



Antibiotic Assay Medium No. 10

MU225

Antibiotic Assay Medium No. 10 is used as seed layer for antibiotic plate assay of Carbenicillin, Colistimethate sodium, Colistin and Polymyxin B in accordance with United States Pharmacopoeia.

Composition**

Ingredients	Gms / Litre
Tryptone #	17.000
Soya peptone ##	3.000
Dextrose	2.500
Sodium chloride	5.000
Dibasic potassium phosphate	2.500
Agar	12.000
pH after sterilization	7.2±0.1

**Formula adjusted, standardized to suit performance parameters

Pancreatic digest of casein

Papaic digest of soybean

Directions

Suspend 42 grams in 1000 ml purified/distilled water containing 10 ml of 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

This medium is used as seed agar for assay of Polymyxin B, Colistimethate sodium, Colistin and Carbenicillin. The medium composition is in accordance to USP and CFR (1,2) and numerically identical with the name assigned by Groove and Randall (3).

Combination of tryptone and soya peptone provide essential nutrients for the growth of test organisms. Dextrose provides the carbon source, enhances the growth of test organism. Phosphates in the medium enhances buffering action and sodium chloride maintains osmotic equilibrium. Polymixins are reported to have slow diffusion in agar giving smaller zone of inhibition (4). Hence the reduced agar concentration (1.2%) in this medium improves the diffusion of polymyxin in the medium. Polysorbate 80 is reported to function synergistically with polymixins on spheroplasts of *Pseudomonas aeruginosa*. Polysorbate 80 enhances the penetration of Polymyxin to the cytoplasmic membrane and hence is an appropriate ingredient in the medium used for assay of Polymyxin (5).

Freshly prepared plates should be used for antibiotic assays. Test organisms are inoculated in sterile seed agar pre-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 steps for good results.

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 very slightly opalescent gel forms in Petri plates.

Reaction

Reaction of 4.2% w/v aqueous solution containing 1% polysorbate 80 (after sterilization). pH : 7.2±0.1

pH

7.10-7.30

Growth Promotion Test

As per United States Pharmacopoeia

Cultural Response

Cultural characteristics observed after an incubation at 32-37°C for 18-24 hours.

Cultural Response

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

Storage and Shelf Life

Store below 30°C in tightly closed container and use freshly prepared medium . Use before expiry date on the label.

Reference

1. United States Pharmacopoeia / National Formulary 2011, US Pharmacopoeial Convention, Inc., Rockville, MD.
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, (April 1).
3. Grove and Randall, 1955, Assay Methods of Antibiotics Medical Encyclopaedia, Inc. New York.
4. Barry, 1991, Procedure and theoretical considerations for testing antimicrobial agents in agar media. Antibiotics in Laboratory medicine, New York pp 3
5. Brown & Winsley, 1968.. J Gen Microbiol. 1968 50(3) Suppl:ix.

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