



Cetrimide Agar

M024B

Cetrimide Agar is a selective medium used for the isolation of *Pseudomonas aeruginosa* from pharmaceutical products in accordance with the microbial limit testing by harmonized methodology of BP.

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 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.

Principle And Interpretation

Cetrimide Agar was described by King et al (1). This media is based on the formulation described in BP(3) and is in accordance with the harmonized method of USP/EP/IP/JP (2,4,5,7). 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 Soyabean Casein Digest Medium (M011B). 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 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

Cultural Response

Growth Promotion is carried out in accordance with the harmonized method of BP. 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

M024B: 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						
<i>Pseudomonas aeruginosa</i> ATCC 9027	50 -100	luxuriant	25 -100	≥ 50 %	30 -35 °C	≤ 18 hrs
Inhibitory						
<i>Escherichia coli</i> ATCC 8739	$\geq 10^3$	inhibited	0	0 %	30 -35 °C	≥ 72 hrs
Additional Microbiological testing						
<i>Pseudomonas aeruginosa</i> ATCC 27853	50 -100	luxuriant	25 -100	≥ 50 %	30 -35 °C	18 -24 hrs
<i>Pseudomonas aeruginosa</i> ATCC 25668	50 -100	luxuriant	25 -100	≥ 50 %	30 -35 °C	18 -24 hrs
<i>Stenotrophomonas maltophilia</i> ATCC 13637	$\geq 10^3$	inhibited	0	0%	30 -35 °C	≥ 72 hrs
<i>Escherichia coli</i> ATCC 25922	$\geq 10^3$	inhibited	0	0%	30 -35 °C	≥ 72 hrs
<i>Escherichia coli</i> NCTC 9002	$\geq 10^3$	inhibited	0	0%	30 -35 °C	≥ 72 hrs
<i>Staphylococcus aureus</i> ATCC 6538	$\geq 10^3$	inhibited	0	0%	30 -35 °C	≥ 72 hrs
<i>Staphylococcus aureus</i> ATCC 25923	$\geq 10^3$	inhibited	0	0%	30 -35 °C	≥ 72 hrs
<i>Salmonella Typhimurium</i> ATCC 14028	$\geq 10^3$	inhibited	0	0%	30 -35 °C	≥ 72 hrs
<i>Proteus mirabilis</i> ATCC 29906	$\geq 10^3$	inhibited	0	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, 2011, The United States Pharmacopoeial Convention. Rockville, MD.
3. British Pharmacopoeia, 2011, The Stationery office British Pharmacopoeia
4. European Pharmacopoeia, 2011, European Dept. for the quality of Medicines.
5. Japanese Pharmacopoeia, 2008
6. Lowbury E J L., 1951, J.Clin.Path., 4:66.
7. Indian Pharmacopoeia, 2010 Ministry of Health and Family Welfare, Govt. of India

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