Glycerol Mannitol Acetamide Cetrimide Agar

M1935

Recommended for the enumeration of *Pseudomonas aeruginosa* from contaminated materials.

Composition**

Ingredients	Gms / Litre
Part A	-
Peptone	0.200
Potassium sulphate	10.000
Magnesium chloride,6H2O	1.400
Cetrimide	0.300
D-Mannitol	5.000
Agar	15.000
Part B	-
Phenol red	0.012
Acetamide	10.000
Final pH (at 25°C)	7.0±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 31.16 grams of part A in 900 ml distilled water containing 5 ml of glycerol. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 118°-121°C for 20 minutes. Suspend 10.012 grams of part B Dispense in tubes or as desired. Sterilize by autoclaving at 15lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Aseptically add contents of Part B to Part A. Mix well and pour into sterile Petri plates.

Principle And Interpretation

Gilardi and others showed that a wide variety of non-fermenting organisms were capable of utilizing acetamide by using basal mineral media (1,2). However very few organisms growing in the medium metabolize acetamide by the process of deamination (acrylamidase activity) (3,4). This unique ability is useful in identification of various non-fermenting gram-negative organisms (5,6,7). This ability is shown by *Pseudomonas aeruginosa* (8). Acetamide deamination leads to the liberation of ammonia, which thereby increases the pH of the medium, leading to a subsequent colour change of the phenol red indicator from yellow orange to cherry red.

The medium was distributed in tubes as slants. The tubes were streaked and then incubated in water bath at 41-43°C for 24-48 hours. *Ps.aeruginosa* grew luxuriantly with a colour change from yellow orange to cherry red. The medium can also be used to enumerate *Ps.aeruginosa* in contaminated materials. The contaminated lake water or sewage were spread directlyon GMAC Agar in Kolle flasks and incubated at 41-43°C for 20-48 hours. After incubation the colonies surrounded by red zones were counted as *Ps.aeruginosa* . This medium does not support the growth of most organisms(9).

Peptone in the medium supports growth. Glycerol serves as a carbon source. Potassium sulphate and magnesium chloride serves as a source of ions that stimulate metabolism. Mannitol is the fermenting sugar. Acetamide is a source of nitrogen and carbon. Phenol red is the indicator dye. Acetamide is deaminated by *Ps.aeruginosa* and mannitol is not fermented which is detected by phenol red indicator. This imparts cherry red colour.

Quality Control

Appearance

Part A :Cream to yellow homogeneous flowing powder Part B : Light yellow to pink-red deliquescent crystals

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

Yellow orange coloured clear to slightly opalescent gel forms in tubes as slants or can be poured into sterile Petri plates.

Reaction
Reaction of the medium (Mixture of 3.16 % w/v Part A and 1.012% Part B)at 25°C. pH : 7.0±0.2

pH
6.80-7.20

Cultural Response
Cultural characteristics observed after an incubation at 35-37°C for 20-48 hours.

Cultural Response

Organism	Inoculum (CFU)	Growth	Deamination	Colour of colony
Cultural Response				
Stenotrophomonas maltophila ATCC 13637	50-100	good-luxuriant	negative reaction ,no cherry red colour.	colonies without red zone
Pseudomonas aeruginosa ATCC 27853	50-100	good-luxuriant	positive reaction, cherry red colour	colonies surrounded by cherry red colour

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
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