Cetrimide Agar Plate

MPH024

Intended use

Recommended for the selective isolation of *Pseudomonas aeruginosa* from pharmaceutical products in accordance with the microbial limit testing by harmonized methodology of USP/EP/BP/JP/IP.

Composition**

Ingredients	g / L
Gelatin peptone #	20.000
Magnesium chloride	1.400
Dipotassium sulphate	10.000
Cetrimide	0.300
Glycerol	10.000
Agar	13.600
pH after sterilization (at 25°C)	7.2±0.2

**Formula adjusted, standardized to suit performance parameters

Pancreatic digest of gelatin

Directions

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

Principle And Interpretation

Cetrimide Agar was described by King et al (1). This media formulation is in accordance with the harmonized method of USP/EP/BP/JP/IP (2,3,4,5,6). It is used as a selective medium for the isolation of *Pseudomonas aeruginosa* from pharmaceutical products. This medium is also used for microbial limit testing for non- sterile products. Lowburry first reported the use of cetrimide as an agent for selective isolation of *Pseudomonas* (7). This medium is also used for determining the ability of an organism to produce fluorescein and pyocyanin. Cetrimide (N-acetyl-N,N,N-trimethylammonium bromide) is incorporated in the medium to inhibit bacteria other than *Pseudomonas aeruginosa*. This compound a cationic detergent acts as a quaternary ammonium compound, which causes nitrogen and phosphorus to be released from bacterial cells other than *Pseudomonas aeruginosa*. Magnesium chloride and potassium sulphate incorporated in the medium enhances the production of pigment pyocyanin, which is a blue-green pigment, diffusing into the medium. This improves detection of *Pseudomonas* on this medium. Presence of magnesium ions can also neutralizes EDTA, if present in the sample. Gelatin peptone provides the essential nutrients for growth of *Pseudomonas,* while glycerin serves as slow and continuous carbon source for the growing cell.

For the isolation of *Pseudomonas aeruginosa*, plates of Cetrimide Agar should be inoculated from non-selective medium such as Soybean Casein Digest Medium (MH011). If the count is high the test sample can be directly inoculated onto this medium. *Pseudomonas aeruginosa* colonies may appear pigmented greenish (under uv light also).

Type of specimen

Pharmaceutical samples

Specimen Collection and Handling

For pharmaceutical samples, follow appropriate techniques for sample collection, processing as per guidelines (2,3,4,5,6). After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions:

Read the label before opening the pack. 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. This medium is a selective medium, some strains may show poor growth as cetrimide is highly toxic.
2. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium.

4. 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.
5. It is recommended to store the plates at 24-30°C to avoid minimum condensation.
6.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

Sterile Cetrimide Agar in 90 mm disposable plates with smooth surface and absence of black particles/ cracks/ bubbles.

Colour of medium

Light amber coloured medium

Quantity of medium

25 ml of medium in 90 mm disposable plates.

pH

7.00-7.40

Sterility Check

Passes release criteria

Growth Promotion Test

Growth Promotion is carried out in accordance with the harmonized method of USP/EP/BP/JP/IP. Cultural response was observed after an incubation at 30-35°C for specified time. Recovery rate is considered as 100% for bacteria growth on 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 (at 30-35°C for <=18 hours).

Inhibitory properties

No growth of the test microorganism occurs for the specified temp for not less than longest period of time specified inoculating >=100 cfu (at least 100 cfu) (at 30-35°C for >= 72 hours).

Cultural Response

Cultural characteristics observed after incubation at 30-35 °C for 18-72 hours. Recovery rate is considered as 100% for bacteria growth on Soyabean Casein Digest Agar.

Organism	Inoculum (CFU)	Growth	Recovery	Incubation temperature	Incubation period	Pigment production
Growth promoting						
^*Pseudomonas paraeruginosa* ATCC 9027 (00026*)	50 -100	luxuriant	>=50 %	30 -35 °C	<=18 hrs	Yellow green
Inhibitory						
Escherichia coli ATCC 8739 (00012*)	>=10^3	inhibited	0 %	30 -35 °C	>=72 hrs	

Key : (*) Corresponding WDCM numbers. (^) Formerly known as *Pseudomonas aeruginosa.*

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 (8,9).

Reference

1.King, Ward and Raney, 1954, J. Lab. Clin. Med., 44:301.
2.The British Pharmacopoeia, 2022, Medicines and Healthcare products Regulatory Agency.

3.European Pharmacopoeia, 2022, 10 th volume, European Directorate for the quality of medicines & Healthcare.
4.Indian Pharmacopoeia, Addendum 2024 Indian Pharmacopoeia Commission, Ministry of Health and Family Welfare Government of India.
5.The Japanese Pharmacopoeia, 17th edition, 2016, The Ministry of Health, Labour and welfare.
6.The United States Pharmacopoeia-National Formulatory (USP-NF), 2022.
7.Lowbury E J L., 1951, J.Clin.Path., 4:66.
8.Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition
9.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.

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