Caffeic Acid Ferric Citrate Test Agar(CFAC Medium) M563

Caffeic Acid Ferric Citrate Test Agar is recommended for selective and presumptive identification of *Cryptococcus neoformans* and its differentiation from other species.

Composition**

Ingredients	Gms / Litre
Yeast extract	2.000
Dextrose	5.000
Ammonium sulphate	5.000
Dipotassium phosphate	0.800
Magnesium sulphate	0.700
Caffeic acid	0.180
Ferric citrate	0.020
Agar	20.000
Final pH (at 25°C)	6.5±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 33.7 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Dispense and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45 to 50°C. If desired aseptically add sterile solution of Chloramphenicol to yield a final concentration of 50µg/ml medium. Mixwell and pour into sterile Petri plates.

Principle And Interpretation

Cryptococcus neoformans is an encapsulated basidiomycete yeast-like fungus.
C. neoformans have affinity for avian habitats and has been isolated from soil contaminated by bird droppings (1). It causes diseases in apparently immunocompetants, as well as immunocompromised hosts (7). The most susceptible are patients with T-Cell deficiencies (7). *C. neoformans* is the fourth most common cause of life-threatening infection in patients with AIDS (1).

Caffeic Acid Ferric Citrate Test Agar is used for the rapid identification and differentiation of *C. neoformans* from other species of *Cryptococcus* . This medium was described by Hopfer and Blank (2). The medium contains caffeic acid which is a selective agent for *C. neoformans* . Caffeic acid is an O-diphenol compound which can be oxidized by phenoloxidase enzyme to produce dark brown melanin pigmentation. *C. neoformans* has a unique ability to produce melanin or melanin-like pigment from p- and o-diphenols (3, 4) and can be differentiated from *Candida albicans* (5). Thus, Caffeic acid causes pigment production of *C. neoformans* in the presence of (iron) ferric citrate (6).

Dextrose is the fermentable carbohydrate in the medium while yeast extract serves as the source of nitrogenous nutrients and B vitamins. Sulphates and phosphate buffer the medium. Ferric citrate aids in pigment production by *C. neoformans* in the presence of caffeic acid. Chloramphenicol, if added, inhibits the accompanying bacterial flora.

Growth of *C. neoformans* on this medium should be compared with same organism on another medium before inoculation to see whether colonial growth is naturally pigmented. False negative reactions may occur. Pigment production is delayed during luxurious growth. Other Cryptococci may become pigmented after 3-4 days of inoculation, but they are not so intensely coloured and can therefore be distinguished from *C. neoformans* (2).

Quality Control

Appearance
Cream to yellow homogeneous free flowing powder

Gelling
Firm,comparable with 2.0% agar gel.

Colour and Clarity of prepared medium
Light blue coloured, clear to slightly opalescent gel forms in Petri plates

Reaction
Reaction of 3.37% w/v aqueous solution at 25°C. pH : 6.5±0.2

pH
6.30-6.70

Cultural Response
M563: Cultural characteristics observed with added 50 mcg/ml Chloramphenicol after an incubation at 25-30°C for 24-48 hours .

Organism	Growth	Colour of colony
Candida albicans ATCC 10231	good	white
Cryptococcus neoformans ATCC 32045	good	brown
Escherichia coli ATCC 25922	inhibited	
Staphylococcus aureus ATCC 25923	inhibited	

Storage and Shelf Life
Store below 30°C in tightly closed container and prepared medium at 2-8°C. Use before expiry period on the label.

Reference
1. Taylor R. L. and Duangmani C., 1968, Am. J. Epidemiol., 87 (2): 318
2. Hopfer R. L. and Blank F., 1975, J. Clin. Microbiol., 2 (2):115.
3. Chaskes S. and Tyndall R., 1975, J. Clin. Microbiol., 1(6):509.
4. MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. I, Williams and Wilkins, Baltimore.
5. Korth H. and Pulverer G., 1971, Appl. Microbiol., 21:541.
6. Pulverer G. and Korth H., 1971, Med. Microbiol. Immunol., 157, 46.
7. Mitchell T. G., Perdect J. R., 1995, 8: 515

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