

Cetrimide Agar Plate

MP024

Intended use

Recommended for selective isolation of *Pseudomonas aeruginosa* from clinical specimens.

Composition**

| Ingredients | g / L |
|---------------------|--------------|
| Gelatin peptone | 20.000 |
| Magnesium chloride | 1.400 |
| Potassium sulphate | 10.000 |
| Cetrimide | 0.300 |
| Glycerol | 10.00ml |
| Agar | 15.000 |
| Final pH (at 25°C) | 7.2±0.2 |

**Formula adjusted, standardized to suit performance parameters

Directions

Either streak, inoculate or surface spread the test inoculum (50-100 CFU) aseptically on the plate.

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

Gelatin peptone 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 BHI Broth (M210) or Tryptone Soya Broth (M011). 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.

Type of specimen

Clinical samples - urine, wound, pus, etc.; Water samples

Specimen Collection and Handling:

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (11,12).

For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (13). After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

In Vitro diagnostic Use. For professional use only. 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 at 24-30°C to avoid 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 with smooth surface and absence of black particles/cracks/bubbles.

Colour of medium

Light amber coloured medium

Quantity of medium

25 ml of medium in 90 mm disposable plates.

pH

7.00-7.40

Sterility Check

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 as 100% for bacteria growth on Soyabean Casein Digest Agar.

| Organism | Inoculum (CFU) | Growth | Observed Lot value (CFU) | Recovery | Incubation temperature | Incubation period |
|---|------------------|-----------|--------------------------|----------|------------------------|-------------------|
| <i>Pseudomonas paraeruginosa</i> ATCC 9027 (00026*) | 50 -100 | luxuriant | 25 -100 | ≥50 % | 30 -35 °C | ≤18 hrs |
| <i>Escherichia coli</i> ATCC 8739 (00012*) | ≥10 ³ | inhibited | 0 | 0% | 30 -35 °C | ≥72 hrs |
| <i>Pseudomonas aeruginosa</i> ATCC 27853 (00025*) | 50 -100 | luxuriant | 25 -100 | ≥50 % | 30 -35 °C | 18 -24 hrs |
| <i>Pseudomonas aeruginosa</i> ATCC 25668 (00114*) | 50 -100 | luxuriant | 25 -100 | ≥50 % | 30 -35 °C | 18 -24 hrs |
| <i>Stenotrophomonas maltophilia</i> ATCC 13637 | ≥10 ³ | inhibited | 0 | 0% | 30 -35 °C | ≥72 hrs |
| <i>Escherichia coli</i> ATCC 25922 (00013*) | ≥10 ³ | inhibited | 0 | 0% | 30 -35 °C | ≥72 hrs |
| <i>Staphylococcus aureus</i> subsp.aureus ATCC 6538 (00032*) | ≥10 ³ | inhibited | 0 | 0% | 30 -35 °C | ≥72 hrs |
| <i>Staphylococcus aureus</i> subsp.aureus ATCC 25923 (00034*) | ≥10 ³ | inhibited | 0 | 0 % | 30 -35 °C | ≥72 hrs |
| <i>Salmonella</i> Typhimurium ATCC 14028 (00031*) | ≥10 ³ | inhibited | 0 | 0% | 30 -35 °C | ≥72 hrs |

Proteus mirabilis ATCC 29906 (00023*) $\geq 10^3$ inhibited 0 0% 30 -35 °C ≥ 72 hrs

Key : (*) Corresponding WDCM numbers.

^ Formerly known as *Pseudomonas aeruginosa*

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 clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (11,12).

Reference

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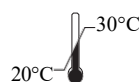
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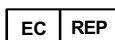
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