS-REFERENCE LIST 2005: Merck - BD/Difco

Merck Cat.No.	BD/Difco Cat.No.	BD/Difco Product Description	Size	UOM
1.10886.0500	244100	Heart Infusion Agar	500	g
1.10886.0500	211839	Heart Infusion Agar	2	kg
1.11681.0500	212211	Hektoen Enteric Agar	500	g
1.11681.0500	212210	Hektoen Enteric Agar	100	g
1.11681.0500	212253	Hektoen Enteric Agar	5	lb
1.10886.0500	212423	Infusion Agar (Blood Agar Base)	100	g
1.10886.0500	211037	Infusion Agar (Blood Agar Base)	500	g
1.10707.0500	211313	KF Streptococcal Agar	500	g
1.03913.0500	212436	Kligler Iron Agar	100	g
1.03913.0500	211317	Kligler Iron Agar	500	g
1.07661.0500	211835	Lactose Broth	500	g
1.07661.2500	241000	Lactose Broth	2	kg
1.00547.0500	299190	LB Broth (Lennox L Broth Base)	500	g
1.00547.0500	240230	LB Broth, Lennox	500	g
1.00547.0500	240210	LB Broth, Lennox	2	kg
1.10285.0500	244620	LB Broth, Miller (Luria-Bertani)	500	g
1.10404.0500	263110	Letheen Agar, Modified	500	g
1.10405.0500	263010	Letheen Broth, Modified	500	g
1.11951.0500	220530	Listeria Enrichment Broth, Modified	500	g
1.05400.0500	211359	Lowenstein-Jensen Medium Base	500	g
1.10283.0500	299191	Luria Agar (Miller's LB Agar)	500	g
1.10285.0500	299192	Luria Broth (Miller's LB Broth Base)	500	g
1.15108.0500	218571	M 17 Agar	500	g
1.10658.0500	298126	M Broth	500	g
1.10658.0500	29420d	M Broth	500	g
1.10658.0500	294010	M Broth	2	kg
1.10750.0500	274930	m Endo Broth ww Millipore Filter	500	g
1.00549.0500	288330	m FC Broth Base	500	g
1.05465.0500	211387	MacConkey Agar	500	g
1.05465.5000	211390	MacConkey Agar	5	lb
1.05396.0500	211397	MacConkey Broth	500	g
1.05396.0500	220100	MacConkey Broth	500	g
1.05391.0500	211885	Malt Extract	500	g
1.05398.0500	211403	Malt Extract Agar	500	g
1.05397.0500	211320	Malt Extract Broth	500	g
1.05404.0500	211407	Mannitol Salt Agar	500	g
1.11277.0500	211203	M-Endo Agar LES	500	g
1.07324.0500	255320	Microbial Content Test Agar	500	g
1.07324.0500	255310	Microbial Content Test Agar	2	kg
1.14582.0500	234020	Modified EC Medium	500	g
1.14582.0500	234010	Modified EC Medium	2	kg
1.10404.0500	263110	Modified Letheen Agar	500	g

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MICROBIOLOGY CROSS-REFERENCE LIST 2005: Merck - BD/Difco

Merck Cat.No.	BD/Difco Cat.No.	BD/Difco Product Description	Size	UOM
1.10405.0500	263010	Modified Letheen Broth	500	g
1.11951.0500	220530	Modified Listeria Enrichment Broth	500	g
1.05712.0500	212445	MR-VP Broth	100	g
1.05712.0500	216200	MR-VP Medium	100	g
1.05712.0500	216100	MR-VP Medium	2	kg
1.10863.0500	218531	Muller Kauffmann Tetrathionate Broth Base	500	g
1.05267.0500	281010	MYP Agar	500	g
1.10456.0500	263510	Nickerson Medium	100	g
1.10456.0500	263520	Nickerson Medium	500	g
1.08190.0500	225710	NIH Thioglycollate Broth	500	g
1.09874.0001	231971	Novobiocin Antimicrobic Supplement	6x10	ml
1.05450.0500	212447	Nutrient Agar	100	g
1.05450.0500	211472	Nutrient Agar	500	g
1.05443.0500	211478	Nutrient Broth	100	g
1.05443.0500	211479	Nutrient Broth	500	g
1.07006.0001	211755	Oxford Antimicrobic Supplement	6x10	ml
1.13300.0001	235501	Oxidase Reagent	50x0.75	ml
1.12122.0001	263710	PALCAM Antimicrobic Supplement	3 x 10	ml
1.11755.0500	263620	PALCAM Medium Base	500	g
1.11755.0500	263610	PALCAM Medium Base	2	kg
1.07224.1000	211840	Peptone, Bacto	100	g
1.10987.0500	212451	Phenol Red Broth Base	100	g
1.10987.0500	211506	Phenol Red Broth Base	500	g
1.07212.0500	211906	Phytone Peptone	454	g
1.05467.0500	211546	Phytone Yeast Extract Agar	500	g
1.09875.0001	232681	Polymyxin B	6 x 10	ml
1.00510.0500	254920	Potato Dextrose Broth	500	g
1.00510.0500	254910	Potato Dextrose Broth	10	kg
1.00414.0500	219200	Presense-Absense Broth	500	g
1.10989.0500	244810	Pseudomonas Agar F	100	g
1.10989.0500	244820	Pseudomonas Agar F	500	g
1.10988.0500	244910	Pseudomonas Agar P	500	g
1.05284.0500	211553	Pseudosel Agar (Cetrimide Agar)	100	g
1.05284.0500	211554	Pseudosel Agar (Cetrimide Agar)	500	g
1.00416.0500	218262	R2A Agar	100	g
1.00416.0500	218263	R2A Agar	500	g
1.00416.0500	218261	R2A Agar	2	kg
1.07315.0500	274720	Sabouraud Agar Modified	500	g
1.07315.0500	274710	Sabouraud Agar Modified	2	kg
1.05438.0500	212456	Sabouraud Dextrose Agar	100	g
1.05438.0500	211584	Sabouraud Dextrose Agar	500	g
1.07315.0500	211589	Sabouraud Dextrose Agar Emmons	500	g

