Antibiotic Assay Medium No. 10 (Polymyxin Seed Agar) M225

Intended Use:

Recommended as a seed layer medium for assaying the products containing Polymyxin-B, also for assaying Carbenicillin, Colistin and Colistimethate sodium.

Composition**

Ingredients	Gms / Litre
Tryptone	17.000
Soya peptone	3.000
Sodium chloride	5.000
Dextrose (Glucose)	2.500
Dipotassium hydrogen phosphate	2.500
Agar	12.000
Final pH (at 25°C)	7.2±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 42 grams in 1000 ml purified / distilled water cotaining10 ml Polysorbate 80. 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 dispense as desired.

Principle And Interpretation

Antibiotic Assay media are used in the performance of antibiotic assays. Grove and Randall have elucidated those antibiotic assays and media in their comprehensive treatise on antibiotic assays (1). Schmidt and Moyer have reported the use of antibiotic assay medium for the liquid formulation used in the performance of antibiotic assay (4). 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. After incubation the concentration of the antibiotic being assayed is determined by measuring the zone of inhibition obtained, with that of reference standard antibiotic. All conditions in the microbiological assay must be carefully controlled. The use of standard culture media in the test is one of the important steps for good results.

Nutrients and growth factors are supplied by the ingredients like Tryptone and Soya peptone. Sodium chloride maintains the osmotic equilibrium. Dipotassium hydrogen phosphate provides the buffering system. Dextrose serves as the source of energy.

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 1.2% Agar gel.

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

Reaction
Reaction of 4.2% w/v aqueous solution containing 1% polysorbate 80 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
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 (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.

4. Schmidt and Moyer, 1944; J. Bact, 47:199.

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