Technical Information

Cetrimide Agar Plate

Product Code: PM 1024

Application: Recommended for selective isolation of Pseudomonas aeruginosa from clinical specimens.

Composition**

Ingredients	Gms / Litre
Gelatin peptone	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 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* (7). Cetrimide Agar developed by Lowburry (8) 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 (9). The incubation was carried out at 37°C for a period of 18-24 hours (2)

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 (11). *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 (12) and clinical specimens (3, 11) for the presence of *P.aeruginosa*, as well as for evaluating the efficacy of disinfectants against this organism (13).

Gelatin peptone provide necessary nutrients for *P.aeruginosa*. Sodium chloride maintains osmotic equilibrium in the medium. Magnesium chloride and potassium sulfate stimulates pyocyanin production (10). 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 (2). Type of peptone used in the base may also affect pigment production (2,4). 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 (9). Biochemical tests and serological procedures should be performed to confirm the findings.

Type of specimen

Clinical samples - Blood, urine; pus Water samples

Specimen Collection and Handling:

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (5,6). For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards(1). After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

In Vitro diagnostic Use. Read the label before opening the pack. 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. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium
2. Each lot of the medium has been tested for the organisms specified on the COA. It is recommended to users to validate the medium for any specific microorganism other than mentioned in the COA based on the user's unique requirement.
3. This medium is highly selective hence certain strains may show poor growth, as cetrimide is highly toxic.
4. Biochemical tests and serological procedures should be performed to confirm the findings.
5. It is recommended to store the plates ta 24-30°C to avoid minimum condensation.5. It is recommended to store the plates ta 24-30°C to avoid minimum condensation.

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
Sterile Cetrimide Agar in 90 mm disposable plates.
pH
7.00-7.40
Quantity of medium
25 ml of medium in 90 mm disposable plates.
Colour of medium
Light amber coloured medium
Sterility Test
Passes release criteria
Cultural Response
Growth Promotion is carried out in accordance with the standard method. Cultural response was observed after incubation at 30-35°C for specified time. Recovery rate is considered an s 100% for bacteria growth on Soyabean Casein Digest Agar.

Oragnism	Inoculum (CFU)	Growth	Observed Lot value (CFU)	Recovery	Incubation temperature	Incubation period
Pseudomonas aeruginosa ATCC 9027 (00026*)	50 -100	luxuriant	25 -100	>=50 %	30 -35 °C	<=18 hrs
Escherichia coli ATCC 8739 (00012*)	>=104	inhibited	0	0%	30 -35 °C	>=72 hrs
Pseudomonas aeruginosa ATCC 27853 (00025*)	50 -100	luxuriant	25 -100	>=50 %	30 -35 °C	18 -24 hrs
Pseudomonas aeruginosa ATCC 25668 (00114*)	50 -100	luxuriant	25 -100	>=50 %	30 -35 °C	18 -24 hrs
Stenotrophomonas maltophila ATCC 13637	>=104	inhibited	0	0%	30 -35 °C	>=72 hrs
Escherichia coli ATCC 25922 (00013*)	>=104	inhibited	0	0%	30 -35 °C	>=72 hrs
Escherichia coli NCTC 9002	>=104	inhibited	0	0%	30 -35 °C	>=72 hrs
Staphylococcus aureus subsp.*aureus* ATCC 6538 (00032*)	>=104	inhibited	0	0%	30 -35 °C	>=72 hrs

Staphylococcus aureus subsp.*aureus* ATCC 25923 (00034*)	>=104	inhibited	0	0%	30 -35 °C	>=72 hrs
Salmonella typhimurium ATCC 14028 (00031*)	>=104	inhibited	0	0%	30 -35 °C	>=72 hrs
Proteus mirabilis ATCC 29906 (00023*)	>=104	inhibited	0	0%	30 -35 °C	>=72 hrs

Storage and Shelf Life

- On receipt store between 20-30°C.
- Use before expiry date on the label.
- 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 sample must be decontaminated and disposed of in accordance with current laboratory techniques (2, 4).

Further Reading

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5. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
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7. King, Ward and Raney, 1954, J. Lab. Clin. Med., 44:301.
8. Lowbury, 1951, J. Clin. Pathol., 4:66.
9. Lowbury and Collins, 1955, J. Clin. Pathol., 8:47
10. MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification -Maintenance of Medical Bacteria, Vol. I, Williams and Wilkins, Baltimore.
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12. USFDA Bacteriological Analytical Manual, 2005, 18th Ed., AOAC, Washington, DC.
13.Williams, (Ed.), 2005, Official Methods of Analysis of the Association of Official Analytical Chemists, 19th Ed., AOAC, Washington, D.C.

Disclaimer :

- User must ensure suitability of the product(s) in their application prior to use.
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