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MICROBIOLOGY CROSS-REFERENCE LIST 2005: Merck - BD/Difco

Merck Cat.No.	BD/Difco Cat.No.	BD/Difco Product Description	Size	UOM
1.08339.0500	210986	Sabouraud Liquid Broth Modified	500	g
1.08339.0500	264210	Sabouraud Medium, Fluid	500	g
1.07667.0500	211596	Salmonella Shigella Agar	100	g
1.07667.0500	211597	Salmonella Shigella Agar	500	g
1.07667.5000	211600	Salmonella Shigella Agar	5	lb
1.07667.5000	293306	Salmonella Shigella Agar	25	lb
1.07709.0500	211606	Selenite Cystine Broth	500	g
1.07709.0500	268730	Selenite Cystine Broth	100	g
1.07709.0500	268740	Selenite Cystine Broth	500	g
1.07709.0500	268710	Selenite Cystine Broth	2	kg
1.07717.0500	211607	Selenite-F Broth	100	g
1.07717.0500	211608	Selenite-F Broth	500	g
1.03032.0500	211576	SF Broth	500	g
1.05470.0500	212457	SIM Medium	100	g
1.05470.0500	211578	SIM Medium	500	g
1.02501.0500	211619	Simmons Citrate Agar	100	g
1.02501.0500	211620	Simmons Citrate Agar	500	g
1.15363.0500	211915	Skim Milk Powder	500	g
1.05459.0500	211824	Soybean - Casein Digest Medium (Tryptic Soy Broth)	100	g
1.05459.0500	211825	Soybean - Casein Digest Medium (Tryptic Soy Broth)	500	g
1.05459.0500	211822	Soybean - Casein Digest Medium (Tryptic Soy Broth)	2	kg
1.05459.0500	211823	Soybean - Casein Digest Medium (Tryptic Soy Broth)	10	kg
1.10235.0500	211580	SPS Agar	500	g
1.05463.0500	212638	Standard Methods Agar	500	g
1.05469.0500	212459	Staphylococcus Agar #110	100	g
1.05469.0500	211647	Staphylococcus Agar #110	500	g
1.08190.0500	225710	Sterility Test Broth (USP Alternate Thioglycollate Medium)	500	g
1.09874.0001	231971	Supplement N	6 x 10	ml
1.09877.0001	232671	Supplement O	10	ml
1.09875.0001	232681	Supplement P	6 x 10	ml
1.11723.0500	298410	TAT Broth Base	500	g
1.10263.0500	211685	TCBS Agar	100	g
1.10263.0500	211686	TCBS Agar	500	g
1.07680.0500	211702	Tergitol 7 Agar	500	g
1.07680.0500	245510	Tergitol 7 Agar	500	g
1.01629.0500	243820	Terrific Broth	500	g
1.05285.0500	210430	Tetrathionate Broth Base	500	g
1.05285.0500	210420	Tetrathionate Broth Base	2	kg
1.08190.0500	225710	Thioglycollate Medium USP Alternative	500	g
1.08191.0500	212461	Thioglycollate Medium, Fluid	100	g
1.08191.0500	211260	Thioglycollate Medium, Fluid	500	g
1.07224.2500	299599	Thiotone, E Peptone	5	lb

Please note: This designation comparison referes solely to the formulations of the compared culture media, nothing is said about their quality.

