Cetrimide Agar Base

Intended Use
Cetrimide Agar Base is a medium used for selective isolation of *Pseudomonas aeruginosa* from pus, sputum, drains etc.

Summary
Pseudomonas aeruginosa 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. Cetrimide inhibits the growth of many microorganisms whilst allowing *Pseudomonas aeruginosa* to develop typical colonies.
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*. King et al developed Medium A for the enhancement of pyocyanin production by *Pseudomonas*. Cetrimide Agar developed by Lowburry is a modification of Tech Agar (Medium A) with addition of 0.1% cetrimide for selective isolation of *P. aeruginosa*. Later, due to the availability of the highly purified cetrimide, its concentration in the medium was decreased. The incubation was carried out at 37°C for a period of 18-24 hours.
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 amino-acetophenone. *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 examination of cosmetics and clinical specimens for the presence of *P. aeruginosa*, as well as for evaluating the efficacy of disinfectants against this organism.

Principle
Cetrimide (Cetyltrimethylammonium bromide) is a quaternary ammonium compound, cationic detergent, which is inhibitory to a wide variety of bacteria including *Pseudomonas* species other than *P. aeruginosa*. It causes nitrogen and phosphorous to be released from bacterial cells other than *Pseudomonas aeruginosa*. The magnesium chloride and potassium sulphate in the medium stimulates the production of pyocyanin. Pancreatic digest of gelatin provides nitrogenous compounds.

Formula*
Ingredients	**g/L**
Pancreatic Digest of Gelatin	20.0
Potassium Sulphate	10.0
Magnesium Chloride	1.4
Cetrimide	0.3
Agar	13.6
Final pH (at 25°C)	7.2 ± 0.2

*Adjusted to suit performance parameters

Storage and Stability
Store dehydrated medium below 30°C in tightly closed container and the prepared medium at 2ºC-8°C. Avoid freezing and overheating. Use before expiry date on the label. Once opened keep powdered medium closed to avoid hydration.

Type of Specimen
Clinical samples - Blood, urine, pus, sputum; Water samples

Specimen Collection and Handling
Ensure that all samples are properly labelled.
Follow appropriate techniques for handling samples as per established guidelines.
Some samples may require special handling, such as immediate refrigeration or protection from light, follow the standard procedure.
The samples must be stored and tested within the permissible time duration.

After use, contaminated materials must be sterilized by autoclaving before discarding.

Directions

1. Suspend 45.30 g of the powder in 1000 mL purified / distilled water containing 10 mL glycerol.
2. Mix thoroughly.
3. Heat with frequent agitation to dissolve the powder completely.
4. Sterilize by autoclaving at 121°C (15 psi) for 15 minutes as per validated cycle.
5. If desired, add rehydrated contents of 1 vial of Nalidixic Selective Supplement (204140370005) aseptically to Sterile molten medium.

Quality Control

Dehydrated Appearance: Light yellow coloured, homogenous, free flowing powder.
Prepared Appearance: Light amber coloured, very slightly opalescent gel with slight precipitate forms in petridishes.
Growth Promotion Test: Growth promotion is carried out in accordance with the harmonized method of USP/EP/JP/IP and growth is observed after an incubation at 30°C-35°C for 18 to 72 hours.
Growth Promoting Properties: The test results observed are within the specified temperature and shortest period of time specified in the test, inoculating ≤ 100 cfu of appropriate microorganism at 30°C-35°C for 18 hours.
Indicative Properties: The test results observed are within the specified temperature and time, inoculating ≤100 cfu of appropriate microorganism.
Inhibitory Properties: No growth of the test microorganism occurs for the specified temperature and not less than the longest period of the time specified, inoculating >100 cfu of the appropriate microorganism at 30°C-35°C for 72 hours.

Organism (ATCC)	**Growth**	**Colour of Colony**
Growth Promoting		
Pseudomonas aeruginosa (9027)	Good	Greenish
Pseudomonas aeruginosa Strain Boston 41501 (27853)	Good	Greenish
Inhibitory		
Escherichia coli (8739)	Inhibited	-

Note: For good growth - Growth observed on test media should be comparable to the growth observed on control media. - For inhibition no growth of test microorganism should occur.

Interpretation of Results

Colonies that are surrounded by a blue green pigment give fluorescence under ultraviolet light (wavelength 254 nm) may be presumptively identified as *Pseudomonas aeruginosa*.

Performance and Evaluation

Performance of the product is dependent on following parameters as per product label claim:

1. Directions
2. Storage
3. Expiry

Precautions/Limitations

1. Certain strains of *P. aeruginosa* may not produce pyocyanin while other species of *Pseudomonas* do not produce pyocyanin but fluoresce under UV light.
2. Most non-*Pseudomonas* species are inhibited, and some species of *Pseudomonas* may also be inhibited.
3. The type of peptone used in the base may affect pigment production.
4. No single medium can be depended upon to exhibit all pigment producing *P. aeruginosa* strains.
5. Occasionally some enterics will exhibit a slight yellowing of the medium: however, this colouration is easily distinguished from fluorescein production since this yellowing does not fluoresce.
6. Some non-fermenters and some aerobic spore formers may exhibit a water-soluble tan to brown pigmentation on this medium. *Serratia* strains may exhibit a pink pigmentation.
7. If swarming colonies of *Proteus* species are a problem in food samples then the incubation temperature can be lowered to 20°C for a period of 3-5 days.

8. Molten agar should not be kept longer than 4 hours. Medium should not be stored and remelted.

Warranty

This product is designed to perform as described on the label and package insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

Reference

1. Atlas R. M., 2004, Handbook of Microbiological Media, 3rd Edition, CRC Press, Washington D. C.
2. FDA Bacteriological Analytical Manual, 2005, 18th Ed., AOAC, Washington, D.C.
3. International Organization for Standardization (ISO), 1993, Draft ISO/DIS 10560.
4. Data on file: Microxpress®, A Division of Tulip Diagnostics (P) Ltd.

Product Presentation:

Cat No.	Product description	Pack Size
201030060100	Dehydrated Culture Media	100 g
201030060500	Dehydrated Culture Media	500 g