PRODUCT SPECIFICATION SHEET

Caffeic Acid Ferric Citrate Test Agar (CAFC Medium) (DM1045)

Intended Use
Caffeic Acid Ferric Citrate Test Agar (CAFC Medium) (DM1045) is recommended for selective isolation, presumptive identification and differentiation of *Cryptococcus neoformans* from other species.

Product Summary and Explanation
Cryptococcus neoformans is an encapsulated basidiomycete, obligate aerobe and yeast-like fungus. *C. neoformans* is often found in soil contaminated by bird excrement as they have affinity for avian habitats.[1] Infections with this fungus are rare in those with fully functioning immune systems. It causes diseases in apparently immunocompetants, as well as immunocompromised hosts.[2] The most susceptible are patients with TCell deficiencies.[2] *C. neoformans* is the fourth most common cause of life-threatening infection in patients with AIDS.[1] Caffeic Acid Ferric Citrate Test Agar was described by Hopfer and Blank for the rapid identification and differentiation of *C. neoformans* from other species of *Cryptococcus*.[3]

Principles of the Procedure
Caffeic Acid Ferric Citrate Test Agar (CAFC Medium) contains yeast extract which provides nitrogenous substances and B complex vitamins. Dextrose is a fermentable carbohydrate source. Sulphates and phosphate acts as buffering agent in the medium. Caffeic acid is an O-diphenol compound and is a selective agent for *C. neoformans* which can be oxidized by phenoloxidase enzyme to produce dark brown melanin pigmentation. *C. neoformans* has a unique ability to produce melanin or melaninlike pigment from p- and o-diphenols[4,5] and can be differentiated from *Candida albicans*.[6] Thus, Caffeic acid causes pigment production of *C. neoformans* in the presence of (iron) ferric citrate.[7] Ferric citrate aids in pigment production by *C. neoformans* in the presence of caffeic acid. Chloramphenicol, if added, inhibits the accompanying bacterial flora.

Formula / Liter

Ingredients	Gms / Liter
Yeast extract	2.00
Dextrose	5.00
Ammonium sulphate	5.00
Dipotassium phosphate	0.80
Magnesium sulphate	0.70
Caffeic acid	0.18
Ferric citrate	0.02
Agar	20.00
Final pH : 6.5 ± 0.2 at 25°C	
Formula may be adjusted and/or supplemented as required to meet performance specifications	

Precautions
1. For Laboratory Use only.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions
1. Suspend 33.7 grams of the medium in one litre distilled water.
2. Heat to boiling to dissolve the medium completely.

3. Dispense and autoclave at 121°C, 15 psi pressure, for 15 minutes / validated cycle.
4. Cool to 45 to 50°C.
5. If desired aseptically add sterile solution of Chloramphenicol to yield a final concentration of 50µg/ml medium.
6. Mix well and pour into sterile Petri plates.

Quality Control Specifications

Dehydrated Appearance	Cream to yellow homogeneous free flowing powder
Prepared Medium	Light blue coloured, clear to slightly opalescent gel forms in Petri plates
Reaction of 3.37% Solution	pH : 6.5 ± 0.2 at 25°C
Gel Strength	Firm, comparable with 2.0% agar gel

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

Sr. No.	Organisms	Results to be achieved	
		Growth	Colour of colony
1.	*Candida albicans ATCC 10231*	good-luxuriant	white
2.	*Cryptococcus neoformans ATCC 32045*	good-luxuriant	brown
3.	*Escherichia coli ATCC 25922*	inhibited	--
4.	*Staphylococcus aureus ATCC 25923*	inhibited	--

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure
Refer to appropriate references for standard test procedures.

Results
Refer to appropriate references for interpretation of results.

Storage
Store the sealed bottle containing the dehydrated medium at 10 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light.

Expiration
Refer to the expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure
1. 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.
2. 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*.[3]
3. For identification, organisms must be in pure culture. Morphological, biochemical and/or serological tests should be performed for final identification.
4. Consult appropriate texts for detailed information and recommended procedures.

Packaging
Product Name : Caffeic Acid Ferric Citrate Test Agar (CAFC Medium)
Product Code : DM1045
Available Pack sizes : 100gm

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References

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

Further Information

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