Cetrimide Agar, Granulated

GMH024

Cetrimide Agar, granulated is used 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
Pancreatic digest of gelatin	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

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 (2,3,4,5,7). 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* (6). 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 neutralize EDTA, if present in the sample. Pancreatic digest of gelatin 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 (GMH011/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). Addition of nalidixic acid can aid in inhibiting the growth of accompanying flora.

Quality Control

Appearance
Cream to yellow coloured granular medium

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 Soyabean 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
Growth promoting					
Pseudomonas aeruginosa ATCC 9027	50 -100	luxuriant	>=50 %	30 -35 °C	<=18 hrs
Inhibitory					
Escherichia coli ATCC 8739	>=10^3	inhibited	0 %	30 -35 °C	>=72 hrs
Additional Microbiological testing					
Pseudomonas aeruginosa ATCC 27853	50 -100	luxuriant	>=50 %	30 -35 °C	18 -24 hrs
Pseudomonas aeruginosa ATCC 25668	50 -100	luxuriant	>=50 %	30 -35 °C	18 -24 hrs
Stenotrophomonas maltophila ATCC 13637	>=10^3	inhibited	0%	30 -35 °C	>=72 hrs
Escherichia coli ATCC 25922	>=10^3	inhibited	0%	30 -35 °C	>=72 hrs
Escherichia coli NCTC 9002	>=10^3	inhibited	0%	30 -35 °C	>=72 hrs
Staphylococcus aureus ATCC 6538	>=10^3	inhibited	0%	30 -35 °C	>=72 hrs
Staphylococcus aureus ATCC 25923	>=10^3	inhibited	0%	30 -35 °C	>=72 hrs
Salmonella Typhimurium *ATCC 14028*	>=10^3	inhibited	0%	30 -35 °C	>=72 hrs
Proteus mirabilis ATCC 29906	>=10^3	inhibited	0%	30 -35 °C	>=72 hrs

Storage and Shelf Life
Store below 30°C in tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label.

Reference
1.King, Ward and Raney, 1954, J. Lab. Clin. Med., 44:301.
2.The United States Pharmacopoeia, 2014, The United States Pharmacopoeial Convention,Rockville, MD.
3.British Pharmacopoeia, 2014, The Stationery Office British Pharmacopoeia.
4.European Pharmacopoeia, 2014, European Department for the Quality of Medicines of Council of Europe.
5.Japanese Pharmacopoeia, 2008, Published by Society of Japanese Pharmacopoeia,Tokyo, Japan.
6.Lowbury E J L., 1951, J.Clin.Path., 4:66.
7.Indian Pharmocoepoeia, 2014 Ministry of Health and Family Welfare, Govt. of India.

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