Columbia Agar

MH144

Intended use

Recommended for detection of *Clostridium sporogenes* from pharmaceutical products in accordance with the microbial limit testing by harmonized methodology of USP/EP/BP/JP/IP (Medium 15).

Composition**

Ingredients	Gms / Litre
Tryptone #	10.000
HM extract ##	5.000
HM hydrolysate ###	3.000
Yeast extract	5.000
Maize starch	1.000
Sodium chloride	5.000
Agar	15.000

If necessary adjust the pH so that after sterilization it is 7.3±0.2

* pH can also be measured after sterilization at 25°C

**Formula adjusted, standardized to suit performance parameters

Pancreatic digest of casein
Meat peptic digest
Heart pancreatic digest

Directions

Suspend 44 grams in 1000 ml purified/distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes or as per validated cycle. Cool to 45-50°C, if required add the rehydrated contents of 1 vial of Gentamicin Selective Supplement (FD252). Mix well and pour into sterile Petri plates.

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 (2). 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/IP (8,1,3,6,4). 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 tryptone, HM extract and HM hydrolysate and yeast extract provides carbonaceous and nitrogenous substances, long chain amino acids, vitamins of B complex groupand other essential nutrients for the 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.

Type of specimen
Pharmaceutical samples; Clinical samples

Specimen Collection and Handling
For pharmaceutical samples, follow appropriate techniques for sample collection, processing as per guidelines (8,1,3,6,4). For clinical samples follow appropriate techniques for handling specimens as per established guidelines (5,7). After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions:
In Vitro diagnostic use only. Read the label before opening the container. Wear protective gloves/ protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations
1. Some *Clostridium* species may show poor growth due to nutritional variations.
2. Further biochemical tests must be carried out for complete identification.

Performance and Evaluation
Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control
Appearance
Cream to yellow homogeneous free flowing powder

Gelling
Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium
Light amber coloured clear to slightly opalescent gel forms in Petri 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 24-48 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).

Cultural Response

Organism	Inoculum (CFU)	Growth	Observed Lot value (CFU)	Recovery	Incubation temperature	Incubation period
Growth Promoting						
Clostridium sporogenes ATCC 19404 (00008)*	50 -100	luxuriant	25 -100	>=50 %	30 -35 °C	<=48 hrs
Clostridium sporogenes ATCC 11437	50 -100	luxuriant	25 -100	>=50 %	30 -35 °C	<=48 hrs
Bacteroides vulgatus ATCC 8482	50 -100	luxuriant	25 -100	>=50 %	30 -35 °C	<=48 hrs
Additional Microbiological testing						
Clostridium perfringens ATCC 13124 (00007)*	50 -100	luxuriant	25 -100	>=50 %	30 -35 °C	<=48 hrs
Bacteroides fragilis ATCC 23745	50 -100	luxuriant	25 -100	>=50 %	30 -35 °C	<=48 hrs

Key : (*) Corresponding WDCM numbers

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 20-30°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Use before expiry date on the label. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (5,7).

Reference

1. British Pharmacopoeia, 2016, The Stationery office British Pharmacopoeia
2. Ellner, Stoessel, Drakeford and Vasi, 1966, Am. J. Clin. Pathol., 45:502.
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4. Indian Pharmacopoeia, 2018, Govt.of India, the Controller of Publication , New Delhi
5. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition
6. Japanese Pharmacopoeia, 2016.
7. Jorgensen,J.H., Pfaller , M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. , 11th Ed., 2015, Manual of Clinical Microbiology,
8. The United States Pharmacopoeia, 2019, The United States Pharmacopeial Convention. Rockville, MD.

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