# Technical Information

## Caffeic Acid Ferric Citrate Test Agar(CFAC Medium)

## Product Code: DM 1563

**Application: -** 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

## Principle & 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*. It 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 act as 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).

## Methodology

Suspend 33.7 grams of dehydrated media powder in 1000 ml distilled water. Mix thoroughly & 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. Mix well and pour into sterile Petri plates.

## Quality Control

**Appearance**
Cream to yellow homogeneous free flowing powder.

**Gelling**
Firm, comparable with 2.0% agar gel.

**Colour and Clarity**
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 Range**
6.30-6.70

**Cultural Response**
DM 1563: 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

**Dried Media:** Store below 30°C in tightly closed container and prepared medium at 2-8°C. Use before expiry period on the label.
**Prepared Media**: 2-8° in sealable plastic bags for 2-5 days.

## Further Reading

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

## Disclaimer :

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