



Technical Data

Antibiotic Assay Medium No.19 (Antibiotic Assay Medium G) MAP101 (MU101/MM101)

Intended Use:

Recommended for microbiological assay of Amphotericin B and Nystatin using *Saccharomyces cerevisiae* as the test organisms in accordance with USP/IP.

Composition**

Ingredients	g / L
Peptone	9.400
Yeast extract	4.700
HM peptone B #	2.400
Dextrose	10.000
Sodium chloride	10.000
Agar	23.500
pH after sterilization	6.1±0.1

**Formula adjusted, standardized to suit performance parameters

Equivalent to Beef extract

Directions

Suspend 60.0 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.

Principle And Interpretation

The medium composition is in accordance to USP, IP and CFR (1,2,3). This medium is used as seed agar for assay of antifungal agents like Amphotericin B and Nystatin. This medium is used for maintenance and inoculum development of *Saccharomyces cerevisiae*. This medium is also used for assaying mycostatic activity in pharmaceutical formulations. This medium is formulated as reported by Kirshbam and Arret (4).

Ingredients like peptone, yeast extract and HM peptone B supplement essential nutrients, minerals and growth factors for the growth of test organism. Dextrose in the medium provides enhanced source of carbon and energy. Osmotic equilibrium in the medium is maintained by sodium chloride which retains the cell integrity and viability. This medium is used as both base and seed medium for agar diffusion assay for antibiotics like Amphotericin B and Nystatin.

Type of specimen

Antibiotics as per USP & IP (1,2)

Specimen Collection and Handling

Freshly prepared plates should be used for antibiotic assays. Test organisms are inoculated in sterile seed agar precooled to 40-45°C and spread evenly over the surface of solidified base agar. Prediffusion of antibiotics for 20 minutes in the agar by incubating at temperature below the optimal growth temperature for microorganism would facilitate better diffusion of antibiotic, followed by incubation of the plates for microbial growth. 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 coloured homogeneous free flowing powder

Gelling

Firm, comparable with 2.35% Agar gel.

Colour and Clarity of prepared medium

Yellow coloured clear to slightly opalescent gel forms in Petri plates

pH

6.00-6.20

Growth Promotion Test

As per USP/IP

Cultural Response

Cultural characteristics observed after an incubation at 29-31°C for 48 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Antibiotics assayed
# <i>Saccharomyces kudriavzevii</i> ATCC 2601	50-100	luxuriant	≥70%	Nystatin
<i>Saccharomyces cerevisiae</i> ATCC 9763 (00058*)	50-100	luxuriant	≥70%	Amphotericin B

Key : *- Corresponding WDCM numbers, # Formerly known as *Saccharomyces cerevisiae*

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 (5,6).

Reference

1. The United States Pharmacopoeia-National Formulary (USP-NF), 2022.
2. Indian Pharmacopoeia, 2022, Indian Pharmacopoeia Commission, Ministry of Health and Family Welfare Government of India.
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 1).
4. Krishbam A and Arret B, 1967, J.Pharma. Sci. 56:512
5. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
6. 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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