TM 1156 – CAFFEIC ACID FERRIC CITRATE TEST AGAR (CAFC MEDIUM)

INTENDED USE

For selective and presumptive identification of *Cryptococcus neoformans* and its differentiation from other species.

PRODUCT SUMMARY AND EXPLANATION

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. It causes diseases in apparently immunocompetants, as well as immunocompromised hosts. The most susceptible are patients with T-Cell deficiencies. *C. neoformans* is the fourth most common cause of life-threatening infection in patients with AIDS.

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. 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 and can be differentiated from *Candida albicans*. Thus, Caffeic acid causes pigment production of *C. neoformans* in the presence of (iron) ferric citrate.

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.*

COMPOSITION

Ingredients	Gms / Ltr
Yeast extract	2.000
Dextrose (Glucose)	5.000
Ammonium sulphate	5.000
Dipotassium hydrogen phosphate	0.800
Magnesium sulphate	0.700
Caffeic acid	0.180
Ferric citrate	0.020
Agar	20.000

PRINCIPLE

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.

INSTRUCTION FOR USE

- Dissolve 33.7 grams in 1000 ml purified / distilled water.
- Heat to boiling to dissolve the medium completely.
- Dispense and sterilize by autoclaving at 15 psi pressure (121°C) for 15 minutes. Cool to 45-50°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 SPECIFICATIONS

Appearance of Powder : Cream to yellow homogeneous free flowing powder.
Appearance of prepared medium : Light blue coloured, clear to slightly opalescent gel forms in Petri plates.
pH (at 25°C) : 6.5±0.2

INTERPRETATION

Cultural characteristics observed after incubation.

Microorganism	ATCC	Inoculum (CFU/ml)	Growth	Recovery	Colour of colony	Incubation Temperature	Incubation Period
Candida albicans	10231	10-100	Good	40-50%	White	25-30°C	24-48 Hours
Cryptococcus neoformans	32045	10-100	Good	40-50%	Brown	25-30°C	24-48 Hours
Escherichia coli	25922	$>10^3$	Inhibited	0%	-	25-30°C	24-48 Hours
Staphylococcus aureus subsp. aureus	25923	$>10^3$	Inhibited	0%	-	25-30°C	24-48 Hours

PACKAGING:

In pack size of 100 gm bottles.

STORAGE

Dehydrated powder, hygroscopic in nature, store in a dry place, in tightly-sealed containers between 25-30°C and protect from direct sunlight. Under optimal conditions, the medium has a shelf life of 4 years. When the container is opened for the first time, note the time and date on the label space provided on the container. After the desired amount of medium has been taken out replace the cap tightly to protect from hydration.
Product Deterioration: Do not use if they show evidence of microbial contamination, discoloration, drying or any other signs of deterioration.

DISPOSAL

After use, prepared plates, specimen/sample containers and other contaminated materials must be sterilized before discarding.

REFERENCES

1. Hopfer R. L. and Blank F., 1975, J. Clin. Microbiol., 2 (2):115.
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3. Mitchell T. G., Perdect J. R., 1995, 8: 515.
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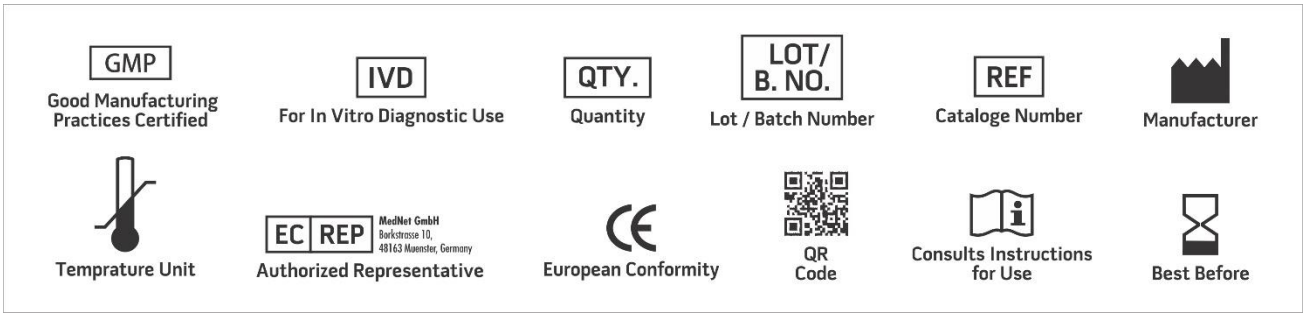
5. Taylor R. L. and Duangmani C., 1968, Am. J. Epidemiol., 87 (2): 318.



NOTE: Please consult the Material Safety Data Sheet for information regarding hazards and safe handling Practices.

***For Lab Use Only**

Revision: 08 Nov., 2019