



Antibiotic Assay Medium No.19

MU101

Antibiotic Assay Medium No.19 is used for the microbiological assay of Amphotericin B and Nystatin using *Saccharomyces cerevisiae* as the test organisms in accordance with United States Pharmacopoeia.

Composition**

Ingredients	Gms / Litre
Peptone	9.400
Yeast extract	4.700
Beef extract	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

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. Mix well before pouring into sterile Petri plates.

Principle And Interpretation

The medium composition is in accordance to USP and CFR (1,2). 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 (3).

Ingredients like peptone, yeast and beef extract 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. Antibiotic assay medium No.19, is used as both base and seed medium for agar diffusion assay for antibiotics like Amphotericin B and Nystatin.

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.

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 United States Pharmacopoeia.

Cultural Response

Cultural characteristics observed after an incubation at 29-31°C for 24-48 hours.

Cultural Response

Organism	Inoculum (CFU)	Growth	Recovery	Antibiotics assayed
Cultural Response <i>Saccharomyces cerevisiae</i> ATCC 2601	50-100	luxuriant	>=70%	Nystatin
<i>Saccharomyces cerevisiae</i> ATCC 9763	50-100	luxuriant	>=70%	Amphotericin B

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 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. Krishbam A and Arret B, 1967, J.Pharma. Sci. 56:512

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