



Nutrient Agar Medium

MM012

Intended Use:

A general culture medium which may be used as enriched medium by incorporating blood or other biological fluids in accordance with as per I.P.1996.

Composition**

Ingredients	Gms / Litre
Peptone	10.000
HM peptone B #	10.000
Sodium chloride	5.000
Agar	12.000
pH after sterilization	7.3±0.1

**Formula adjusted, standardized to suit performance parameters

Equivalent to Beef extract

Directions

Suspend 37.0 grams in 1000 ml purified/distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at $\Delta 115^{\circ}\text{C}$ for 30 minutes or alternatively at 15 lbs pressure (121°C) for 15 minutes or as per validated cycle. Δ Corresponds to 10 lbs pressure.

Note: Directions specified are as per the concurrent edition of pharmacopoeia in force. Specified expiry period corresponds to this. User must ensure its compatibility with the latest edition.

Principle And Interpretation

Nutrient Agar is a basic culture medium used for maintaining microorganisms (1), for purity checking prior to biochemical or serological testing. It is used for the cultivation and enumeration of bacteria, which are not particularly fastidious. In semisolid form it is used for maintenance of control or standard organisms. Indian Pharmacopoeia has recommended it for microbial limit tests of viable aerobic microorganism present in pharmaceutical substances (2).

Peptone and HM peptone B provide the necessary nitrogen compounds, carbon, vitamins and also some trace ingredients. Sodium chloride maintains osmotic equilibrium. Nutrient media may be used as enriched media by the addition of 10% v/v blood or other biological fluids like ascitic fluid, serum etc.

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) 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.
3. Other biochemical tests must be carried out for confirmation.

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 1.2% Agar gel

Colour and Clarity of prepared medium

Light yellow coloured clear to slightly opalescent gel forms in Petri plates

pH

7.20-7.40

Growth Promotion Test

Growth Promotion is carried out as per Indian Pharmacopoeia

Cultural Response

Cultural characteristics observed after an incubation at 35-37°C for 18-48 hours.

Organism	Inoculum (CFU)	Observed Lot value (CFU)	Recovery	Incubation temperature	Incubation period
<i>Escherichia coli</i> ATCC 8739 (00012*)	50 -100	35 -100	≥70 %	35 -37 °C	18 -24 hrs
<i>Staphylococcus aureus</i> subsp <i>aureus</i> ATCC 6538 (00032*)	50 -100	35 -100	≥70 %	35 -37 °C	18 -24 hrs
<i>Salmonella</i> Typhimurium ATCC 14028 (00031*)	50 -100	35 -100	≥70 %	35 -37 °C	18 -24 hrs
<i>Salmonella</i> Abony NCTC 6017 (00029*)	50 -100	35 -100	≥70 %	35 -37 °C	18 -24 hrs
<i>Pseudomonas aeruginosa</i> ATCC 9027 (00026*)	50 -100	35 -100	≥70 %	35 -37 °C	18 -24 hrs

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 sample must be decontaminated and disposed of in accordance with current laboratory techniques (3,4).

Reference

- Lapage S., Shelton J. and Mitchell T., 1970, 'Methods in Microbiology', Norris J. and Ribbons D. (ed.), Vol. 3A., Academic Press, London.
- Indian Pharmacopoeia, 2022, Indian Pharmacopoeia Commission, Ministry of Health and Family Welfare Government of India.
- Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
- 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.

Disclaimer :

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