

Columbia Agar Plate

MPH144

For the selection and subculture of *Clostridium sporogenes* in accordance with the harmonized method of USP/EP/BP/JP/IP.

Composition**

Ingredients	Gms / Litre
Pancreatic digest of casein	10.000
Meat peptic digest	5.000
Heart pancreatic digest	3.000
Yeast extract	5.000
Maize starch	1.000
Sodium chloride	5.000
Agar	15.000

**Formula adjusted, standardized to suit performance parameters

Directions

Either streak, inoculate or surface spread the test inoculum (50-100 CFU) aseptically on the plate.

Principle And Interpretation

Columbia Blood Agar Base used as a general-purpose nutritious medium was devised by Ellner et al from Columbia University, which was further enriched by the addition of sheep blood (1). It can also be used for the isolation of organisms by addition of various supplements. Columbia Agar is prepared in accordance with the microbial limit testing harmonized methodology of USP/EP/BP/JP (2,3,4,5). This medium is recommended to check the presence of *Clostridium* in non-sterile products like food, dietary, nutritional supplements related products. The genus *Clostridium* belongs to the family Clostridiaceae in the class Clostridia.

The product to be examined is initially enriched in Reinforced medium for clostridia . This medium contains 0.05% Agar and cysteine, which creates anaerobic conditions, thereby allowing anaerobic organisms to grow. The enriched sample is then subcultured on Columbia Agar. Columbia Agar is used as a base for media containing blood and for selective media formulations in which different combinations of antimicrobial agents are used as additives.

.,This medium is highly nutritious as it contains pancreatic digest of casein, meat peptic digest, heart pancreatic digest and yeast extract which supports rapid and luxuriant growth of fastidious as well as non-fastidious organisms. Sodium chloride maintains osmotic balance of medium. Maize starch acts as an energy source and also neutralizes toxic metabolites if produced. It is used in detection of Clostridia from pharmaceutical products. Gentamicin (FD252) inhibits a number of contaminating gram-negative organisms and Staphylococcus species.

Clostridia grows under anaerobic conditions as gram positive rods giving a catalase negative test. Further confirmation is carried out by identification tests.

Quality Control

Appearance

Sterile Columbia Agar in 90 mm disposable plates.

Colour of medium

Light amber coloured medium

Quantity of medium

25 ml of medium in 90 mm disposable plates.

pH

7.10- 7.50

Growth Promotion Test

Growth Promotion was carried out in accordance with the harmonized method of USP/EP/BP/JP, and growth was observed under anaerobic conditions after an incubation at 30-35°C for 48 hours. Recovery rate is considered as 100% for bacteria growth on Casein Soybean Digest Agar (Soybean Casein Digest Agar).

Growth promoting properties

Growth of microorganism comparable to that previously obtained with previously tested and approved lot of medium occurs at the specified temperature for not more than the shortest period of time specified inoculating ≤ 100 cfu under anaerobic conditions (at 30-35°C for 48 hours).

Sterility Test

Passes release criteria

Cultural Response

Organism	Growth	Inoculum	Observed Lot value (CFU)	Recovery	Incubation Temp.	Incubation period
Growth Promoting <i>Clostridium sporogenes</i> ATCC 19404	luxuriant	50-100	25 -100	≥ 50 %	30 -35 °C	≤ 48 hrs
<i>Clostridium sporogenes</i> ATCC 11437	good-luxuriant	50-100	25 -100	≥ 50 %	30 -35 °C	≤ 48 hrs
<i>Bacteroides vulgatus</i> ATCC 8482	luxuriant	50-100	25 -100	≥ 50 %	30 -35 °C	≤ 48 hrs
Additional Microbiological testing						
<i>Bacteroides fragilis</i> ATCC 23745	luxuriant	50-100	25 -100	≥ 50 %	30 -35 °C	≤ 48 hrs
<i>Streptococcus pyogenes</i> ATCC 19615	inhibited	$\geq 10^3$	0	0 %	30 -35 °C	≥ 72 hrs
<i>Neisseria meningitidis</i> ATCC 13090	inhibited	$\geq 10^3$	0	0 %	30 -35 °C	≥ 72 hrs
<i>Staphylococcus epidermidis</i> ATCC 12228	inhibited	$\geq 10^3$	0	0 %	30 -35 °C	≥ 72 hrs
<i>Clostridium perfringens</i> ATCC 13124	luxuriant	50-100	25 -100	≥ 50 %	30 -35 °C	≤ 48 hrs
<i>Staphylococcus aureus</i> ATCC 6538	inhibited	$\geq 10^3$	0	0 %	30 -35 °C	≥ 72 hrs
<i>Staphylococcus aureus</i> ATCC 25923	inhibited	$\geq 10^3$	0	0 %	30 -35 °C	≥ 72 hrs

Storage and Shelf Life

Store between 15-25°C. Use before expiry date on the label.

Reference

1. Ellner P. P., Stoessel C. J., Drakeford E. and Vasi F., 1966, Am. J. Clin. Pathol., 45:502.
2. Fildes P., 1920, Br. J. Exp. Pathol., 1:129.
3. Fildes P., 1921, Br. J. Exp. Pathol., 2:16.
4. Chapin K. C. and Doern G. V., 1983, J. Clin. Microbiol., 17:1163.
5. Bailey R. K., Voss J. L. and Smith R. F., 1979, J. Clin. Microbiol., 9 ; 65-71

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