PRODUCT SPECIFICATION SHEET

Cetrimide Agar Plate (RP002H)

Intended Use
Medium for selective isolation and subculture of Clostridium sporogenes in compliance with the harmonized method of USP/EP/BP/JP.

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
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. Prepared plated media are For *in vitro* Diagnostic Use or For Laboratory Use as labeled.
2. Directions for use should be read and followed carefully.
3. If excessive moisture is observed, invert the bottom over an off-set lid and allow to air dry in order to prevent formation of a seal between the top and bottom of the plate during incubation.

4. Observe aseptic techniques and established precautions against microbiological hazards throughout all procedures, since it must be assumed that all specimens/samples collected might contain infectious microorganisms.

Product Deterioration

Do not use plates if they show evidence of microbial contamination, discoloration, drying, cracking or other signs of deterioration.

Quality Control Specifications

Appearance	Sterile Cetrimide Agar in 90mm plates
Colour	Light amber coloured medium
Reaction	pH : 7.2 ± 0.2 at 25°C
Quantity of medium	25ml of medium in 90mm plates

Sterility Check: Passes release criteria.

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.

Expected Cultural Response:

Sr. No.	Organisms	Results to be achieved				
		Inoculum (CFU)	Growth	Observed Lot value (CFU)	Recovery	Incubation Temperature & Time
1.	*Pseudomonas aeruginosa ATCC 9027*	50 - 100	good-luxuriant	25 -100	>=50 %	30-35°C <=18 hrs
2.	*Escherichia coli ATCC 8739*	>=10^3	inhibited	0	0%	30-35°C >=72 hrs
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

1. 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*. 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

On receipt, store plates at 20-25°C.

Expiration

Refer to the expiration date stamped on the pack. Prepared plates stored in their original sleeve wrapping at 20-25°C until just prior to use may be inoculated up to the expiration date and incubated for recommended incubation times.

Product Disposal

After use, prepared plates, specimen/sample containers and other contaminated materials must be sterilized before discarding.

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.
7. .

Packaging

Product Name : Cetrimide Agar Plate
Product Code : RP002H
Available Pack sizes : Pack of 10 plates

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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	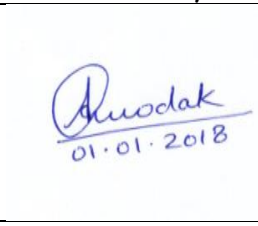	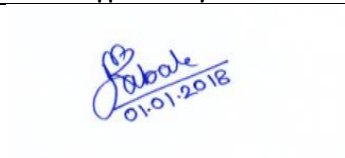
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