PRODUCT SPECIFICATION SHEET

Cetrimide Agar (DM057H)

Intended Use

Cetrimide Agar (DM057H) is recommended as a selective medium for the isolation of *pseudomonas aeruginosa* from pharmaceutical products in compliance with the microbial limit testing using harmonized methodology of USP/EP/BP/IP.

Product Summary and Explanation

King et al.[1] developed Medium A (Tech Agar) for the enhancement of pyocyanin production by *Pseudomonas*. Cetrimide Agar has the formula for Tech Agar but is modified by the addition of cetrimide (cetyl trimethyl ammonium bromide) for the selective inhibition of organisms other than *P. aeruginosa*. In 1951, Lowburry first reported the use of cetrimide as an agent for selective isolation of *Pseudomonas*.[2] The concentration was later reduced, because of the increased purity of the inhibitory agent, as reported by Lowbury and Collins in 1955.[3] Brown and Lowbury employed incubation at 37°C with examination after 18 and 42 hours of incubation.[4] Strains of *P. aeruginosa* are identified from specimens by their production of pyocyanin, a blue, water-soluble, nonfluorescent, phenazine pigment in addition to their colonial morphology[5] and the characteristic grapelike odor of aminoacetophenone.[6] *P. aeruginosa* is the only species of *Pseudomonas* or gram-negative rod known to excrete pyocyanin. Cetrimide Agar, therefore, is a valuable culture medium in the identification of this organism.

This media formulation is in accordance with the harmonized method of USP/EP/BP/JP/IP.[7-11] It is used as a selective medium for the isolation of *Pseudomonas aeruginosa* from pharmaceutical products. Cetrimide Agar is widely recommended for use in the examination of cosmetics,[12] clinical specimens[5,13] for the presence of *P. aeruginosa*, as well as for evaluating the efficacy of disinfectants against this organism.[14]

Principles of the Procedure

Cetrimide Agar contains pancreatic digest of gelatine which supplies the nutrients necessary to support growth, while glycerin serves as slow and continuous carbon source for the growing cell. 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. Cetrimide (N-acetyl-N,N,Ntrimethylammonium bromide) is a quaternary ammonium, cationic detergent compound, which is inhibitory to a wide variety of bacterial species including *Pseudomonas* species other than *P. aeruginosa*. *Pseudomonas aeruginosa* colonies may appear pigmented greenish (under UV light also). Addition of nalidixic acid can aid in inhibiting the growth of accompanying flora.

Formula / Liter

Ingredients	Gms / Liter
Pancreatic digest of gelatin	20.00
Magnesium chloride	1.40
Dipotassium sulphate	10.00
Cetrimide	0.30
Agar	13.60
Final pH: 7.2 ± 0.2 at 25°C	
Formula may be adjusted and/or supplemented as required to meet performance specifications	

Precautions

1. For Laboratory Use only.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Suspend 45.3 grams of medium in one liter purified/distilled water containing 10 ml glycerin/glycerol.
2. Heat to boiling to dissolve the medium completely.
3. Autoclave at 121°C, 15 psi pressure, for 15 minutes / validated cycle.

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Quality Control Specifications

Dehydrated Appearance	Cream to yellow homogeneous free flowing powder
Prepared Medium	Light amber coloured opalescent gel with a slight precipitate forms in Petri plates
Reaction of % Solution	Not Applicable
Gel Strength	Firm, comparable with 1.36% Agar gel

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).

Expected 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.

Sr. No.	Organisms	Results to be achieved					
		Inoculum (CFU)	Growth	Observed Lot value (CFU)	Recovery	Incubation Tempera-ture	Incubation period
	Growth promoting						
1.	*Pseudomonas aeruginosa ATCC 9027*	50-100	good-luxuriant	25 -100	>=50%	30 -35 °C	<=18 hrs
	Inhibitory						
2.	*Escherichia coli ATCC 8739*	>=10^3	inhibited	0	0%	30 -35 °C	>=72 hrs
	Additional Microbiological Testing						
3.	*Pseudomonas aeruginosa ATCC 27853*	50-100	good-luxuriant	25 -100	>=50%	30 -35 °C	18 -24 hrs
4.	*Pseudomonas aeruginosa ATCC 25668*	50-100	good-luxuriant	25 -100	>=50%	30 -35 °C	18 -24 hrs
5.	*Stenotrophomonas maltophila ATCC 13637*	>=10^3	inhibited	0	0%	30 -35 °C	>=72 hrs
6.	*Escherichia coli ATCC 25922*	>=10^3	inhibited	0	0%	30 -35 °C	>=72 hrs
7.	*Escherichia coli NCTC 9002*	>=10^3	inhibited	0	0%	30 -35 °C	>=72 hrs
8.	*Staphylococcus aureus ATCC 6538*	>=10^3	inhibited	0	0%	30 -35 °C	>=72 hrs
9.	*Staphylococcus aureus ATCC 25923*	>=10^3	inhibited	0	0%	30 -35 °C	>=72 hrs
10.	*Salmonella Typhimurium ATCC 14028*	>=10^3	inhibited	0	0%	30 -35 °C	>=72 hrs

11.	*Proteus mirabilis* ATCC 29906	>=10^3	inhibited	0	0%	30 -35 °C	>=72 hrs

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Refer to appropriate references for standard test procedures. For the isolation of *Pseudomonas aeruginosa*, plates of Cetrimide Agar should be inoculated from non-selective medium such as Soybean Casein Digest Medium (DM277H). If the count is high the test sample can be directly inoculated onto this medium.

Results

1. Colonies that are surrounded by a blue-green pigment and fluoresce under short wavelength (254 nm) ultraviolet light may be presumptively identified as *Pseudomonas aeruginosa*.
2. Note, however, that certain strains of *P. aeruginosa* may not produce pyocyanin.
3. Other species of *Pseudomonas* do not produce pyocyanin, but fluoresce under UV light.
4. Most non-Pseudomonas species are inhibited, and some species of Pseudomonas may also be inhibited.
5. Gram staining, biochemical tests and serological procedures should be performed to confirm findings.
6. Refer to appropriate references and standard test procedures for interpretation of results.

Storage

Store the sealed bottle containing the dehydrated medium at 10 -30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light.

Expiration

Refer to the expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. The type of peptone used in the base may affect pigment production.
2. No single medium can be depended upon to exhibit all pigment-producing *P. aeruginosa* strains.
3. Occasionally some enterics will exhibit a slight yellowing of the medium; however, this coloration is easily distinguished from fluorescin production since this yellowing does not fluoresce.
4. Some nonfermenters and some aerobic sporeformers may exhibit a water-soluble tan to brown pigmentation on this medium. *Serratia* strains may exhibit a pink pigmentation.
5. Studies of Lowbury and Collins showed *P. aeruginosa* may lose its fluorescence under UV light if the cultures are left at room temperature for a short time. Fluorescence reappears when plates are reincubated.
6. Consult appropriate texts for detailed information and recommended procedures.

Packaging

Product Name : Cetrimide Agar
Product Code : DM057H
Available Pack sizes : 100gm/500gm

References

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14. Horwitz, (ed). 2002. AOAC Official Method 955.13. Official methods of analysis of AOAC International, 17th ed, vol. 1, Rev. 1. AOAC International, Gaithersburg, Md.

Further Information

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