Cryptococcus Differential Agar

M1814

Intended Use:

Recommended for a differentiation of *Cryptococcus* species.

Composition**

Ingredients	Gms / Litre
Dextrose (Glucose)	20.000
Glycine	0.500
DL- Tryptophan	2.000
Potassium dihydrogen phosphate	4.000
Magnesium sulphate	2.500
Thiamine hydrochloride	0.005
Trypan Blue	0.030
Agar	15.000
Final pH (at 25°C)	5.4±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 44.04 grams in 1000 ml purified / distilled water. Heat to boiling to dissolve the medium completely. DO NOT AUTOCLAVE. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

Principle And Interpretation

Cryptococcus is the etiological agent of cryptococcosis, a systemic mycosis of humans and animals with a worldwide distribution. Cryptococcosis (earlier called European blastomycosis) commonly starts following inhalation of the organism, which is considered opportunistic infections as it affects mainly immunosupressed individuals. (5)

This medium was based on the formulation of m-FDTG medium except the sugar fructose was replaced by glucose as it supported better growth of *Cryptococcus* species. Glucose supports growth as well as strong pigment production by nearly all *C. gattii* strains. *C. gaitii* can while *C. neoformans* cannot assimilate D-tryptophan (1), thereby producing a brown diffusible pigment (6). Pigmentation is not apparent on the first day of growth but is usually noticeable after 5 days of incubation, intensity gradually increases with time after 2-3 weeks. (2).

Glycine serves as a sole source of carbon and nitrogen which is utilized by *Cryptococcus gaitti, Cryptococcus laurentii* and not by *Cryptococcus neoformans*. Salts in the medium help in pigment induction by D-tryptophan. Pigment production was more intense at 25-30°C as compared to 37°C. Dyes in media for the isolation of fungi have not been commonly utilized, although many such media are available for the isolation of bacteria. Trypan blue in the medium allows suspected *C. neoformans* colonies to be subcultured before mold overgrowth becomes a problem (7).

Type of specimen

Enviromental sample - air sample; Food samples - fruits and vegetables.

Specimen Collection and Handling

For enviromental samples follow appropriate techniques for handling specimens as per established guidelines (7).
For food samples, follow appropriate techniques for sample collection and processing as per guidelines (5, 8).
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

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 specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1. Further biochemical and serological tests must be carried out for complete identification.
2. Some organism may show poor growth due to nutritional variation.

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
Light yellow to yellow with bluish tinge homogeneous free flowing powder

Gelling
Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium
Light blue coloured, opalescent gel with white precipitate forms in Petri plates

Reaction
Reaction of 4.4 % w/v aqueous solution at 25°C. pH : 5.4±0.2

pH
5.20-5.60

Cultural Response
Cultural characteristics observed after an incubation at 25- 30°C for 5 to 6 days.

Organism	Inoculum (CFU)	Growth	Colony Characteristics
Cyptococcus neoformans ATCC 32045	50-100	luxuriant	Light blue, dry colony
Cryptococcus laurentii ATCC 18803	50-100	luxuriant	Brown, dry colony
Cryptococcus gattii ATCC MYA- 4566	50-100	luxuriant	Brown, mucoid colony

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 20-30°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 (3,4).

Reference

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