Technical Information

Cetrimide Agar Base

Product Code: DM 1024

Application: - Cetrimide Agar Base is used for the selective isolation of *Pseudomonas aeruginosa* from clinical specimens.

Composition**

Ingredients	Gms / Litre
Pancreatic digest of gelatin	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

Principle & Interpretation

Pseudomonas aeruginosa grows well on all normal laboratory media but for specific isolation of the organism, from environmental sites or from human, animal or plant sources, medium, which contains a selective agent and also constituents to enhance pigment production is required. 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 has 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).

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).

Pancreatic digest of gelatin provide 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 Agar should be inoculated from non-selective medium such as Brain Heart Infusion Broth (DM1210) or Soyabean Casein Digest Medium (DM1011). 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 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.

Methodology

Suspend 46.7 grams of powder media in 1000 ml distilled water containing 10 ml glycerol. Shake well & heat 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 Acid Selective Supplement (MS2130) may be added aseptically to 1000 ml medium. Mix well and pour into sterile Petri plates.

Quality Control

Physical 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 : 7.2±0.2

pH range 7.00-7.40

Cultural Response/ characteristics

DM 1024: 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.

Organism	Inoculum (CFU)	Growth	Observed Lot value (CFU)	Recovery	Incubation temperature	Incubation period
Pseudomonas aeruginosa ATCC 9027	50-100	luxuriant	25 -100	>=50%	30-35 °C	<=18 hrs
Escherichia coli ATCC 8739	>=10^3	inhibited	0	0%	30-35 °C	>=72 hrs
Pseudomonas aeruginosa ATCC 27853	50-100	luxuriant	25 -100	>=50%	30-35 °C	18 -24 hrs
Pseudomonas aeruginosa ATCC 25668	50-100	luxuriant	25 -100	>=50%	30-35 °C	18 -24 hrs
Stenotrophomonas maltophila ATCC 13637	>=10^3	inhibited	0	0%	30-35 °C	>=72 hrs
Escherichia coli ATCC 25922	>=10^3	inhibited	0	0%	30-35 °C	>=72 hrs
Escherichia coli NCTC 9002	>=10^3	inhibited	0	0%	30-35 °C	>=72 hrs
Staphylococcus aureus ATCC 6538	>=10^3	inhibited	0	0%	30-35 °C	>=72 hrs
Staphylococcus aureus ATCC 25923	>=10^3	inhibited	0	0%	30-35 °C	>=72 hrs
Salmonella Typhimurium ATCC 14028	>=10^3	inhibited	0	0%	30-35 °C	>=72 hrs
Proteus mirabilis ATCC 29906	>=10^3	inhibited	0	0%	30-35 °C	>=72 hrs

Storage and Shelf Life

Dried Media: Store below 30°C in tightly closed container and use before expiry date as mentioned on the label.
Prepared Media: 2-8° in sealable plastic bags for 2-5 days.

Further Reading

1. King, Ward and Raney, 1954, J. Lab. Clin. Med., 44:301.
2. Lowbury, 1951, J. Clin. Pathol., 4:66.
3. Lowbury and Collins, 1955, J. Clin. Pathol., 8:47
4. Brown and Lowbury, 1965, J. Clin. Pathol., 18:752.
5. Murray P. R., Baron J. H., Pfaller M. A., Jorgensen J. H. and YolkenR. H., (Ed.), 2003, Manual of Clinical Microbiology, 8th Ed., American Society for Microbiology, Washington, D.C.
6. USFDA Bacteriological Analytical Manual, 2005, 18th Ed., AOAC, Washington, DC.
7. Forbes B. A., Sahm A. S. and Weissfeld D. F., Bailey & Scotts Diagnostic Microbiology, 10th Ed., 1998, Mosby, Inc., St. Louis, Mo.
8. Williams, (Ed.), 2005, Official Methods of Analysis of the Association of Official Analytical Chemists, 19th Ed., AOAC, Washington, D.C.
9. MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification -Maintenance of Medical Bacteria, Vol. I, Williams and Wilkins, Baltimore.
10. Goto and Enomoto, 1970, Jpn. J. Microbiol., 14:65.

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