Antibiotic Assay Medium E

M1347

Intended Use:

Recommended in the microbiological assay of Neomycin sulphate and Framycetin sulphate using *Bacillus subtilis* and *Bacillus pumilis.*

Composition**

Ingredients	Gms / Litre
Peptone	5.000
HM extract #	3.000
Disodium hydrogen phosphate.12H_2O	26.900
Agar	10.000
Final pH (at 25°C)	7.9±0.2

**Formula adjusted, standardized to suit performance parameters
Equivalent to Meat extract

Directions

Suspend 28.67 grams (the equivalent weight of dehydrated medium per litre) in 1000 ml purified/distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

Advice: *Recommended for the microbiological assay of Framycetin sulphate and Neomycin sulphate.*

Principle And Interpretation

Antibiotic Assay Medium E is widely used as seed agar in the plate assay of Framycetin sulphate and Neomycin sulphate using *Bacillus subtilis* and *Bacillus pumilus* as test organism.

Peptone and HM extract supplies nutrients essential for microbial growth. Phosphates are incorporated in the medium to provide good buffering action. The low concentration of agar facilitates proper diffusion of antibiotic in the seed agar. This medium is formulated in accordance to British Pharmacopoeia (1) and European Pharmacopoeia (2).

Freshly prepared plates should be used for antibiotic assays. Test organisms are inoculated in sterile seed agar cooled to 40-45°C and spread evenly over the surface of solidified base agar. Zones of inhibition around the antibiotic are then measured. All conditions in the microbiological assay must be controlled carefully. The use of standard culture media in the test is one of the important steps for good results.

Type of specimen

Pharmaceutical sample

Specimen Collection and Handling

For pharmaceutical sample follow appropriate techniques for handling specimens as per established guidelines (1,2). 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. Freshly prepared medium plates must be used or it may result in erroneous results.

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 1.0% Agar gel.

Colour and Clarity of prepared medium
Light yellow coloured clear to slightly opalescent gel forms in Petri plates

Reaction
Reaction of 2.87 % w/v aqueous solution after sterilization. pH : 7.9±0.2

pH
7.70 - 8.10

Cultural Response
Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Antibiotics assayed
Bacillus pumilus NCTC 8241	50-100	luxuriant	>=70%	Neomycin sulphate, Framycetin sulphate
Bacillus subtilis subsp. *spizizenii* ATCC 6633 (00003*)	50-100	luxuriant	>=70%	Neomycin sulphate, Framycetin sulphate

Key : *Corresponding WDCM numbers.

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and use freshly prepared medium. 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

1. The British Pharmacopoeia, 2022, Medicines and Healthcare products Regulatory Agency.
2. European Pharmacopoeia, 2022, 10 th volume, European Directorate for the quality of medicines & Healthcare.
3. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
4. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual Clinical Microbiology, 11th Edition. Vol. 1.

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