

## CM 20399 – CETRIMIDE AGAR BASE (W 1.3% AGAR)

### INTENDED USE

For the selective isolation of *Pseudomonas aeruginosa* from various materials.

### PRODUCT SUMMARY AND EXPLANATION

Cetrimide AgarBase w / 1.3% Agar is recommended as a selective medium for isolation of *Pseudomonas aeruginosa*. It is similar in composition as cited in various pharmacopoeias except that the concentration of agar in this medium is 1.3%. The original formula was described by King et al. It can also be used for determining the ability of an organism to produce fluorescein and pyocyanin.

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, nonfluorescent phenazine pigment coupled with their colonial morphology and the characteristic grape like odour of aminocetophenone.

For the isolation of *P. aeruginosa*, plates of cetrimide agar should be inoculated from non-selective medium such as Brain Heart infusion Broth or Soyabean Casein Digest Medium. If the count is high, the test sample can be directly inoculated onto Cetrimide Agar. *P. aeruginosa* colonies may appear blue, blue-green or nonpigmented. Colonies exhibiting fluorescence at 250 nm and a blue green pigmentation are considered as presumptive positive. *P. aeruginosa* may lose its fluorescence under UV if the cultures are left at room temperature for short time. Fluorescence reappears after the plates are re-incubated. Goto and Enomoto recommended that addition of nalidixic acid aids in inhibiting the growth of accompanying flora.

### COMPOSITION

Ingredients	Gms / Ltr
Peptone from gelatin	20.000
Magnesium chloride	1.400
Potassium sulphate	10.000
Cetrimide	0.300
Agar	13.000

### PRINCIPLE

Cetrimide (N-acetyl-N,N,N-trimethylammonium bromide) in the medium acts as a selective agent inhibiting bacteria other than *Pseudomonas aeruginosa*. It 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*. 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. Peptone from gelatin provides the essential nutrients for growth of *Pseudomonas*, while glycerin/glycerol serves as slow and continuous carbon source for the growing cell.

### INSTRUCTION FOR USE

Dissolve 44.7 grams in 1000 ml distilled water containing 10 ml glycerin/glycerol.

Heat to boiling to dissolve the medium completely.



Sterilize by autoclaving at 15 psi pressure (121°C) for 15 minutes.

If desired, rehydrated contents of 1 vial of Nalidixic Selective Supplement may be added aseptically to 1000 ml medium previously cooled to 45- 50°C.

Mix well and pour into sterile Petri plates.

#### QUALITY CONTROL SPECIFICATIONS

Appearance of Powder	: Cream to yellow homogeneous free flowing powder.
Appearance of prepared medium	: Light amber coloured, opalescent gel with a slight precipitate forms in Petri plates.
pH (at 25°C)	: 7.0±0.2

#### INTERPRETATION

Cultural characteristics observed after incubation with added Nalidixic Selective Supplement.

Microorganism	ATCC	Inoculum (CFU/ml)	Growth	Recovery	Incubation Temperature	Incubation Period
Pseudomonas aeruginosa	9027	50-100	Luxuriant (with yellow green pigment)	≥70%	35-37°C	24-48 Hours
Pseudomonas aeruginosa	27853	50-100	Luxuriant (with yellow green pigment)	≥70%	35-37°C	24-48 Hours
Pseudomonas aeruginosa	25668	50-100	Luxuriant (with yellow green pigment)	≥70%	35-37°C	24-48 Hours
Escherichia coli	25922	≥10 <sup>3</sup>	Inhibited	0%	35-37°C	24-48 Hours
Proteus mirabilis	29906	≥10 <sup>3</sup>	Inhibited	0%	35-37°C	24-48 Hours
Stenotrophomonas maltophilia	13637	≥10 <sup>3</sup>	Inhibited	0%	35-37°C	24-48 Hours
Staphylococcus aureus	25923	≥10 <sup>3</sup>	Inhibited	0%	35-37°C	24-48 Hours
Escherichia coli	8739	≥10 <sup>3</sup>	Inhibited	0%	35-37°C	24-48 Hours
Salmonella Typhimurium	14028	≥10 <sup>3</sup>	Inhibited	0%	35-37°C	24-48 Hours



Staphylococcus aureus	6538	$\geq 10^3$	Inhibited	0%	35-37°C	24-48 Hours
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**PACKAGING:**

In pack size of 100 gm and 500 gm bottles.

**STORAGE**

Dehydrated powder, hygroscopic in nature, store in a dry place, in tightly-sealed containers between 25-30°C and protect from direct sunlight. Under optimal conditions, the medium has a shelf life of 4 years. When the container is opened for the first time, note the time and date on the label space provided on the container. After the desired amount of medium has been taken out replace the cap tightly to protect from hydration.













Product Deterioration: Do not use if they show evidence of microbial contamination, discoloration, drying or any other signs of deterioration.

**DISPOSAL**

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

**REFERENCES**

1. The United States Pharmacopoeia, 2011, The United States Pharmacopoeial Convention. Rockville, MD.
2. British Pharmacopoeia, 2011, The Stationery office British Pharmacopoeia.
3. European Pharmacopoeia, 2011, European Dept. for the Quality of Medicines.
4. Japanese Pharmacopoeia, 2008.
5. King, Ward and Raney, 1954, J. Lab. Clin. Med., 44:301.
6. Lowbury, 1951, J. Clin. Pathol., 4:66.
7. Lowbury and Collins, 1955, J. Clin. Pathol., 8:47.
8. Brown and Lowbury, 1965, J. Clin. Pathol., 18: 752.
9. Murray, P.R, Baron J.H., Tenover F.C., Tenover P.C. (Ed.) 2003, Manual of Clinical Microbiology, 8th Ed., American Society for Microbiology, Washington, D.C.
10. Goto, S. and Enomoto, S., 1970. Japan. J. Microbiol., 14: 65.

 GMP Good Manufacturing Practices Certified	 IVD For In Vitro Diagnostic Use	 QTY. Quantity	 LOT/ B. NO. Lot / Batch Number	 REF Catalogue Number	 Manufacturer
 Temperature Unit	 EC REP Authorized Representative <small>MedNet GmbH Birkstrasse 10, 48153 Münster, Germany</small>	 European Conformity	 QR Code	 Consults Instructions for Use	 Best Before

NOTE: Please consult the Material Safety Data Sheet for information regarding hazards and safe handling Practices.

\*For Lab Use Only

