Antibiotic Assay Medium No.9

MU147

Intended Use:

Used as base layer for microbiological plate assay of Carbenicillin, Colistimethate sodium and Polymyxin B in accordance with USP.

Composition**

Ingredients	g / L
Tryptone	17.000
Soya peptone	3.000
Dextrose (Glucose)	2.500
Sodium chloride	5.000
Dipotassium hydrogen phosphate	2.500
Agar	20.000
pH after sterilization	7.2±0.1

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 50.0 grams in 1000 ml purified/distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

Principle And Interpretation

This medium is widely recommended for assay of Polymyxin B, Colistimethate sodium and Colistin using *Bordetella bronchiseptica* as test organims. Carbenicillin assay is also performed using this medium with *Pseudomonas aeruginosa*.The medium is formulated in accordance with USP and CFR (1,2) and numerically identical with the name assigned by Groove and Randall (3).

Tryptone and soya peptone serves as source for essential nutrients. Dextrose stimulates the growth by providing carbon and energy. Phosphates in the medium enhance buffering action and sodium chloride maintains osmotic equilibrium in the medium. Agar concentration provides control over the diffusion activity of Polymixin B antibiotics and provides solid substratum to support the seed agar layer.

Type of specimen

Pharmaceutical preparations

Specimen Collection and Handling

To perform the antibiotic assay the Base Agar should be prepared on the same day as the test. For the cylinder method, a base layer of 21 ml is required. Once the base medium has solidified, seed layer inoculated with the standardized culture can be overlaid. Even distribution of the layer is important. 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 2.0% agar gel.

Colour and Clarity of prepared medium

Light amber coloured clear to slightly opalescent gel forms in Petri plates.

pH

7.10-7.30

Cultural Response

Cultural characteristics observed after an incubation at 36-37.5°C for 18-24 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Antibiotics assayed
Bordetella bronchiseptica ATCC 4617	50-100	luxuriant	>=50%	Polymyxin B, Colistimethate sodium, Colistin
Pseudomonas aeruginosa ATCC 25619	50-100	luxuriant	>=70%	Carbenicillin

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 (4,5).

Reference

1. The United States Pharmacopoeia-National Formulatory (USP-NF), 2022.
2. 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, (April1).
3. Grove and Randall, 1955, Assay Methods of Antibiotics Medical Encyclopaedia, Inc. New York.
4. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
5. 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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