MICROBIOLOGY CROSS-REFERENCE LIST 2005: Merck - BD/Difco

Merck Cat.No.	BD/Difco Cat.No.	BD/Difco Product Description	Size	UOM
1.03915.0500	212462	Triple Sugar Iron Agar	100	g
1.03915.0500	211749	Triple Sugar Iron Agar	500	g
1.07324.0500	255320	Tryptic Soy Agar w/Lecithin and Polysorbate 80	500	g
1.07324.0500	255310	Tryptic Soy Agar w/Lecithin and Polysorbate 80	2	kg
1.05459.0500	211824	Tryptic Soy Broth (Soybean - Casein Digest Medium)	100	g
1.05459.0500	211825	Tryptic Soy Broth (Soybean - Casein Digest Medium)	500	g
1.05459.0500	211822	Tryptic Soy Broth (Soybean - Casein Digest Medium)	2	kg
1.05459.0500	211823	Tryptic Soy Broth (Soybean - Casein Digest Medium)	10	kg
1.10128.0500	212465	Trypticase Glucose Extract Agar	100	g
1.10128.0500	211760	Trypticase Glucose Extract Agar	500	g
1.07213.1000	211920	Trypticase Peptone	100	g
1.07213.1000	211921	Trypticase Peptone	454	g
1.07213.2500	211922	Trypticase Peptone	5	lb
1.05458.0500	211043	Trypticase Soy Agar	500	g
1.07324.0500	211764	Trypticase Soy Agar with Lecithin and Polysorbate 80	500	g
1.00800.0500	296264	Trypticase Soy Broth, Sterile	500	g
1.11723.0500	298410	Tryptone Azolectin Tween Broth Base	500	g
1.10128.0500	222000	Tryptone Glucose Extract Agar	100	g
1.10128.0500	223000	Tryptone Glucose Extract Agar	500	g
1.10128.0500	221000	Tryptone Glucose Extract Agar	2	kg
1.05463.0500	212638	Tryptone Glucose Yeast Agar	500	g
1.05264.0500	211690	TSN Agar (Tryticase Sulfite Neomycin)	500	g
1.08492.0500	212466	Urea Agar Base	100	g
1.08492.0500	211795	Urea Agar Base	500	g
1.08492.0500	228310	Urea Agar Base	100	g
1.08492.0500	228320	Urea Agar Base	500	g
1.08483.0500	212467	Urease Test Broth	100	g
1.08190.0500	225710	USP Thioglycollate Medium Alternative	500	g
1.10824.0500	212348	UVM Modified Listeria Enrichment Broth	500	g
1.01406.0500	212468	Violet Red Bile Agar	100	g
1.01406.0500	211807	Violet Red Bile Agar	500	g
1.04030.0500	229100	Violet Red Bile Agar w/MUG	500	g
1.04030.0500	298081	Violet Red Bile Agar with MUG	500	g
1.05405.0500	211812	Vogel & Johnson Agar	500	g
1.05287.0500	211838	XLD Agar	500	g
1.03753.0500	211928	Yeast Extract	100	g
1.03753.0500	211929	Yeast Extract	454	g
1.16434.0500	218171	Yersinia Selective Agar Base (CIN Agar Base, Modified)	10	kg

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MICROBIOLOGY CROSS-REFERENCE LIST 2005: Merck - Oxoid

Merck Cat.No.	Oxoid Cat.No.	Oxoid Product Description	Size	UOM
1.10230.0500	CM0833B	Aeromonas Medium Base (Ryan)	500	g
1.01614.0500	LP0011	Agar Bacteriological (Agar No. 1)	500	g
1.01614.1000	LP0011	Agar Bacteriological (Agar No. 1)	500	g
1.11925.9025	LP0013	Agar Technical (Agar No. 3)	5	kg
1.11925.1000	LP0013	Agar Technical (Agar No.3)	500	g
1.01800.0500	BO0035	Alkaline Peptone Water	500	g
1.13677.0001	BR0038B	Anaerobic Gas Generating Kit	10	env
1.13675.0001	BR0055B	Anaerobic Indicator	100	strips
1.13681.0001	HP0011A	Anaerobic Jar	3,5	litre
1.13681.0001	HP0031A	Anaerobic Jar	3,5	litre
1.13677.0001	AN0025A	AnaeroGen 2.5 litre	10	env
1.13677.0001	AN0035A	AnaeroGen 3.5 litre	10	env
1.13677.0001	AN0020C	AnaeroGen Compact	20	env
1.14255.0001	AN005C	AnaeroGen Compact Sealing Bars	5	clips
1.13681.0001	AG0025A	AnaeroJar	1	ea
9.57008.0000	AG0027A	AnaeroJar Lid	1	ea
1.13674.0001	AG0029A	AnaeroJar Plate Carrier	1	ea
1.05272.0500	CM0327B	Antibiotic Medium No. 1 (Seed Agar)	500	g
1.05273.0500	CM0287B	Antibiotic Medium No. 3 (Assay Broth)	500	g
1.05273.0500	CM0287B	Assay Broth (Antibiotic Medium No. 3)	500	g
1.05267.0500	CM0929B	Bacillus Cereus Selective Agar Base	500	g
1.09875.0001	SR0099E	Bacillus Cereus Selective Supplement	10	vials
1.05406.0500	CM0275B	Baird-Parker Agar Base	500	g
1.10456.0500	CM0589B	Biggy Agar (Nickerson Medium)	500	g
1.11432.0500	CM0888B	Bile Aesculin Agar	500	g
1.03756.0500	LP0055J	Bile Salts	250	g
1.03756.0500	LP0056J	Bile Salts No. 3	250	g
1.05418.0500	CM0201B	Bismuth Sulphite Agar (Modified Wilson & Blair Medium)	500	g
1.10886.0500	CM0055B	Blood Agar Base	500	g
1.10886.0500	CM0055T	Blood Agar Base	5	kg
1.10328.0500	CM0271B	Blood Agar Base No. 2	500	g
1.10328.0500	CM0271R	Blood Agar Base No. 2	2,5	kg
1.10328.0500	CM0271T	Blood Agar Base No. 2	5	kg
1.13825.0500	CM0375B	Brain Heart Infusion Agar	500	g
1.10493.0500	CM0225B	Brain Heart Infusion Broth	500	g
1.10493.0500	CM0225R	Brain Heart Institute Broth	2,5	kg
1.07232.0500	CM0263B	Brilliant Green Agar (Kauffmann Medium)	500	g
1.10747.0500	CM0329B	Brilliant Green Agar (Modified)(Edel-Kampelbacher Medium)	500	g
1.10747.0500	CM0329R	Brilliant Green Agar (Modified)(Edel-Kampelbacher Medium)	2,5	kg

