



Antifungal Assay Agar

M164

Intended Use:

Recommended for assaying antifungal activity of pharmaceutical products and other materials by the cylinder plate or disc method.

Composition**

Ingredients	Gms / Litre
Dextrose (Glucose)	50.000
Sodium citrate	4.500
Potassium dihydrogen phosphate	0.550
Citric acid	1.000
Tryptone	4.000
Pyridoxine hydrochloride	0.00025
Thiamine	0.00025
Inositol	0.025
Calcium pantothenate	0.0025
Niacin	0.0025
Potassium chloride	0.425
Calcium chloride	0.125
Magnesium sulphate	0.125
Ferric chloride	0.0025
Manganese sulphate	0.0025
Biotin	0.000008
Agar	15.000
Final pH (at 25°C)	5.5±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 75.76 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

Fungal infections have been reported to have dramatically increased in the past decade, and these often occur as systemic infections or as co-infections with other diseases, such as AIDS or cancer, or in patients who are immunocompromised (2,3). Unfortunately, in addition to the limited number of antifungal drugs currently available, fungal infections tend to rapidly develop resistance to these drugs. For these reasons, fungal infections now show much higher mortality rates than bacterial infections (7). The rapid increase in fungal infections and the growing number of new antifungal agents indicate an increasing need for rapid and accurate methods for antifungal screening and susceptibility testing. Antifungal Assay Agar was formulated by Berger and Lazecka for convenience in assaying antifungal activity of pharmaceutical products and other materials by both base and seed layers for assays by cylinder plate or disc methods. The defined ingredients in the medium provide the necessary nutrients and growth factors required for the development of the test culture. Phosphate is included in this medium for good buffering action. Dextrose in the medium serves as a carbon and energy source. Other ingredients like the sulphates; vitamins, growth factors etc are added to enhance the growth of the test organisms, so that the inhibition obtained is always due to the antifungal agents and not due to nutrient depletion.

Assay Methods

Cylinder plate method: This method was first devised by Abraham et al (1) and later modified by Schmidt and Moyer (6) and it depends upon diffusion of the antibiotic from vertical steel cylinders placed on the surface of inoculated agar medium. This produces zones of inhibition around the cylinder containing antibiotic solution depending upon the concentration of the antibiotic in the cylinder. This method is commonly employed in the assay of pharmaceutical

preparations of Penicillin and other antibiotics. For assay, use Petri plates with 20 x 100 mm dimension and stainless steel or porcelain cylinders with the outside diameter 8 mm, inside diameter 6 mm and length 10 mm. All dimensions should have a tolerance of 0.1 mm. The cylinders should be carefully cleaned to remove all the impurities. For assays requiring base and seed layer, the base layer is allowed to solidify first and then overlaid with the seed agar containing the proper concentration of the test organism. Most assays require base layer of 21 ml and seed layer of 4 ml. Generally 6 cylinders are used per plate. The cylinders are placed on inoculated plates at equal distance.

Paper-disc method: Paper discs with a diameter of 9 mm are impregnated with the antibiotic solution and placed on the culture medium. Antibiotic can also be applied to the disc after it has been placed on the medium. Plates containing a single layer of medium with 2 mm thickness may be used for these tests. All other steps are similar to the cylinder plate method.

Type of specimen

Pharmaceutical products

Specimen Collection and Handling

For pharmaceutical samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (4,5)

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 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 beige homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

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

Reaction

Reaction of 7.57% w/v aqueous solution at 25°C. pH : 5.5±0.2

pH

5.30-5.70

Cultural Response

Cultural characteristics observed after an incubation at 25-30°C for 18-48 hours.

Organism	Inoculum (CFU)	Growth	Recovery
<i>Saccharomyces cerevisiae</i> ATCC 9763 (00058*)	50-100	luxuriant	≥70%
# <i>Aspergillus brasiliensis</i> ATCC 16404 (00053*)	50-100	luxuriant	

Key - *Corresponding WDCM numbers.

Formerly known as *Aspergillus niger*

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. Abraham, Chain, Fletcher, Florey, Gardner, Heatley and Jennings, 1941, Lancet ii: 177.
2. Beck-Sague C. and Jarvis W. R., 1993, J. Infect. Dis., 167:1247-1251.
3. Berrouane Y. F., Herwaldt L. A., and Pfaller M. A., 1999, J. Clin. Microbiol., 37:531-537.
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 of Clinical Microbiology, 11th Edition. Vol. 1.
6. Schmidt and Moyer, 1944, J. Bacteriol., 47:199.
7. Weinstein, M. P., Towns M. L., Quartey S. M., Mirrett S., Reimer L. G., Parmigiani G. and Reller L. B., 1997., Clin. Infect. Dis., 24:584-602.

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