Columbia Agar Plate (γ-irradiated, Triple Pack) MPH144GT

Intended Use

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

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

**Formula adjusted, standardized to suit performance parameters

Pancreatic digest of casein ## Meat peptic digest ### Heart pancreatic digest

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/IP (2-6). 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, HM hydrolysate 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. 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

Specimen Collection and Handling

For pharmaceutical samples, follow appropriate techniques for sample collection, processing as per guidelines (2-6).
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/face protection. Follow 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) Individual strain of a microorganism may have unique growth requirements with respect to nutrients and physical conditions. Based on which the growth pattern of each varies on a medium and some even may display significant delay in development.

2) Each lot of the medium has been tested for the organisms specified on the COA. It is recommended to users to validate the medium for any specific microorganism other than mentioned in the COA based on the user's unique requirement.

3) It is recommended to store the plates ta 24-30°C to avoid minimum condensation.

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

Sterile Columbia Agar in 90 mm disposable plates (γ-irradiated, Triple Pack)

Colour of medium

Light amber coloured medium

Quantity of medium

25 ml of medium in 90 mm disposable plates

Dose of irradiation (Kgy)

13.00- 20.00

pH

7.10- 7.50

Growth Promotion Test

Growth Promotion was carried out in accordance with the harmonized method of USP/EP/BP/JP/IP, 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 Promoting**	**Inoculum**	**Growth**	**Observed Lot Value**	**Recovery**	**Incubation Temp.**	**Incubation period**
Clostridium sporogenes ATCC 19404 (00008*)	50-100	luxuriant	25 -100	>=50 %	30 -35 °C	<=48 hrs
Clostridium sporogenes ATCC 11437	50-100	good-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						
Bacteroides fragilis ATCC 23745	50-100	luxuriant	25 -100	>=50 %	30 -35 °C	<=48 hrs
Streptococcus pyogenes ATCC 19615	>=10^4	inhibited	0	0 %	30 -35 °C	>=72 hrs
Neisseria meningitidis ATCC13090	>=10^4	inhibited	0	0 %	30 -35 °C	>=72 hrs
Staphylococcus epidermidis ATCC 12228 (00036*)	>=10^4	inhibited	0	0 %	30 -35 °C	>=72 hrs
Clostridium perfringens ATCC 13124 (00007*)	50-100	luxuriant	25 -100	>=50 %	30 -35 °C	<=48 hrs

Staphylococcus aureus subsp. aureus ATCC 6538 (00032)*	>=10^4	inhibited	0	0 %	30 -35 °C	>=72 hrs
Staphylococcus aureus subsp. aureus ATCC 25923 (00034)*	>=10^4	inhibited	0	0 %	30 -35 °C	>=72 hrs

Key : (*) Corresponding WDCM numbers

Storage and Shelf Life

On receipt store between 20-30°C 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 (7,8).

Reference

1. Ellner, Stoessel, Drakeford and Vasi, 1966, Am. J. Clin. Pathol., 45:502.
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6. Indian Pharmacopoeia, 2018, Govt.of India, the Controller of Publication , New Delhi
7. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition
8. 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, 11th Edition. Vol. 1.

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