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MICROBIOLOGY CROSS-REFERENCE LIST 2005: Merck - Oxoid

Merck Cat.No.	Oxoid Cat.No.	Oxoid Product Description	Size	UOM
1.05454.0500	CM0031B	Brilliant Green Bile 2% Broth	500	g
1.12587.0500	CM0031B	Brilliant Green Bile 2% Broth	500	g
1.10490.0500	CM0169B	Brucella Medium Base	500	g
1.07228.0500	CM0509B	Buffered Peptone Water	500	g
1.01638.0500	CM0301B	C.L.E.D. Medium	500	g
1.01638.0500	CM0301R	C.L.E.D. Medium	2,5	kg
1.13678.0001	CN0025	CampyGen	10	env
1.13678.0001	CN0035	CampyGen	10	env
1.13699.0001	CN0020	CampyGen Compact	20	env
1.02248.0500	CM0689B	Campylobacter Agar Base	500	g
1.13678.0001	BR0056	Campylobacter Gas Generating Kit	10	env
1.13678.0001	BR0060	Campylobacter Gas Generating Kit	10	env
1.02249.0001	SR0069E	Campylobacter Selective Supplement (Skirrow)	10	vials
1.02249.0001	SR0069H	Campylobacter Selective Supplement (Skirrow)	10	vials
1.02245.0500	LP0041B	Casein Hydrolysate (Acid)	500	g
1.02245.5000	LP0041B	Casein Hydrolysate (Acid)	500	g
1.05284.0500	CM0579B	Cetrimide Agar	500	g
1.01639.0500	CM0209B	China Blue Lactose Agar	500	g
1.10426.0500	CM0956A	Chromogenic E. Coli/Coliform Medium	100	g
1.10426.0500	CM0956B	Chromogenic E. Coli/Coliform Medium	500	g
1.11708.0500	CM0353B	Clausen Medium - Dithionite Thioglycollate (HS-T) Medium	500	g
1.10455.0500	CM0331B	Columbia Blood Agar Base	500	g
1.10455.5000	CM0331R	Columbia Blood Agar Base	2,5	kg
1.10455.5000	CM0331T	Columbia Blood Agar Base	5	kg
1.09202.0001	SR0172E	CT Supplement	10	vials
1.09202.0001	SR0172H	CT Supplement	10	vials
1.09202.0001	SR0172N	CT Supplement	10	vials
1.05460.0500	CM0097B	Czapek Dox Agar Modified	500	g
1.10270.5000	CM0393B	D.C.L.S Agar (Desoxycholate Lactose Sucrose)	500	g
1.10896.0500	CM0539B	Dermasel Medium	500	g
1.02894.0500	CM0163B	Desoxycholate Agar	500	g
1.02896.0500	CM0035B	Desoxycholate Citrate Agar	500	g
1.02896.0500	CM0227B	Desoxycholate Citrate Agar (Hynes)	500	g
1.10860.0500	CM0075B	Dextrose Tryptone Agar	500	g
1.00465.0500	CM0729B	Dichloran Glycerol (DG-18) Selective Medium	500	g
1.00466.0500	CM0727B	Dichloran Rose-Bengal Chloramphenicol Agar	500	g
1.10449.0500	CM0321B	DNase Agar	500	g
1.10765.0500	CM0853B	E.C. Broth	500	g
1.05394.0500	CM0317B	E.E. Broth (Buffered Brilliant Bile Broth)	500	g

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MICROBIOLOGY CROSS-REFERENCE LIST 2005: Merck - Oxoid

