Antibiotic Assay Medium F

M923

Intended Use:

Recommended for microbiological assay of Amphotericin B and Nystatin using *Saccharomyces cerevisiae* & *Candida tropicalis* respectively.

Composition**

Ingredients	Gms / Litre
Peptone	9.400
Yeast extract	4.700
HM peptone B #	2.400
Sodium chloride	10.000
Dextrose (Glucose)	10.000
Agar	23.500
Final pH (at 25°C)	6.0±0.2

**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 or dispense as desired.

Advice: *Recommended for the microbiological assay of Amphotericin B and Nystatin.*

Principle And Interpretation

Grove and Randall have elucidated the antibiotic assays and medias in their comprehensive treatise on antibiotic assays (1). Antibiotic assay Medium F is recommended for the microbiological assay of Nystatin and Amphotericin B using *Saccharomyces cerevisiae* and *Candida tropicalis*. This medium is formulated in accordance with the European Pharmacopoeia and British Pharmacopoeia (2,3). 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. After incubation the concentration of the antibiotic being assayed is determined by measuring the zone of inhibition obtained, with that of reference standard antibiotic. All conditions in the microbiological assay must be carefully controlled. The use of standard culture media in the test is one of the important steps for good results. Peptone, yeast extract and HM Peptone B supply essential nutrients, minerals and growth factors for the growth of the test organisms. Glucose monohydrate in the medium provides enhanced source of carbon and energy. Osmotic equilibrium in the medium is provided by sodium chloride thereby maintaining the cell viability and integrity. Higher agar concentration provides solid substratum for growth of colonies and controls the diffusion of antibiotics.

Type of specimen

Pharmaceutical sample

Specimen Collection and Handling

For pharmaceutical sample samples follow appropriate techniques for handling specimens as per established guidelines (1,2). After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precaution

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.
2. Use of this method is appropriate only when test samples are clear.

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 2.35% Agar gel.

Colour and Clarity of prepared medium

Light yellow coloured clear to slightly opalescent gel forms in Petri plates.

pH

5.80-6.20

Growth promotion test

In accordance with EP/BP

Cultural Response

Cultural characteristics observed after an incubation at specified temperature for 18 - 48 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Antibiotics assayed	Incubation Temperature
Saccharomyces cerevisiae ATCC 9763	50-100	luxuriant	>=70%	Amphotericin B, Nystatin	35-37°C 30-32°C
Candida tropicalis CIP 1433-83	50-100	luxuriant	>=70%	Nystatin	30-37°C

Key : (*) Corresponding WDCM numbers.

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 (4,5).

Reference

1. The British Pharmacopoeia, 2020, Medicines and Healthcare products Regulatory Agency.
2. European Pharmacopoeia, 2020, 10 th volume, European Directorate for the quality of medicines & Healthcare.
3. Grove and Randall, 1955, Assay Methods of Antibiotics Medical Encyclopedia, Inc, New York.
4. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
5. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual Clinical Microbiology, 11th Edition. Vol. 1.

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