Modified Pseudomonas Selective Agar w/ Cetrimide (Twin Pack) M1273

Recommended for the detection and enumeration of *Pseudomonas aeruginosa* in water.

Composition**

Ingredients	g / L
Part A	-
SM powder	133.330
Part B	-
Peptone	3.330
Sodium chloride	1.670
Yeast extract	1.000
Cetrimide	0.400
Agar	20.000
Final pH (at 25°C)	7.3±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 26.4 grams of Part B in 250 ml purified / distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 20 minutes. Suspend 133.33 grams of Part A in 750 ml of purified / distilled water and sterilize by autoclaving at 15 lbs pressure (121°C) for 5 minutes. Cool to 45-50°C. Mix Part A and B and pour into sterile Petri plates.

Principle And Interpretation

Modified Pseudomonas Selective Agar w/Cetrimide was modified by Brown and Scott (1) for the confirmation of *Pseudomonas aeruginosa* in swimming pool waters. Swimming pool water is generally chlorinated potable water but it can also be from thermal springs or salt water. Microorganisms of concern are typically those from the body of the bathers, including the orifices. As *P.aeruginosa* can survive for longer time in water compared to other microorganisms, it is one of the major indicator organisms in the swimming pool. This organism is mainly responsible for ear and eye infection and is very likely to get disseminated in the swimming pool water due to constant contact of ears and eyes with the water. Modified Pseudomonas Selective Agar w/Cetrimide is formulated in accordance with ISO Committee under the specifications ISO 8360-1:1988 for the detection and enumeration of P.aeruginosa from water (2). Strains of *P.aeruginosa* are identified by their pigment production i.e. pyocyanin. *P.aeruginosa* is the only species of *Pseudomonas* or gram-negative rod known to excrete pyocyanin.

P.aeruginosa hydrolyzes casein and produces a yellowish to green diffusible pigment on Modified Pseudomonas Selective Agar w/ Cetrimide. For isolation, filter 200ml or less water of the swimming pool through sterile membrane filters. Place each membrane filter on M-PA Agar (M1121). Incubate the plates at 41.5±0.5°C for 72 hours. Typical *P.aeruginosa* colonies are 0.8-2.2 mm in diameter, flat in appearance with brownish to greenish centers. For confirmation, using make a single streak from an isolated colony on a Modified Pseudomonas Selective Agar w/ Cetrimide plate and incubate at 35-37° C for 24 hours. After incubation *P.aeruginosa* forms pigmented colonies.

SM powder, peptone and yeast extract provide all the necessary nutrients mainly nitrogenous for the multiplication of *P.aeruginosa*. *P.aeruginosa* forms yellowish green colonies on this medium. Cetrimide acts as a quaternary ammonium, cationic detergent that causes release of nitrogen and phosphorus from bacterial cells other than *P.aeruginosa.*

Type of specimen

Water samples: Chlorinated potable water, Swimming pool, Thermal springs, Salt water.

Specimen Collection and Handling

For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (3). After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

Read the label before opening the container. 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 specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1. Some species may show poor growth due to nutritional variations.

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

Part A : White to cream homogeneous free flowing powder Part B : Cream to yellow homogeneous free flowing powder

Gelling

Firm, comparable with 2.0% Agar gel.

Colour and Clarity of prepared medium

Light amber coloured opalescent gel forms in Petri plates

Reaction

Reaction of 2.64% w/v aqueous solution of Part B at 25°C. pH : 7.3±0.2

pH

7.10-7.50

Cultural Response

Cultural characteristics observed after an incubation at 35-37°C for 24-48 hours.

Organism	Inoculum (CFU)	Growth	Pigment
Escherichia coli ATCC 25922 (00013)*	>=10^4	inhibited	
Pseudomonas aeruginosa ATCC 27853 (00025)*	50-100	good-luxuriant	blue green
Stenotrophomonas maltophilia ATCC 13637	>=10^4	inhibited	

Key : (*) Corresponding WDCM numbers.

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 20-30°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. 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 (4,5).

Reference

1. Brown M. R. W. and Scott F. J. H., 1970, J. Clin. Pathol., 23:172.
2. International Organization for Standardization (ISO), Draft ISO/DIS 8360-1:1988.
3. Lipps WC, Braun-Howland EB, Baxter TE,eds. Standard methods for the Examination of Water and Wastewater, 24th ed. Washington DC:APHA Press; 2023.
4. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
5. 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.

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Disclaimer :

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