Medium 21. Pseudomonas Agar Medium for Detection of Pyocyanin

MM119

Pseudomonas Agar for detection of Pyocyanin is recommended for the detection of pyocyanin production by *Pseudomonas* species in accordance with Indian Pharmacopoeia, 2007.

Composition**

Ingredients	Gms / Litre
Pancreatic digest of gelatin	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

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 15 lbs pressure (121°C) for 15 minutes or as per validated cycle.

Principle And Interpretation

Pseudomonas Agar is based on the formulation described by King et al (1) and as recommended in Indian Pharmacopoeia (2) for detecting pyocyanin, a water soluble pigment by *Pseudomonas* species from pharmaceutical preparation and clinical specimens such as stools, wounds, and urine. (3). *Pseudomonas* species are commonly isolated pathogen and is the significant causative agent of nosocomial, skin and burn infections. 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. Pyocyanin is readily recovered in large quantities in sputum from patients with cystic fibrosis, an infection caused by *Pseudomonas* (4,5). This medium enhances the formation of Pyocyanin and/or pyorubin and reduces that of fluorescein

Pancreatic digest of gelatin provides essential nutrients for growth of *Pseudomonas* , while glycerol 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.

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 the harmonized method of 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.

Cultural Response

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

Storage and Shelf Life

Store below 30°C in tightly closed container and the prepared medium at 2 - 8°C. Use before expiry date on the label.

Reference

1.King, Ward and Raney, 1954, J.Lab. and Clin. Med., 44:301
2.Indian Pharmacopoeia, 2007, Govt. of India, Ministry of Health and Family Welfare, New Delhi, India.
3.MacFaddin J., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. I, Williams and Wilkins, Baltimore.
4. Daly J A, Boshard R, and Matsen J M, 1984, J Clin Microbiol. 19: 742
5. Lau GW, Hassett DJ, Ran H, Kong F., 2004. Trends Mol Med. 10:599

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