Antifungal Assay Agar

M164

Antifungal Assay Agar is recommended for assaying antifungal activity of pharmaceutical products and other materials by the cylinder plate or disc method.

Composition**

Ingredients	Gms / Litre
Dextrose	50.000
Sodium citrate	4.500
Potassium phosphate	0.550
Citric acid	1.000
Casein enzymic hydrolysate	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 distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. 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 (1,2). 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 (3). 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 (4) and later modified by Schmidt and Moyer (5) 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 x100 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.

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
M164: Cultural characteristics observed after an incubation at 25-30°C for 18-48 hours.

Organism	Inoculum (CFU)	Growth	Recovery
Saccharomyces cerevisiae ATCC 9763	50-100	luxuriant	>=70%
**Aspergillus brasiliensis* ATCC 16404	50-100	luxuriant	

*Key * - Formerly known as Aspergillus niger*

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. Beck-Sague C. and Jarvis W. R., 1993, J. Infect. Dis., 167:1247-1251.
2. Berrouane Y. F., Herwaldt L. A., and Pfaller M. A., 1999, J. Clin. Microbiol., 37:531-537.
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.
4. Abraham, Chain, Fletcher, Florey, Gardner, Heatley and Jennings, 1941, Lancet ii: 177.
5. Schmidt and Moyer, 1944, J. Bacteriol., 47:199.

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