



Ready Prepared Media

Technical Information

Pseudomonas Isolation Agar Plate

Product Code: PM 1406

Application: Recommended for selective isolation and identification of *Pseudomonas aeruginosa* from clinical and non clinical specimens.

Composition**

Ingredients	Gms / Litre
Peptone	20.000
Magnesium chloride	1.400
Potassium sulphate	10.000
Triclosan (Irgasan)	0.025
Glycerol	20.000ml
Agar	13.600
Final pH (at 25°C)	7.0±0.2

**Formula adjusted, standardized to suit performance parameters

Principle & Interpretation

Pseudomonas aeruginosa is an important human pathogen commonly found in nosocomial infections. It successfully combines adaptability to a variety of moist environments with a collection of potent virulence factors (1). *Pseudomonas* infections usually occur at any site where moisture tends to accumulate e. g. tracheostomies, in-dwelling catheters, burns, the external ear and weeping cutaneous wounds (2). *Pseudomonas* Isolation Agar Base, used for the selective isolation and identification of *P. aeruginosa*, is a modification of Medium A, originally formulated by King, Ward and Raney (3). The medium contains pigment-enhancing components and the selective agents, triclosan (4) which selectively inhibits non-pseudomonads. The pigment-enhancers i.e. potassium sulphate and magnesium chloride enhance the blue or blue-green pigment production by *P. aeruginosa*, thus aiding in its identification. Peptone provides nitrogenous compounds and other essential growth nutrients. Glycerol is a source of energy and promotes pyocyanin i.e. pigment production which is characteristic of *Pseudomonas* (5,6). Potassium sulphate and magnesium chloride enhance pyocyanin production. Triclosan (7) selectively inhibits gram-positive and gram negative bacteria but *Pseudomonas* species are resistant to it. Some pyocyanin producing strains may also produce small amounts of fluorescein, resulting in the production of a blue-green to green pigment. Presumptive *Pseudomonas* should be further confirmed by performing biochemical tests, as some strains of *Pseudomonas* do not produce pyocyanin (8).

Type of specimen

Clinical specimen: Pus, wound, urine, etc.; food samples; water samples.

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (9,10).

For food samples, follow appropriate techniques for sample collection and processing as per guidelines (11).

For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (12). 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.



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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. Some strains may show poor pigmentation. In such case isolation of enriched culture will enhance pigmentation.
4. Developed colonies can be confirmed as *Pseudomonas aeruginosa* by development of blue-green pigment and confirmation by oxidase test.
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.

Methodology

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

Quality Control

Appearance

Sterile *Pseudomonas* Agar in 90 mm disposable plate with smooth surface and absence of black particles/cracks/bubbles

Colour

Light yellow coloured medium

Quantity of medium

25 ml of medium in 90 mm Petri plate

pH

6.80-7.20

Sterility Check

Passes release criteria

Cultural Response

Cultural characteristics observed after an incubation at 35-37°C for 18-48 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of colony
<i>Escherichia coli</i> ATCC 25922 (00013*)	$\geq 10^3$	inhibited	0%	
<i>Proteus mirabilis</i> ATCC 25933	$\geq 10^3$	inhibited	0%	
<i>Pseudomonas aeruginosa</i> ATCC 10145 (00024*)	50-100	luxuriant	$\geq 50\%$	green
<i>Pseudomonas aeruginosa</i> ATCC 27853 (00025*)	50-100	luxuriant	$\geq 50\%$	blue to blue green

Key : (*) Corresponding WDCM numbers.

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.



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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 (9,10).

Further Reading

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2. Koneman E. W., Allen S. D., Janda W. M., Schreckenberger P. C., Winn W. C. Jr., 1992, Colour Atlas and Textbook of Diagnostic Microbiology, 4th Ed., J. B. Lippincott Company.
3. King F. O., Ward M. K. and Raney D. E., 1954, J. Lab. Clin. Med., 44 :301.
4. Collee J. G., Fraser A. G., Marmion B. P., Simmons A., (Eds.), Mackie and McCartney, Practical Medical Microbiology, 1996, 14th Edition, Churchill Livingstone
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6. MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. I, Williams and Wilkins, Baltimore.
7. Furia T. E. and Schenkel A. G., 1968, Soap and Chemical Specialties 44:47.
8. Gaby W. L. and Free E., 1958, J. Bacteriol., 76:442
9. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
10. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W.(2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.
11. Salfinger Y., and Tortorello M.L., 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
12. Lipps WC, Braun-Howland EB, Baxter TE, eds. Standard methods for the Examination of Water and Wastewater, 24th ed. Washington DC:APHA Press; 2023.

Disclaimer :

- User must ensure suitability of the product(s) in their application prior to use.
- The product conform solely to the technical information provided in this booklet and to the best of knowledge research and development work carried at **CDH** is true and accurate
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