



Technical Data

Antibiotic Assay Medium No. 9 (Polymyxin Base Agar)

M147

Intended Use:

Recommended as a base layer medium for assaying the products containing Polymyxin-B.

Composition**

Ingredients	Gms / Litre
Tryptone	17.000
Soya peptone	3.000
Sodium chloride	5.000
Dipotassium hydrogen phosphate	2.500
Dextrose (Glucose)	2.500
Agar	20.000
Final pH (at 25°C)	7.2±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 50 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 or as desired..

Principle And Interpretation

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

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

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.

Type of specimen

Pharmaceutical preparations

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 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

Reaction

Reaction of 5% w/v aqueous solution at 25°C. pH : 7.2±0.2

pH

7.00-7.40

Cultural Response

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

Organism	Inoculum (CFU)	Growth	Recovery	Antibiotics assayed
<i>Bordetella bronchiseptica</i> ATCC 4617	50-100	good	50-70%	Colistimethate sodium, Colistin, Polymyxin B Carbenicillin
<i>Pseudomonas aeruginosa</i> ATCC 25619	50-100	luxuriant	≥70%	Carbenicillin
<i>Pseudomonas aeruginosa</i> ATCC 27853 (00025*)	50-100	luxuriant	≥70%	Carbenicillin

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 (2,3).

Reference

1. Grove and Randall, 1955, Assay Methods of Antibiotics Medical Encyclopedia, Inc. New York.
2. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
3. 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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Disclaimer :

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