Merck Cat.No.	Oxoid Cat.No.	Oxoid Product Description	Size	UOM
1.03784.0001	SR0047C	Egg Yolk Emulsion	100	ml
1.03785.0001	SR0054C	Egg Yolk Tellurite Emulsion	100	ml
1.04044.0500	CM0479B	Endo Agar Base	500	g
1.01342.0500	CM0069B	Eosin Methylene Blue Agar (Levine)	500	g
1.08191.0500	CM0173B	Fluid Thiglycollate Medium (USP)	500	g
1.10398.0500	CM0895B	Fraser Broth	500	g
1.10399.0001	SR0156E	Fraser Supplement	10	vials
1.00800.0500	CM1016B	Gamma Irradiated Tryptone Soya Broth (Gamma Irradiated TSB)	500	g
1.00800.0500	CM1016T	Gamma Irradiated Tryptone Soya Broth (Gamma Irradiated TSB)	5	kg
1.04070.0500	LP0008B	Gelatin Bacteriological	500	GM
1.10675.0500	CM0523B	Giolitti-Cantoni Broth	500	g
1.10399.0001	SR0166E	Half Fraser Supplement	10	vials
1.10399.0001	SR0166G	Half Fraser Supplement	10	vials
1.11681.0500	CM0419B	Hektoen Enteric Agar	500	g
1.10864.0500	CM0079B	Iron Sulphite Agar	500	g
1.05222.0500	CM0591B	Kanamycin Acsculin Azide Agar Base	500	GM
1.10707.0500	CM0701B	K-F Streptococcus Agar	500	g
1.03913.0500	CM0033B	Kligler Iron Agar	500	g
1.05450.0500	CM0017B	Lab-Lemco Agar (Nutrient Agar)	500	g
1.05443.0500	CM0015B	Lab-Lemco Broth (Nutrient Broth)	500	g
1.03979.0500	LP0029B	Lab-Lemco Powder (Beef Extract)	500	g
1.03979.2500	LP0029T	Lab-Lemco Powder (Beef Extract)	5	kg
1.12523.1000	LP0048B	Lactalbumin Hydrolysate	500	g
1.07661.0500	CM0137B	Lactose Broth	500	g
1.07661.1000	CM0137B	Lactose Broth	500	g
1.07661.2500	CM0137B	Lactose Broth	500	g
1.10266.0500	CM0451B	Lauryl Tryptose Broth (Lauryl Sulphate Broth)	500	g
1.10266.5000	CM0451B	Lauryl Tryptose Broth (Lauryl Sulphate Broth)	500	g
1.10240.0001	SR0110A	Legionella BCYE Growth Supplement	10	vials
1.10240.0001	SR0110C	Legionella BCYE Growth Supplement	10	vials
1.10242.0001	CM0655A	Legionella CYE Agar Base	500	g
1.10241.0001	SR0152E	Legionella GVPC Selective Supplement	10	vials
1.10549.0500	CM0862B	Listeria Enrichment Broth	500	g
1.11951.0500	CM0862B	Listeria Enrichment Broth	500	g
1.10824.0500	CM0863B	Listeria Enrichment Broth Base (UVM formulation)	500	g
1.04039.0001	SR0143E	Listeria Secondary Selective Enrichment Supplement (UVM II)	10	vials
1.07004.0500	CM0856B	Listeria Selective Agar Base (Oxford)	500	g
1.11883.0001	SR0141E	Listeria Selective Enrichment Supplement	10	vials

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MICROBIOLOGY CROSS-REFERENCE LIST 2005:
Merck - Oxoid

Merck Cat.No.	Oxoid Cat.No.	Oxoid Product Description	Size	UOM
1.11781.0001	SR0149E	Listeria Selective Enrichment Supplement (modified with 10mg/liter Acriflavine)	10	vials
1.07006.0001	SR0140E	Listeria Selective Supplement	10	vials
1.07006.0001	SR0140B	Listeria Selective Supplement	10	vials
1.11640.0500	CM0381B	Lysine Iron Agar	500	g
1.15108.0500	CM0785B	M17 Agar	500	g
1.15029.0500	CM0817B	M17 Broth	500	g
1.05465.5000	CM0109B	MacConkey No. 2	500	g
1.05465.0500	CM0115B	MacConkey No. 3 (US formulation)	500	g
1.05465.5000	CM0115B	MacConkey No. 3 (US formulation)	500	g
1.11929.1000	LP0039B	Malt Extract	500	g
1.05398.5000	CM0059B	Malt Extract Agar	500	g
1.05398.0500	CM0059B	Malt Extract Agar	500	g
1.05397.0500	CM0057B	Malt Extract Broth	500	g
1.05404.0500	CM0085B	Mannitol Salt Agar (Chapman Medium)	500	g
1.12535.0500	CM0733B	Maximum Recovery Diluent (Peptone Salt Broth)	500	g
1.12535.0500	CM0733R	Maximum Recovery Diluent (Peptone Salt Broth)	2,5	kg
1.11277.0500	MM0551B	Membrane Endo Agar LES	500	g
1.15338.0500	CM0681B	Milk Plate Count Agar (with Antibiotic Free Skimmed Milk)	500	g
1.12588.0500	CM0967B	Modified Lauryl Tryptose Broth with MUG and added Tryptophane	500	g
1.09878.0500	CM0910B	Modified Semisolid Rappaport Vassiliadis (MSRV) Medium	500	g
1.09205.0500	CM0989B	Modified Tryptone Soya Broth (mTSB)	500	g
1.10660.0500	CM0361B	MRS Agar	500	g
1.10661.0500	CM0359B	MRS Broth	500	g
1.05712.0500	CM0043B	MRVP Medium (Clarks and Lubs Medium)	500	g
1.09878.0500	CM0910B	MSRV Medium Base	500	g
1.09874.0001	SR0161E	MSRV Selective Supplement	10	vials
1.10863.0500	CM0343B	Muller-Kauffmann Tetrathionate Broth Base	500	g
1.10863.0500	CM0343R	Muller-Kauffmann Tetrathionate Broth Base	2,5	kg
1.05267.0500	CM0929B	MYP Agar (Mannitol Egg Yolk Polymyxin Agar)	500	g
1.09874.0001	SR0181E	Novobiocin Supplement	10	vials
1.07883.0500	CM0003B	Nutrient Agar	500	g
1.05443.0500	MCM0001B	Nutrient Broth	500	g
1.10685.0500	CM0635B	Nutrient Gelatin	500	g
1.09877.0001	SR0073A	OGY Selective Supplement	10	vials
1.10673.0500	CM0657B	Orange Serum Agar	500	g
1.13300.0001	BR0064A	Oxidase Identification Sticks	100	sticks
1.10877.0500	CM0545B	Oxytetracycline-Glucose-Yeast Extract Selective Medium (OGYE)	500	g
1.11755.0500	CM0877B	PALCAM Agar Base	500	g

