Cetrimide Agar

MH024

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	Gms / Litre
Gelatin peptone #	20.000
Magnesium chloride	1.400
Dipotassium sulphate	10.000
Cetrimide	0.300
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

Suspend 45.3 grams in 1000 ml purified/distilled water containing 10 ml glycerin/glycerol. 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. Mix well and pour into sterile Petri plates.

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 (2-6). 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 (2-6). 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 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 organisms differ in their growth requirement and may show variable growth patterns on the medium.
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. This medium is a selective medium, some strains may show poor growth as cetrimide is highly toxic.
4. 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 1.36% Agar gel

Colour and Clarity of prepared medium

Light amber coloured opalescent gel with a slight precipitate forms in Petri plates

pH

7.00-7.40

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	Observed Lot value (CFU)	Recovery	Incubation temperature	Incubation period
Growth promoting						
Pseudomonas aeruginosa ATCC 9027 (00026*)	50 -100	luxuriant	25 -100	>=50 %	30 -35 °C	<=18 hrs
Inhibitory						
Escherichia coli ATCC 8739 (00012*)	>=10^3	inhibited	0	0 %	30 -35 °C	>=72 hrs
Additional Microbiological testing						
Pseudomonas aeruginosa ATCC 27853(00025*)	50 -100	luxuriant	25 -100	>=50 %	30 -35 °C	18 -24 hrs
Pseudomonas aeruginosa ATCC 25668 (00114*)	50 -100	luxuriant	25 -100	>=50 %	30 -35 °C	18 -24 hrs
Stenotrophomonas maltophila ATCC 13637	>=10^3	inhibited	0	0%	30 -35 °C	>=72 hrs
Escherichia coli ATCC 25922 (00013*)	>=10^3	inhibited	0	0%	30 -35 °C	>=72 hrs
Escherichia coli NCTC 9002	>=10^3	inhibited	0	0%	30 -35 °C	>=72 hrs
Staphylococcus aureus subsp. aureus ATCC 6538 (00032*)	>=10^3	inhibited	0	0%	30 -35 °C	>=72 hrs
Staphylococcus aureus subsp. aureus ATCC 25923 (00034)*	>=10^3	inhibited	0	0%	30 -35 °C	>=72 hrs
Salmonella Typhimurium *ATCC 14028* (00031*)	>=10^3	inhibited	0	0%	30 -35 °C	>=72 hrs
Proteus mirabilis ATCC 29906 (00023)*	>=10^3	inhibited	0	0%	30 -35 °C	>=72 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. 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 (4,8).

Reference

1.King, Ward and Raney, 1954, J. Lab. Clin. Med., 44:301.
2.British Pharmacopoeia, 2022, The Stationery office British Pharmacopoeia
3.European Pharmacopoeia, 2022 European Dept. for the quality of Medicines.
4.Indian Pharmacopoeia, 2020, Govt. of India, Ministry of Health and Family Welfare, New Delhi
5.Japanese Pharmacopoeia, 2016
6.The United States Pharmacopoeia, 2022, The United States Pharmacopeial Convention. Rockville, MD.
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.

Revision : 05/ 2022