



PRODUCT SPECIFICATION SHEET

Cetrimide Agar Base (DM057)

Intended Use

Cetrimide Agar Base (DM057) 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

Pseudomonas aeruginosa is a gram-negative, aerobic bacterium which grows well on all normal laboratory media but specific isolation of the organism, from environmental sites or from human, animal or plant sources, is best carried out on a medium, which contains a selective agent and also constituents to enhance pigment production. Most selective media depend upon the intrinsic resistance of the species to various antibacterial agents. 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*. Cetrimide inhibits the growth of many microorganisms whilst allowing *Pseudomonas aeruginosa* to develop typical colonies. In 1951, Lowbury⁽²⁾ described the use of 0.1% cetrimide in a selective medium for *P. aeruginosa*. Because of the increased purity of the inhibitory agent, the concentration was later reduced, as reported by Lowbury and Collins in 1955.⁽³⁾

Cetrimide is a quaternary ammonium salt, which acts as a cationic detergent that reduces surface tension in the point of contact and has precipitant, complexing and denaturing effects on bacterial membrane proteins. It exhibits inhibitory actions on a wide variety of microorganisms including *Pseudomonas* species other than *Pseudomonas aeruginosa*. Brown and Lowbury employed incubation at 37°C with examination after 18 and 42 hours of incubation.⁽⁴⁾

P.aeruginosa can be identified due to their characteristic production of pyocyanin, a blue, water-soluble, non-fluorescent phenazine pigment coupled with their colonial morphology and the characteristic grape-like odor of aminoacetophenone.⁽⁵⁾ *P.aeruginosa* is the only species of *Pseudomonas* or gram-negative rod known to excrete pyocyanin. These media are therefore, important in the identification of *P.aeruginosa*. These media are used for the microbiological examination of cosmetics⁽⁶⁾ and clinical specimens^(5,7) for the presence of *P.aeruginosa*, as well as for evaluating the efficacy of disinfectants against this organism.⁽⁸⁾

Principles of the Procedure

Cetrimide Agar Base contains gelatin peptone which supplies the nutrients necessary to support growth. The production of pyocyanin is stimulated by the magnesium chloride and potassium sulfate in the medium. Cetrimide is a quaternary ammonium, cationic detergent compound, which is inhibitory to a wide variety of bacterial species including *Pseudomonas* species other than *P. aeruginosa*. Cetrimide Agar Base is supplemented with 1% glycerol as a source of carbon.

Formula / Liter

Ingredients	Gms / Liter
Pancreatic digest of gelatin	20.00
Magnesium chloride	1.40
Potassium sulphate	10.00
Cetrimide	0.30
Agar	15.00
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.





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2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Suspend 46.4 grams of medium in one liter of distilled water water containing 10 ml glycerol.
2. Heat to boiling, to dissolve the medium completely.
3. Autoclave at 121°C, 15 psi pressure, for 15 minutes / validated cycle.
4. If desired, rehydrated contents of 1 vial of Nalidixic Selective Supplement (MS206) may be added aseptically to 1000 ml medium.
5. Mix well and pour into sterile petri plates.

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 4.67% Solution	pH : 7.2 ± 0.2 at 25°C
Gel Strength	Firm, comparable with 1.5% Agar gel

Cultural response	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.
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Expected Cultural Response:

Sr. No.	Organisms	Results to be achieved				
		Inoculum (CFU)	Growth	Observed Lot value (CFU)	Recovery	Incubation Temperature & Time
1.	<i>Pseudomonas aeruginosa</i> ATCC 9027	50 - 100	good-luxuriant	25 - 100	≥50 %	30-35°C <=18 hrs
2.	<i>Escherichia coli</i> ATCC 8739	≥10 ³	inhibited	0	0%	30-35°C ≥72 hrs
3.	<i>Pseudomonas aeruginosa</i> ATCC 27853	50 - 100	good-luxuriant	25 - 100	≥50 %	30-35°C 18-24 hrs
4.	<i>Pseudomonas aeruginosa</i> ATCC 25668	50 - 100	good-luxuriant	25 - 100	≥50 %	30-35°C 18-24 hrs
5.	<i>Stenotrophomonas maltophilia</i> ATCC 13637	≥10 ³	inhibited	0	0%	30-35°C ≥72 hrs
6.	<i>Escherichia coli</i> ATCC 25922	≥10 ³	inhibited	0	0%	30-35°C ≥72 hrs
7.	<i>Escherichia coli</i> NCTC 9002	≥10 ³	inhibited	0	0%	30-35°C ≥72 hrs
8.	<i>Staphylococcus aureus</i> ATCC 6538	≥10 ³	inhibited	0	0%	30-35°C ≥72 hrs
9.	<i>Staphylococcus aureus</i> ATCC 25923	≥10 ³	inhibited	0	0%	30-35°C ≥72 hrs
10.	<i>Salmonella typhimurium</i> ATCC 14028	≥10 ³	inhibited	0	0%	30-35°C ≥72 hrs
11.	<i>Proteus mirabilis</i> 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.





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Test Procedure

1. For the isolation of *P.aeruginosa*, plates of Cetrimide Agar should be inoculated from non-selective medium such as Brain Heart Infusion Broth (DM811) or Soyabean Casein Digest Medium (DM277).
2. If the count is high, the test sample can be directly inoculated onto Cetrimide Agar.
3. Refer to appropriate references for standard test procedures.

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*. Note, however, that certain strains of *P. aeruginosa* may not produce pyocyanin.
2. Other species of *Pseudomonas* do not produce pyocyanin, but fluoresce under UV light.
3. Most non-*Pseudomonas* species are inhibited, and some species of *Pseudomonas* may also be inhibited.
4. Gram staining, biochemical tests and serological procedures should be performed to confirm findings.

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 fluorescein 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 Base

Product Code : DM057

Available Pack sizes : 100gm / 500gm

References

1. King, Ward and Raney, 1954, J. Lab. Clin. Med., 44:301.
2. Lowbury, 1951, J. Clin. Pathol., 4:66.
3. Lowbury and Collins, 1955, J. Clin. Pathol., 8:47
4. Brown and Lowbury, 1965, J. Clin. Pathol., 18:752.
5. Murray P. R., Baron J. H., Tenover F. C., Tenover P. C., (Eds.), 2003, Manual of Clinical Microbiology, 8th Ed., American Society for Microbiology, Washington, D.C.
6. USFDA Bacteriological Analytical Manual, 2005, 18th Ed., AOAC, Washington, DC.
7. Forbes B. A., Sahm A. S. and Weissfeld D. F., Bailey & Scotts Diagnostic Microbiology, 10th Ed., 1998, Mosby, Inc., St. Louis, Mo.
8. Williams, (Ed.), 2005, Official Methods of Analysis of the Association of Official Analytical Chemists, 19th Ed., AOAC, Washington, D.C.





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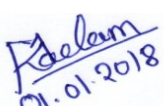


Further Information

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