



Antibiotic Assay Medium No.10 (Antibiotic Assay Medium H) MAP225 (MU225/MM225)

Intended use

Antibiotic Assay Medium No.10 (Antibiotic Assay Medium H) is used as seed layer for microbiological assay of Carbenicillin, Colistimethate sodium, Colistin sulphate and Polymyxin B in accordance with USP/IP.

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	12.000
Final pH (after sterilization)	7.2±0.1

**Formula adjusted, standardized to suit performance parameters

Pancreatic digest of casein ## Papaic digest of soyabean

Directions

Suspend 42.0 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 or as per validated cycle. 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 Carbenicillin, Colistimethate sodium, Colistin sulphate and Polymyxin B. The medium composition is in accordance to IP, USP and CFR (1,2,3) and numerically identical with the name assigned by Groove and Randall (4).

Combination of tryptone and soya peptone provides nitrogenous and carbonaceous compounds, long chain amino acids, vitamins and other essential nutrients for the growth of test organisms. Natural soya sugars enhance microbial growth. Dextrose (Glucose) provides the carbon source, enhances the growth of test organism. Phosphates in the medium enhance buffering action and sodium chloride maintains osmotic equilibrium. Polymyxin are reported to have slow diffusion in agar giving smaller zone of inhibition (5). Hence the reduced agar concentration (1.2%) in this medium improves the diffusion of polymyxin in the medium. Polysorbate 80 are reported to function synergistically with polymyxin 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 (6).

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 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 must be used for testing or it may result in erroneous results.
2. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium.
3. Each lot of the medium has been tested for the organisms specified on the COA. It is recommended to users to validate the medium for any specific microorganism other than mentioned in the COA based on the user's unique requirement.

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.

pH

7.10-7.30

Cultural Response

Cultural characteristics observed after an incubation at

As per IP: different temperatures for 24 hours.

As per USP: 32-35°C for 24 hours

As per USP

Organism	Inoculum (CFU)	Incubation Temperature	Growth	Recovery	Antibiotics assayed
<i>Bordetella bronchiseptica</i> ATCC 4617	50-100	32-35°C	luxuriant	>=70%	Polymyxin B, Colistimethate, Colistin

As per IP

Organism	Inoculum (CFU)	Incubation Temperature	Growth	Recovery	Antibiotics assayed
<i>Bordetella bronchiseptica</i> ATCC 4617	50-100	32-35°C	luxuriant	>=70%	Polymyxin B, Colistimethate sodium, Colistin sulphate
<i>Pseudomonas aeruginosa</i> ATCC 25619	50-100	36-37.5°C	luxuriant	>=70%	Carbenicillin
<i>Escherichia coli</i> ATCC 10536	50-100	35-39°C	luxuriant	>=70%	Colistimethate sodium, Colistin sulphate

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 (7,8).

Reference

1. Indian Pharmacopoeia, 2022, Indian Pharmacopoeia Commission, Ministry of Health and Family Welfare Government of India.
2. The United States Pharmacopoeia-National Formulary (USP-NF), 2022.
3. 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 11).
4. Grove and Randall, 1955, Assay Methods of Antibiotics Medical Encyclopedia, Inc. New York.
5. Barry, 1991, Procedure and theoretical considerations for testing antimicrobial agents in agar media. Antibiotics in Laboratory medicine, New York pp 3

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6. Brown & Winsley, 1968. J Gen Microbiol. 1968 50(3) Suppl:ix.
 7. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
 8. 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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