



Antibiotic Assay Medium B

M1346

Intended Use:

Recommended for microbiological assay of Colistin sulphate and Polymyxin B sulphate using *Bordetella bronchiseptica*.

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	15.000
Final pH (at 25°C)	7.3±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 45.0 grams in 1000 ml purified/distilled water containing 10.0 grams 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.

Principle And Interpretation

Antibiotic Assay Medium B is used for the microbiological assay of Colistimethate sodium. Combination of tryptone and soya peptone provides essential nutrients for the growth of test organisms. Dextrose provides fermentable source of carbon, and enhances the growth of test organisms. Phosphates in the medium enhances buffering action and sodium chloride maintains osmotic equilibrium. It is recommended by various pharmacopoeia (1,2).

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. All conditions in the microbiological assay must be controlled carefully. The use of standard culture media in the test is one of the important step for the good results.

Type of specimen

Pharmaceutical samples

Specimen Collection and Handling

For pharmaceutical samples, follow appropriate techniques for sample collection, processing as per 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 1.5% Agar gel

Colour and Clarity of prepared medium

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

Reaction

Reaction of 4.5% w/v aqueous solution at 25°C. pH : 7.3±0.2

pH

7.10-7.50

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	luxuriant	≥70%	Colistimethate sodium
<i>Escherichia coli</i> ATCC 10536	50-100	luxuriant	≥70%	Colistimethate sodium

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 of Clinical Microbiology, 11th Edition. Vol. 1.

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