



Cetrimide Agar Base (w/1.3% Agar)

M1742

Intemded Use:

Recommended for the selective isolation of *Pseudomonas aeruginosa* from various materials.

Composition**

Ingredients	g / L
Gelatin peptone	20.000
Magnesium chloride	1.400
Potassium sulphate	10.000
Cetrimide	0.300
Agar	13.000
Final pH (at 25°C)	7.0±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 44.7 grams in 1000 ml purified / distilled water containing 10 ml glycerin/glycerol. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. If desired, rehydrated contents of 1 vial of NA Selective Supplement (FD130) may be added aseptically to 1000 ml medium. Mix well and pour into sterile Petri plates.

Principle And Interpretation

Cetrimide Agar Base 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 (1,2,3,4) except that the concentration of agar in this medium is 1.3%.The original formula was described by King et al (5). It can also be used for determining the ability of an organism to produce fluorescein and pyocyanin.

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

King et al developed Medium A for the enhancement of pyocyanin production by *Pseudomonas* (5). Cetrimide agar developed by Lowburry (6) 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 (7). The incubation was carried out at 37°C for a period of 18-24 hours (8). *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 (9).

For the isolation of *P. aeruginosa*, plates of cetrimide agar should be inoculated from non-seelctive medium such as Brain Heart infusion Broth (M210) or Soyabean Casein Digest Medium (M011).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 (10).

Type of specimen

Clinical samples - pus, urine

Specimen Collection and Handling

Please refer disclaimer Overleaf.

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (11,12).
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

In Vitro diagnostic Use only. For professional use only. Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1. Some strains of *Pseudomonas* other than *aeruginosa* species may show poor growth as cetrimide is highly toxic.
2. Further biochemical and serological tests must be carried out for complete identification.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.3% Agar gel

Colour and Clarity of prepared medium

Light amber coloured, opalescent gel with a slight precipitate forms in Petri plates

Reaction

Reaction of 4.47% w/v aqueous solution containing 1.0% glycerol at 25°C . pH : 7.0±0.2

pH

6.80-7.20

Cultural Response

Cultural characteristics observed with added NA Selective Supplement (FD130) after an incubation at 35-37°C for 24-48 hours.

Organism	Inoculum (CFU)	Growth	Recovery
<i>Pseudomonas aeruginosa</i> ATCC 9027 (00026*)	50-100	Luxuriant(with yellow green pigment)	>=50 %
<i>Pseudomonas aeruginosa</i> ATCC 27853 (00025*)	50-100	Luxuriant(with yellow green pigment)	>=50 %
<i>Pseudomonas aeruginosa</i> ATCC 25668 (00114*)	50-100	Luxuriant(with yellow green pigment)	<=0 %
<i>Escherichia coli</i> ATCC 25922 (00013*)	>=10 ⁴	Inhibited	
<i>Proteus mirabilis</i> ATCC 29906 (00023*)	>=10 ⁴	Inhibited	
<i>Stenotrophomonas maltophilia</i> ATCC 13637	>=10 ⁴	Inhibited	
<i>Staphylococcus aureus</i> subsp. <i>aureus</i> ATCC 25923 (00034*)	>=10 ⁴	Inhibited	
<i>Escherichia coli</i> ATCC 8739 (00012*)	>=10 ⁴	Inhibited	
<i>Salmonella</i> Typhimurium ATCC 14028 (00031*)	>=10 ⁴	Inhibited	
<i>Escherichia coli</i> NCTC 9002	>=10 ⁴	Inhibited	
<i>Staphylococcus aureus</i> NCIMB 9518	>=10 ⁴	Inhibited	
<i>Staphylococcus aureus</i> subsp. <i>aureus</i> ATCC 6538 (00032*)	>=10 ⁴	Inhibited	

Key : (*) Corresponding WDCM numbers.

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (11,12).

Reference

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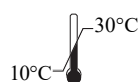
Revision :05 / 2024



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