Pseudomonas Agar Medium for Detection of Fluorescein

MU120

Pseudomonas Agar Medium for Detection of Fluorescein is used for the detection of fluorescein production by *Pseudomonas* species, and is in accordance with United States Pharmacopoeia 2008.

Composition**

Ingredients	Gms / Litre
Pancreatic digest of casein	10.000
Peptic digest of animal tissue	10.000
Anhydrous dibasic potassium phosphate	1.500
Magnesium sulphate, 7H2O	1.500
Agar	15.000
pH after sterilization (at 25°C)	7.2±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 37.23 grams (the equivalent of dehydrated medium per litre) 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.

Principle And Interpretation

Pseudomonas Agar (For Fluorescein) is based on the formula described by King et al (1) and as modified in the U.S. Pharmacopeia (2) for the detection of fluorescein production a water soluble, chloroform insoluble fluorescent pigment by *Pseudomonas* species (3). *Pseudomonas* is ubiquitous in environment and is a common causative agent of burn, skin and nosocomial infections. They are also common contaminant of pharmaceutical and cosmetics related preparations. 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 elaboration of fluorescein by *Pseudomonas* and inhibits the pyocyanin formation. The fluorescein pigment diffuses from the colonies of Pseudomonas into the agar and shows yellow fluorescent colouration. Some Pseudomonas strains produce small amounts of pyocyanin resulting in a yellow-green colouration. Peptic digest of animal tissue provides the essential nitrogenous nutrients, carbon, sulfur and trace elements for the growth of *Pseudomonas* . These nutrients are also conducive to the production of fluroescein. Peptone and phosphorous in the medium enhance the production of pyoverdin/ fluorescein pigment. Dipotassium phosphate buffers the medium while magnesium sulphate provides necessary cations for the activation of fluorescein production.

Salt concentration exceeding 2% affects pigment production. UV illumination may be bactericidal, so make sure that there is good growth before placing culture under UV light (3).

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 USP. 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

MU120: Cultural characteristics observed after incubation at 33-37°C for 18-48 hours. 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	Oxidase
Test for Pseudomonas aeruginosa						
Pseudomonas aeruginosa ATCC 9027	50 -100	35 -100	>=70 %	Generally colourless to yellowish	positive	positive
Additional Microbiological Testing						
Pseudomonas aeruginosa ATCC 27853	50 -100	35 -100	>=70 %	Generally colourless to yellowish	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. Clin. Med., 44 : 301.
2.United States Pharmacopoeia, 2008 United States Pharmacopoeia Convention, Inc., Rockville, MD.
3. MacFaddin J., 1985, Media for Isolation-Cultivation-Identification and Maintenance of Medical Bacteria, Vol. I, Williams and Wilkins, Baltimore.

Revision : 2 / 2015