Cetrimide HiCynth™ Agar Base

MCD024

Cetrimide HiCynth™ Agar Base is used for the selective isolation of *Pseudomonas aeruginosa* from clinical specimens.

Composition**

Ingredients	Gms / Litre
HiCynth™ Peptone No.3*	20.000
Magnesium chloride	1.400
Potassium sulphate	10.000
Cetrimide	0.300
Agar	15.000
Final pH (at 25°C)	7.2±0.2

**Formula adjusted, standardized to suit performance parameters
*Chemically defined peptone

Directions

Suspend 46.7 grams in 1000 ml distilled water containing 10 ml 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 Nalidixic Selective Supplement (FD130) may be added aseptically to 1000 ml medium. Mix well and pour into sterile Petri plates.

Principle And Interpretation

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* (1). Cetrimide Agar developed by Lowburry (2) 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 (3). The incubation was carried out at 37°C for a period of 18-24 hours (4). Cetrimide HiCynth™ Agar Base is the modification prepared by replacing animal and vegetable based peptones with chemically based peptones to avoid BSE/TSE risks associated with animal peptones.

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 aminoacetophenone (5). *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 (6) and clinical specimens (5, 7) for the presence of *P.aeruginosa*, as well as for evaluating the efficacy of disinfectants against this organism (8).

HiCynth™ Peptone No.3 provides nitrogen and carbon source, long chain amino acids, vitamins and necessary nutrients for *P.aeruginosa*. Sodium chloride maintains osmotic equilibrium in the medium. Magnesium chloride and potassium sulfate stimulates pyocyanin production. (9).

For the isolation of *P.aeruginosa*, plates of Cetrimide HiCynth™ Agar base 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 pigmented blue, blue-green or non-pigmented. Colonies exhibiting fluorescence at 250nm 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 a short time. Fluorescence reappears after the plates are re-incubated (4). Type of peptone used in the base may also affect pigment production (4, 10).Certain strains of *P.aeruginosa* may not produce pyocyanin. Other species of *Pseudomonas* do not produce pyocyanin but fluoresce under UV light. Most non- *Pseudomonas* species are inhibited on Cetrimide HiCynth™ Agar, and some species of *Pseudomonas* may also be inhibited. Some non-fermenters and some aerobic spore formers may exhibit a water-soluble tan to brown pigmentation on this medium. *Serratia* may exhibit pink pigmentation (3). Biochemical tests and serological procedures should be performed to confirm the findings.

Quality Control

Appearance
Cream to yellow homogeneous free flowing powder

Gelling
Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium
Light amber coloured opalescent gel with a slight precipitate forms in Petri plates

Reaction
Reaction of 4.67% w/v aqueous solution containing 1% glycerol at 25°C . pH : 7.2±0.2

pH
7.00-7.40

Cultural response
Cultural response was observed after an incubation at 30-35°C for specified time. Recovery rate is considered as 100% for bacteria growth on Soyabean Casein Digest Agar.

Cultural Response
MCD024: 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.

Organism	Inoculum (CFU)	Growth	Observed Lot value (CFU)	Recovery
Cultural response				
Pseudomonas aeruginosa ATCC 9027	50 -100	luxuriant	25 -100	>=50 %
Escherichia coli ATCC 8739	>=10^3	inhibited	0	0%
Pseudomonas aeruginosa ATCC 27853	50 -100	luxuriant	25 -100	>=50 %
Pseudomonas aeruginosa ATCC 25668	50 -100	luxuriant	25 -100	>=50 %
Stenotrophomonas maltophila ATCC 13637	>=10^3	inhibited	0	0%
Escherichia coli ATCC 25922	>=10^3	inhibited	0	0%
Escherichia coli NCTC 9002	>=10^3	inhibited	0	0%
Staphylococcus aureus ATCC 6538	>=10^3	inhibited	0	0%
Staphylococcus aureus ATCC 25923	>=10^3	inhibited	0	0 %
Salmonella Typhimurium ATCC 14028	>=10^3	inhibited	0	0%
Proteus mirabilis ATCC 29906	>=10^3	inhibited	0	0%

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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6.USFDA Bacteriological Analytical Manual, 2005, 18th Ed., AOAC, Washington, DC.
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