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MICROBIOLOGY CROSS-REFERENCE LIST 2005: Merck - Oxoid

Merck Cat.No.	Oxoid Cat.No.	Oxoid Product Description	Size	UOM
1.11755.0500	CM0877R	PALCAM Agar Base	2,5	kg
1.12122.0001	SR0150E	PALCAM Selective Supplement	10	vials
1.12122.0001	SR0150B	PALCAM Selective Supplement	10	vials
1.07214.1000	LP0037B	Peptone Bacteriolgocal	500	g
1.07214.2500	LP0037T	Peptone Bacteriological	5	kg
1.07224.1000	LP0049B	Peptone P	500	g
1.07224.2500	LP0049B	Peptone P	500	g
1.00888.0001	SR0088E	Perfringens (TSC) Selective Supplement B	10	vials
1.10235.0500	CM0543B	Perfringens Agar Base (OPSP)	500	g
1.11972.0500	CM0587B	Perfringens Agar Base (TSC/SFP)	500	g
1.05463.0500	CM0325B	Plate Count Agar	500	g
1.15338.0500	CM0681B	Plate Count Agar with Antibiotic Free Skim Milk	2,5	kg
1.05164.0100	SR0030J	Potassium Tellurite 3.5%	2	ml
1.10130.0500	CM0139B	Potato Dextrose Agar	500	g
1.10988.0500	CM0559B	Pseudomonas Agar Base	500	g
1.01613.1000	LP0028B	Purified Agar	500	g
1.00416.0500	CM0906B	R2A Agar	500	g
1.10236.0500	CM669B	Rappaport Vassiliadis (RV) Enrichment Broth	500	g
1.07700.0500	CM0866B	Rappaport Vassiliadis Soya (RVS) Peptone Broth	500	g
1.07700.9025	CM0866B	Rappaport Vassiliadis Soya (RVS) Peptone Broth	500	g
1.05410.0500	CM0151B	Reinforced Clostridial Agar	500	g
1.05411.0500	CM0149B	Reinforced Clostridial Medium (RCM) (semi solid)	500	g
1.15525.0001	BR0049G	Ringers Solution Tablets	100	tablets
1.05413.0500	CM0627B	Rogosa Agar	500	g
1.00467.0500	CM0549B	Rose-Bengal Chloraphenicol Agar Base	500	g
1.00467.0500	CM0549R	Rose-Bengal Chloraphenicol Agar Base	500	g
1.05470.0500	CM0435B	S.I.M. Medium	500	g
1.05438.0500	CM0041B	Sabouraud Dextrose Agar	500	g
1.08339.0500	CM0147B	Sabouraud Liquid Medium	500	g
1.05439.0500	CM0541B	Sabouraud Maltose Agar	500	g
1.05272.0500	CM0327	Seed Agar (Antibiotic Medium No. 1)	500	g
1.07717.0500	CM0395B	Selenite Broth Base	500	g
1.07709.0500	CM0699B	Selenite Cystine Broth Base	500	g
1.02501.0500	CM0155B	Simmons Citrate Agar	500	g
1.15363.0500	LP0031B	Skim Milk Powder	500	g
1.05262.0500	CM0377B	Slanetz and Bartley Medium (Enterococcus Agar)	500	g
1.04036.0500	CM0813B	Sorbitol MacConkey Agar	500	g
1.07212.0500	LP0044B	Soya Peptone Neutralized	500	g
1.07667.0500	CM0099B	SS Agar	500	g

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MICROBIOLOGY CROSS-REFERENCE LIST 2005:
Merck - Oxoid

