# Glycerol Mannitol Acetamide Cetrimide Agar (Twin Pack) M1935

## Intended Use:

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

## Composition**

| Ingredients | g / L |
|---|---|
| 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 purified/distilled water containing 5 ml of glycerol. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at Δ 118-121°C for 20 minutes. Cool to 45-50°C. Suspend 10.012 grams of Part B in 100 ml purified/distilled water. Dispense in tubes or flasks 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. Δ corresponds to 12-15 lbs pressure.

## 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. *P.aeruginosa* grew luxuriantly with a colour change from yellow orange to cherry red. The contaminated lake water or sewage were spread directly on GMAC Agar in Kolle flasks and incubated at 41-43°C for 20-48 hours. The medium can also be used to enumerate *P. aeruginosa* in contaminated materials. After incubation the colonies surrounded by red zones were counted as *P.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 *P. aeruginosa* and mannitol is not fermented which is detected by phenol red indicator. This imparts cherry red colour.

## Type of specimen

Clinical sample : pus, wound samples; Water samples - lake water or sewage water samples.

## Specimen Collection and Handling:

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (10,11).

For water samples follow appropriate techniques for handling specimens as per established guidelines (12).

After use, contaminated materials must be sterilized by autoclaving before discarding.

## Warning and Precautions

In Vitro diagnostic Use. For professional use only. 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 clinical 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**

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.

| Organism | Inoculum (CFU) | Growth | Deamination | Colour of colony |
|---|---|---|---|---|
| *Stenotrophomonas maltophila* ATCC 13637 | 50-100 | good-luxuriant | negative reaction ,no cherry red colour. | colonies without red zone |
| *Pseudomonas aeruginosa* ATCC 27853 (00025*) | 50-100 | good-luxuriant | positive reaction, cherry red colour | colonies surrounded by cherry red colour |

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 clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (10,11).

## Reference

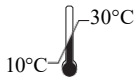
1. Gilardi, 1974, Antonie Van Leeuwenhoek, J. Microbiology Serol., 39:229.
2. Stainier, Palleroni and Doudoroff, 1966, J. Gen Microbiol., 43:159.
3. Pickett M. J. and Pedersen M.M., 1970,Can.J. Microbiol., 16:351.

4. Pickett M. J. and Pedersen M.M., 1970, Can. J.Microbiol., 16:401.
5. Buhlmann, Vischer and Bruhin, 1961, J. Bacteriol., 82:787
6. Hedberg, 1969, Appl. Microbiol., 17: 481
7. Smith and Dayton, 1972, Appl. Microbiol., 24: 143
8. Oberhofer and Rowen, 1974, Appl. Microbiol., 28:720.
9. Mossel, D.A.A. and Lourdes Indacochea. 1970 A new Cetrimide Medium for the detection of Pseudomonas aeruginosa.
10. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
11. 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.
12. Baird R.B., Eaton A.D., and Rice E.W., (Eds.), 2015, Standard Methods for the Examination of Water and Wastewater, 23rd ed., APHA, Washington, D.C.

Revision :05/ 2024

HiMedia Laboratories Pvt. Limited,
Plot No.C-40, Road No.21Y,
MIDC, Wagle Industrial Area,
Thane (W) -400604, MS, India

IVD *In vitro* diagnostic medical device




**Storage temperature**

EC REP

CEpartner4U, Esdoornlaan 13,
3951DB Maarn, NL
www.cepartner4u.eu

CE **CE Marking**

**Do not use if package is damaged**

Disclaimer :

User must ensure suitability of the product(s) in their application prior to use. Products conform solely to the information contained in this and other related HiMedia™ publications. The information contained in this publication is based on our research and development work and is to the best of our knowledge true and accurate. HiMedia™ Laboratories Pvt Ltd reserves the right to make changes to specifications and information related to the products at any time. Products are not intended for human or animal or therapeutic use but for laboratory,diagnostic, research or further manufacturing use only, unless otherwise specified. Statements contained herein should not be considered as a warranty of any kind, expressed or implied, and no liability is accepted for infringement of any patents.