Cryptococcus Differential Agar

M1814

Cryptococcus Differential Agar is recommended for a differentiation of *Cryptococcus* species.

Composition**

Ingredients	Gms / Litre
Glucose	20.000
Glycine	0.500
DL- Tryptophan	2.000
Potassium dihydrogen phosphate	4.000
Magnesium sulphate	2.500
Thiamine HCl	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 distilled water. Heat to boiling to dissolve the medium completely. DO NOT AUTOCLAVE. Cool to 50°C 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. (3)

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 (4). 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 medium allows suspected *C. neoformans* colonies to be subcultured before mold overgrowth becomes a problem (5).

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
M1814: Cultural characteristics observed after an incubation at 25- 30°C for 5 to 6 days.

Organism	Inoculum (CFU)	Growth	Colony Characteristics
Cultural Response			
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 below 30°C in tightly closed container and the prepared medium at 2 - 8°C. Use before expiry date on the label.

Reference

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3.Misral, V.C, and Randhawa. H.S (2000). Occurrence and Significance of Cyptococcus neoformans in Vegetables and Fruits. The Indian Journal of Chest Diseases 6 Allied Sciences. 42:317-322.

4.Mukamurangwa,P., C.Raes- Wuytack, and C. De Vroey.1995. Cryptococcus neoformans var. gattii can be separated from ! var. neoformans by its ability to assimilate D-tryptophan. J.Med. Vet. Mycol.33:419–420.

5. Racicot,T.A, and Bulmer,G.S. (1985). Comparison of Media for the Isolation of Cryptococcus neoformans . Appl. and Environ. Microbiol. 50(2) : 548-549.

Revision : 2 / 2015

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