Cetrimide Agar Base (w 1.3% Agar)

M1742

Cetrimide Agar Base w/1.3% Agar is a selective medium used for the isolation of *Pseudomonas aeruginosa* from various samples such as food, beverages, clinical samples, water, pharmaceutical products etc.

Composition**

Ingredients	Gms / Litre
Peptone from gelatin	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 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. If desired, rehydrated contents of 1 vial of Nalidixic Selective Supplement (FD130) may be added aseptically to 1000 ml medium previously cooled to 45- 50°C. 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 pyocyanain. Cetrimide (N-acetyl-N-N,N-trimethylammaonium 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. 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.

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 370C 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).

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 Nalidixic Selective Supplement (FD130) after an incubation at 35-37°C for 24-48 hours.

Cultural Response

Organism	Inoculum (CFU)	Growth	Recovery
Pseudomonas aeruginosa ATCC 9027	50-100	Luxuriant(with yellow green pigment)	>=50 %
Pseudomonas aeruginosa ATCC 27853	50-100	Luxuriant(with yellow green pigment)	>=50 %
Pseudomonas aeruginosa ATCC 25668	50-100	Luxuriant(with yellow green pigment)	<=0 %
Escherichia coli ATCC 25922	>=10^3	Inhibited	
Proteus mirabilis ATCC 29906	>=10^3	Inhibited	
Stenotrophomonas maltophilia ATCC 13637	>=10^3	inhibited	
Staphylococcus aureus ATCC 25923	>=10^3	inhibited	
Escherichia coli ATCC 8739	>=10^3	Inhibited	
Salmonella Typhimurium ATCC 14028	>=10^3	inhibited	
Escherichia coli NCTC 9002	>=10^3	Inhibited	
Staphylococcus aureus NCIMB 9518	>=10^3	Inhibited	
Staphylococcus aureus ATCC 6538	>=10^3	Inhibited	

Storage and Shelf Life

Store below 30°C in tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label.

Reference

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8.Brown and Lowbury ,1965. .J. Clin. Pathol., 18: 752.

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