Fluconazole Testing Medium (Twin Pack)

M1209

Fluconazole Testing Medium is recommended for fluconazole susceptibility testing using *Candida* species.

Composition**

Ingredients	Gms / Litre
Part A	-
Agar	10.000
Part B	-
Dextrose	19.980
Potassium dihydrogen phosphate	1.990
Ammonium sulphate	4.990
L-Glutamine	0.580
Magnesium sulphate anhydrous	0.990
Sodium chloride	0.200
Calcium chloride	0.200
L-Lysine monohydrochloride	0.073
Valine	0.047
L-Arginine monohydrochloride	0.042
L-Histidine	0.023
DL-Methionine	0.0189
Tryptophan	0.020
Nicotinic acid	0.00079
Inositol	0.00397
Pyridoxine hydrochloride	0.00079
Boric acid	0.00099
Calcium D-pantothenic acid	0.00079
Aneurine hydrochloride	0.00079
Manganous sulphate	0.00079
Zinc sulphate	0.0014
p-Amino benzoic acid (PABA)	0.000395
Riboflavin	0.000395
Ferric chloride	0.000395
Cupric sulphate	0.00012
Biotin crystalline	0.000004
Folic acid	0.000395
L-Isoleucine	0.052
Sodium molybdate	0.00047
Potassium iodide	0.0002
L-Leucine	0.052
Threonine	0.0476

**Formula adjusted, standardized to suit performance parameters

Directions

Part A and Part B are sterilized separately as follows:

Part A: - Suspend 2.0 grams of Part A in 100 ml distilled water, add 0.1 ml phosphate buffer to adjust the pH to 7.5. Heat to boiling to dissolve the agar particles completely and then sterilize by autoclaving at 115°C for 10 minutes.

Part B: - Suspend 29.31 grams of Part B in 900 ml distilled water. Mix well, add 2 gram of sodium bicarbonate, after stirring make up the total volume to 1 litre with distilled water. Sterilize by filtration. The medium can be kept at 4°C for two weeks. Complete medium is prepared by aseptically adding equal volume of molten Part A (previously cooled to 50°C) and Part B. Mix thoroughly and dispense.

Principle And Interpretation

To carry out standardization of antifungal drug susceptibility assays as a standard reference method, CLSI had recommended broth macro dilution testing of yeasts. Fluconazole Testing Medium is a chemically defined medium specifically developed for the in-vitro testing of fluconazole by using *Candida* species. Inhibitory concentration values obtained by using this medium correlate well with the clinical outcome (1, 2, 3).

The medium contains dextrose and a variety of amino acids, salts and vitamins to support the growth of *Candida* and other fungi.

The inoculum size varies with different fungi. *Candida* species are grown in Sabouraud Dextrose Broth (M033) at 37°C for 16-18 hours and then diluted with normal saline to give following dilutions:

Candida albicans ,,,,,, 105 / ml

Candida tropicalis ,,,,,, 105 / ml

Candida krusei ,,,,,,,, 105 / ml

Candida guillermondii ,,,, 106 / ml

Candida parapsilosis ,,,, 106 / ml

Candida pseudotropicalis 106 / ml

Surface inoculate the above diluted cultures and incubate at 28°C for 48 hours to determine MIC value of fluconazole.

Dermatophytes are grown in Sabouraud Dextrose Agar (M063) at 28°C for 5-10 days. The mycelial growth is homogenized in 2 ml of 0.85% saline using glass beads. The density of the suspension is adjusted with 0.85% saline to get a 65% light transmission. Inoculate the plates and incubate for about 5-6 days at 28°C.

Check the control plates to ensure that all isolates have grown adequately and determine the Minimum Inhibitory Concentration (MIC).,,

Quality Control

Appearance

Part A : Cream to light yellow homogeneous coarse powder Part B : White to light yellow homogeneous free flowing powder

Colour and Clarity of prepared medium

Light yellow coloured, opalescent solution may be with fine precipitate

Cultural Response

Cultural characteristics observed after an incubation at 28-30°C for 48 hours .

Cultural Response

Organism	**MIC of Fluconazole**
Cultural Response	
Candida albicans ATCC 10231	1.56 µg/ml

Storage and Shelf Life

Store powder medium and prepared medium at 2-8°C.Use before expiry date on the label.

Reference

1. Hoeprich P. D. and Finn. P. D., 1972, J. Infect, Dis., 126: 353
2. Cook R. A., McIntyre K. A. and Galgiani J. N., 1990, Antimicrob. Agents and Chemother., 34:1542.
3. Pfaller M. A. et al, 1992, Antimicrob. Agents and Chemother.,36:1805.

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