Caffeic Acid Ferric Citrate Test Agar (CAFC Medium)

M563

Intended Use:

Recommended for selective isolation and presumptive identification of *Cryptococcus neoformans* and its differentiation from other species.

Composition**

Ingredients	g / L
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
Final pH (at 25°C)	6.5±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 33.7 grams in 1000 ml purified/distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs 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 50 mcg/ml medium. Mix well 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 (2). The most susceptible are patients with T-Cell 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 is used for the rapid identification and differentiation of *C. neoformans* from other species of *Cryptococcus*. This medium was described by Hopfer and Blank (3). 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 (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).

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* (3).

Type of specimen

Clinical samples - Tissue or body fluid , sputum samples

Specimen Collection and Handling:

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (8,9).
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

In Vitro diagnostic Use only. For professional use only. Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations :

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.
2. Pigment production is delayed during luxurious growth.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Gelling

Firm,comparable with 2.0% agar gel.

Colour and Clarity of prepared medium

Medium amber with purple tinge 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

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 (00054*)	good	white
Cryptococcus neoformans ATCC 32045	good	brown
Escherichia coli ATCC 25922 (00013*)	inhibited	
Staphylococcus aureus subsp. aureus ATCC 25923 (00034*)	inhibited	

Key : *Corresponding WDCM numbers.

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 15-30°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (8,9).

Reference

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IVD ***In vitro* diagnostic medical device**

10°C – 30°C **Storage temperature**

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CE Marking

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