Merck Cat.No.	Oxoid Cat.No.	Oxoid Product Description	Size	UOM
1.07667.5000	CM099B	SS Agar	500	g
1.05463.0500	CM0463B	Standard Plate Count Agar (APHA)	500	g
1.05469.0500	CM0145B	Staphylococcus Medium No.110	500	g
1.10263.0500	CM0333B	TCBS Cholera Medium	500	g
1.07680.0500	CM0793B	Tergitol-7 Agar	500	g
1.05285.0500	CM0671B	Tetrathionate Broth (USA)	500	g
1.08191.0500	CM0391B	Thiglycollate Medium (USP), alternative fluid	500	g
1.08190.0500	CM0173B	Thioglycollate Medium	500	g
1.03915.0500	CM0277B	Triple Sugar Iron Agar	500	g
1.07213.1000	LP0042B	Tryptone	500	g
1.07213.2500	LP0042T	Tryptone	5	kg
1.10128.0500	CM0127B	Tryptone Glucose Extract Agar	500	g
1.10676.0500	CM0283B	Tryptone Phosphate Broth	500	g
1.05458.0500	CM0131B	Tryptone Soya Agar	500	g
1.05459.9025	CM129R	Tryptone Soya Broth	2,5	kg
1.05459.0500	CM0129B	Tryptone Soya Broth (Gamma irradiated)	500	g
1.05459.0500	CM0129B	Tryptone Soya Broth (Soybean Casein Digest Medium USP)	500	g
1.11931.1000	LP0043B	Tryptone T	500	g
1.11931.9025	LP0043B	Tryptone T	500	g
1.10859.0500	CM0087B	Tryptone Water	500	g
1.10213.1000	LP0047B	Tryptose	500	g
1.10213.9025	LP0047B	Tryptose	500	g
1.08492.0500	CM0053B	Urea Agar Base (Christensen Agar Base)	500	g
1.08483.0500	CM0071B	Urea Broth Base (Christensen Broth Base)	500	g
1.00525.0500	VG0101B	Vegetable Peptone Broth	500	g
1.01406.0500	CM0107B	Violet Red Bile (Lactose) Agar	500	g
1.04030.0500	CM0978B	Violet Red Bile Agar (VRBA) with MUG	500	g
1.10275.0500	CM0485B	Violet Red Bile Glucose Agar	500	g
1.10275.2500	CM0485B	Violet Red Bile Glucose Agar	2,5	kg
1.05405.0500	CM0641B	Vogel-Johnson Agar	500	g
1.10866.0500	CM0309B	W.L. Nutrient Agar (Medium)	500	g
1.05448.0500	CM0247B	Wort Agar	500	g
1.05287.0500	CM0469B	XLD Medium	500	g
1.03750.0500	CM0019B	Yeast Extract Agar	500	g
1.03750.0500	CM0019R	Yeast Extract Agar	2,5	kg
1.03753.0500	LP0021B	Yeast Extract Powder	500	g
1.16434.0500	CM0653B	Yersina Selective Agar Base (CIN Agar Base/Schiemann Medium Base)	500	g
1.16466.0001	SR0109E	Yersinal Selective Supplement	10	vials

Please note: This designation comparison referes solely to the formulations of the compared culture media, nothing is said about their quality.

Collection of microorganisms

Abbreviations	Addresses	Important microorganisms
DSMZ	Deutsche Sammlung von Mikroorganismen und Zellkulturen Gesellschaft für Biotechnologische Forschung mbH Mascheroder Weg 1 B D-38124 Braunschweig, Germany	Apathogenic funghi and bacteria
ATCC	American Type Culture Collection 12 301 Parklawn Drive Rockville, Maryland 20 852, USA	All types
NCIB	National Collection of Industrial Bacteria 135 Abbey Road Aberdeen, AB9 8DG, Scotland	Apathogenic bacteria
NCTC	National Collection of Type Cultures Central Public Health Laboratory Colindale Ave. London NW9 5HT, Great Britain	Bacteria
NRRL	Northern Regional Research Laboratory ARS Culture Collection US Department of Agriculture 1815 North University Street Peoria, Illinois 61604, USA	Agriculture strains
DBDR	Division of Bacteriology and Dairy Research Science Service Ottawa, Canada	Funghi, bacteria
NCYC	National Collection of Yeast Cultures Agricultural Council Food Research Institute Colney Lane Norwich NR4 7UA, Great Britain	Yeasts
MRL	Mycological Reference Laboratory London School of Hygiene and Tropical Medicine Keppel Street London WC 1E 7HT, Great Britain	Pathogenic funghi
CBS	Centraalbureau voor Schimmelcultures Baarn Oosterstraat 1, P.O. Box 273 3740 Baarn, The Netherlands	Funghi, actinomyces

More information about specific strains and the above listed organizations can be obtained by:
International Center for Information and Distribution of Type Cultures, 19 Avenue Cesar Roux, 1000 Lausanne, Switzerland.


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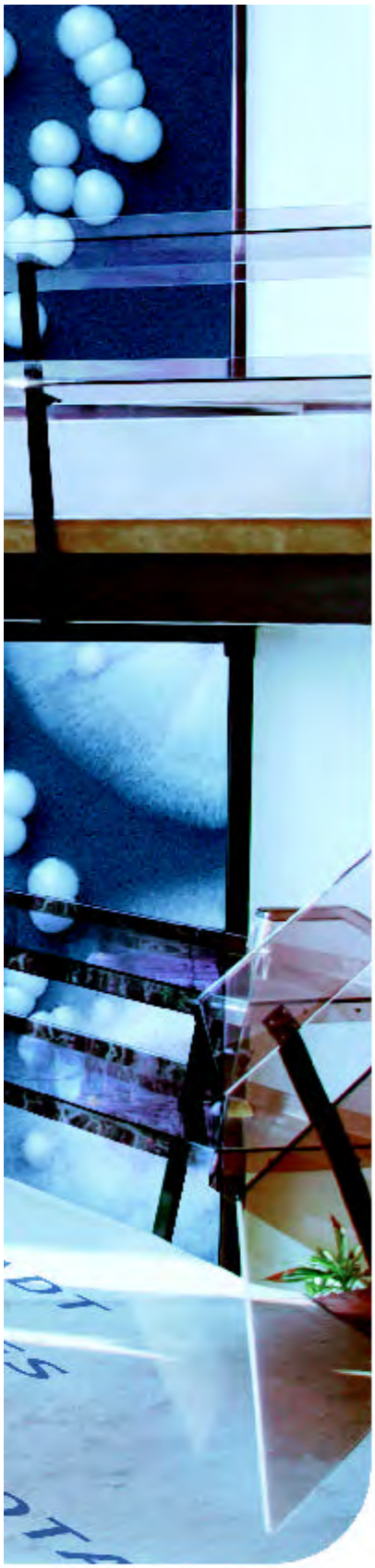

