TOC Agar

M1055

TOC Agar is a differential medium used for the presumptive identification and differentiation of *Candida albicans* and *Cryptococcus neoformans* .

Composition**

Ingredients	Gms / Litre
Ox bile	10.000
Sorbitan monooleate 80	10.000
Caffeic acid	0.300
Agar	20.000
Final pH (at 25°C)	6.5±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 40.3 grams in 1000 ml distilled water. Mix thoroughly. Gently heat and bring to boiling. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 50°C and pour into sterile Petri plates.

Principle And Interpretation

Candida are yeast-like fungus forming normal flora inhabiting the mouth and throat, the intestinal tract and the genital tract. Under certain conditions, they cause life-threatening diseases particularly in immunocompromised patients.

Candida albicans is the species most commonly isolated from patients with nearly all forms of candidiasis. *Cryptococcus neoformans* is often cultured from the urine of patients with disseminated infection. Cryptococcosis is one of the defining diseases associated with AIDS (1). TOC Agar is a multi-purpose medium developed by Fleming et al (2) for the rapid, presumptive identification of *C. albicans* and *C. neoformans* . Both species are common clinical isolates that may be presumptively identified by specific morphological characteristics (3-7).

C. albicans and *C. stellatoides* may be presumptively identified on this medium by the formation of germ tubes and chlamydospores (2, 5). A combination of sorbitan monooleate 80 and oxbile promotes their rapid, sequential development. *C. neoformans* may be identified by the production of a characteristic brown pigment on this medium (2, 5). Caffeic acid is the substrate for phenol oxidase, an enzyme produced only by *C. neoformans* (2). The subsequent enzymatic reaction produces melanin, which is absorbed by the yeast cell wall resulting in tan to brown pigmentation.

For the germ tube test, lightly touch a single colony from TOC Agar with a loop or Pasteur pipette; remove excess inoculum and then emulsify the yeast cells in 0.5 ml of horse or other serum in a small test tube with a loose cotton-wool plug. Failure to achieve a light inoculum inhibits germ-tube formation. Incubate at 37°C in a water bath for 2-4 hours (8). A drop of suspension is then placed on a glass slide and covered with coverslip. Microscopic examination of typical *C. albicans* reveals thin germ tubes 3 to 4 mm in diameter and up to 20 mm long; unlike pseudohyphae that are not constricted at their point of origin.

Quality Control

Appearance
Cream to yellow homogeneous free flowing powder

Gelling
Firm, comparable with 2.0% Agar gel.

Colour and Clarity of prepared medium
Yellow coloured clear to slightly opalescent gel forms in Petri plates.

Reaction
Reaction of 4.03% w/v aqueous solution at 25°C. pH : 6.5±0.2

pH
6.30-6.70

Cultural Response

M1055: Cultural characteristics observed after an incubation at 30°C for 24-48 hours.

Organism	Growth
Cultural Response	
Candida albicans ATCC 10231	luxuriant(Formation of germ tubes within 3-4 hours and chlamydospores within 48 hours)
Cryptococcus neoformans ATCC 32045	luxuriant(Brown colony growth within 48 hours of incubation)

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

1. Murray P. R., Baron J. H., Pfaller M. A., Jorgensen J. H. and Yolken R. H., (Ed.), 2003, Manual of Clinical Microbiology, 8th Ed., American Society for Microbiology, Washington, D.C.
2. Fleming W. H., Hopkins J. M. and Land, 1977, J. Clin. Microbiol., 5:236.
3. Lennette E. H., Balows A., Hausler W. J. and Shadomy H. J.,(Eds.), 1985, Manual of Clinical Microbiology, 4th Ed., ASM, Washington, D.C.
4. Finegold S. M. and Baron E. J., 1986, Bailey and Scotts Diagnostic Microbiology, 7th Ed., The C.V. Mosby Company, St. Louis.
5. MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. 1, Williams and Wilkins, Baltimore.
6. Campbell M. C. and Stewart J. L., 1980, The Medical Mycology Handbook, John Wiley and sons, New York.
7. Ajello L., Georg L. K., Kaplan W. and Kaufman L., 1963, Laboratory Manual for Medical Mycology, DHEW Publication No. 994, US Govt. Printing Office, Washington, D.C.
8. Collee J. G., Fraser A. G., Marmion B. P., Simmons A., (Eds.), Mackie and McCartney, Practical Medical Microbiology, 1996, 14th Edition, Churchill Livingstone.

Revision : 02/ 2015