Pseudomonas Agar for Detection of Pyocyanin

MAP119

(MU119 / MM119)

Intended Use:

Recommended for detection of pyocyanin production by *Pseudomonas* species in accordance with USP/IP.

Composition**

Ingredients	g / L
Gelatin peptone#	20.000
Anhydrous potassium sulphate	10.000
Anhydrous magnesium chloride	1.400
Agar	15.000
pH after sterilization(at 25°C)	7.2±0.2

**Formula adjusted, standardized to suit performance parameters
Pancreatic digest of gelatin

Directions

Suspend 46.4 grams in 1000 ml purified/distilled water containing 10 ml glycerin. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

Principle And Interpretation

Pseudomonas Agar is based on the formulation described by King et al (1) and as recommended by USP (2) and Indian Pharmacopoeia (3) for detecting pyocyanin, a water soluble pigment by *Pseudomonas* species from pharmaceutical preparation. *Pseudomonas* strains are reported to produce phenazine pigments like Pyocyanin-blue green redox-active secondary metabolite pigment, pyorubin-rust brown pigment, oxyphenzine-a breakdown product of Pyocyanin, pyoverdin-a water soluble yellow green pigments also known as fluorescein. This medium enhances the formation of Pyocyanin and/or pyorubin and reduces that of fluorescein.

Gelatin peptone provides essential nutrients for growth of *Pseudomonas*, while glycerin provides carbon and energy to the cell. The pyocyanin pigment diffuses from the colonies of *Pseudomonas* into the agar and shows blue colouration. Potassium sulphate and magnesium chloride enhances the pyocyanin production and suppresses the fluorescein production. Low content of phosphorous in the medium also aids in inhibiting the production of fluorescein. Some *Pseudomonas* strains produce small amounts of fluorescein resulting in a blue-green colouration. Strains of *Pseudomonas aeruginosa* that may fail to produce Pyocyanin are not detected in this medium. Production of other pigments may mask the presence of Pyocyanin.

Type of specimen

Pharmaceutical samples

Specimen Collection and Handling

For pharmaceutical products, follow appropriate techniques for sample processing in case of viscous materials as mentioned under sterility (2,3). 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. 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.

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

Cream to yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

Yellow coloured clear to slightly opalescent gel forms in Petri plates

pH

7.00-7.40

Cultural Response

Growth Promotion is carried out in accordance with USP//IP. Cultural response was observed after an incubation at 33-37°C for not less than 3 days. Recovery rate is considered as 100% for bacteria growth on Soyabean Casein Digest Agar.

Organism	Inoculum (CFU)	Observed Lot value (CFU)	Recovery	Characteristic colonial morphology	Fluorescence in UV light	Growth
Test for *Pseudomonas aeruginosa*						
^*Pseudomonas paraeruginosa* ATCC 9027 (00026*)	50 -100	35 -100	>=70 %	Generally greenish	positive	positive
Additional Microbiological Testing						
Pseudomonas aeruginosa ATCC 27853 (00025*)	50 -100	35 -100	>=70 %	Generally greenish	positive	positive

Key : *Corresponding WDCM numbers. ^ Formerly known as *Pseudomonas aeruginosa*

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.King, Ward and Raney, 1954, J.Lab. and Clin. Med., 44:301

2.The United States Pharmacopoeia-National Formulatory (USP-NF), 2022.

3.Indian Pharmacopoeia, 2022, Indian Pharmacopoeia Commission, Ministry of Health and Family Welfare Government of India.

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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