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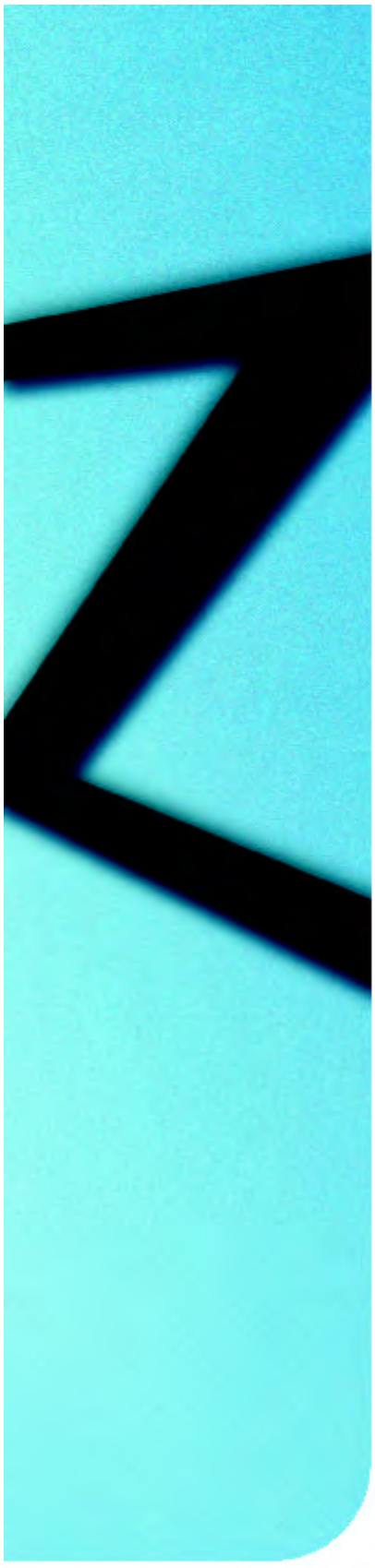


Review Index

Review Index

A

B

C

D

E

F

G

H

K

L

M

N

O

P

R

S

T

U

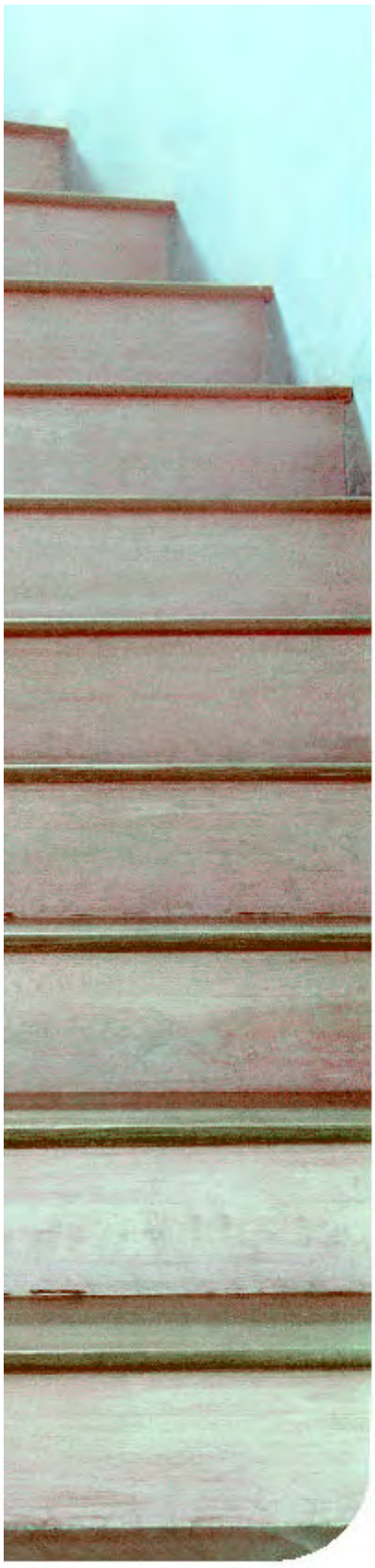
V

W

X

Y


11
10
09
08
07
06
05
04
03
02
01



Numerical Index

Numerical Index

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