Antibiotic Assay Medium No. 5 (Antibiotic Assay Medium E) MAP006

(MU006/MM006)

Intended Use:

Recommended for microbiological assay of Dihydrostreptomycin using *Bacillus subtilis* in accordance with USP and Framycetin, Kanamycin B and Teicoplanin in accordance with IP.

Composition**

Ingredients	g / L
Peptone	6.000
Yeast extract	3.000
HM peptone B #	1.500
Agar	15.000
pH after sterilization	7.9±0.1

**Formula adjusted, standardized to suit performance parameters

Equivalent to Beef extract

Directions

Suspend 25.5 grams 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 Dihydrostreptomycin, Framycetin, Kanamycin B and T*eicoplanin*

Principle And Interpretation

This medium is used in the assay of commercial preparations of antibiotics, as well as for antibiotics in body fluids, feeds etc. Medium composition is in accordance to the specifications detailed in the FDA, USP, IP, (1,2,3) and numerically identical to the name assigned by Grove and Randall (4).

Peptone, yeast and HM peptone B provides necessary growth nutrients for the test organisms like *Bacillus subtilis*. This medium provides solidified substratum for growth of organims. The pH 7.9 maintained in this medium provides optimum growth conditions for *Bacillus subtilis* (5). This medium is used to prepare the base as well as seed layer in the microbiological assay of Dihydrostreptomycin in accordance with USP (2) and Framycetin, Kanamycin B and Teicoplanin in accordance with IP (3).

Type of specimen

Pharmaceutical samples

Specimen Collection and Handling

For pharmaceutical samples follow appropriate techniques for handling specimens as per established guidelines (2,3).

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 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 coloured homogeneous free flowing powder

Gelling
Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium
Medium amber coloured slightly opalescent gel forms in Petri plates.

pH
7.80-8.00

Cultural Response
Cultural characteristics observed after an incubation at 32-35°C for 5 days.

Organism	Inoculum (CFU)	Growth	Recovery	Antibiotics assayed
In accordance with USP				
** *Bacillus spizizenii* ATCC 6633 (00003*)	50-100	good-luxuriant	>=70%	Dihydrostreptomycin
In accordance with IP				
** *Bacillus spizizenii* ATCC 6633 (00003*)	50-100	good-luxuriant	>=70%	Framycetin, Kanamycin B andTeicoplanin

Key : *- Corresponding WDCM numbers **Formerly known as *Bacillus subtilis* subsp. *spizizenii*

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 (5,6).

Reference

1. Tests and Methods of Assay of Antibiotics and Antibiotic containing Drugs, FDA, CFR, 1983 Title 21, Part 436, Subpart D, Washington, D.C.: U.S. Government Printing Office, paragraphs 436, 100-436, 106, p. 242-259.
2. The United States Pharmacopoeia-National Formulatory (USP-NF), 2022.
3. Indian Pharmacopeia 2020, Ministry of Health and Family welfare, Government of India, New Delhi.
4. Grove and Randall, 1955, Assay Methods of Antibiotics Medical Encyclopaedia, Inc. New York.
5. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
6. 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.

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