

Department of Microbiology
Corn Meal Agar with Tween 80 Procedure

I. Purpose and Test Principle

Cornmeal agar with Tween 80 is used for the cultivation and differentiation of *Candida* species on the basis of mycelial characteristics. Tween 80, a surfactant, is specifically incorporated in lieu of dextrose for the demonstration of pseudohyphal, chlamydospore, and arthrospore formation. Chlamydospore production is best obtained if the yeast inoculum is placed under a coverslip or following subsurface inoculation creating a microaerophilic environment. The basic nutrients for yeast growth are provided by cornmeal infusion.

II. Specimen Information

This medium is intended for use with pure cultures of yeast isolates.

III. Reagents & Equipment

- Cornmeal with Tween 80 Agar - Storage: Upon receipt store plates at 2-8°C away from direct light. Protect from heat, moisture, and freezing.
- Sterile inoculating needle
- Coverslips
- Tape for sealing culture plates
- Incubator with ambient air at 25 ± 2°C

IV. Procedure

- A. Using a sterile inoculating needle, harvest a portion of a young, actively growing yeast colony.
- B. Make a streak on the agar surface without cutting into the agar. Make three or four streaks perpendicular to the first streak to dilute the inoculum.
- C. Cover with a 22 x 22-mm coverslip.
- D. Seal the plate with tape and incubate aerobically at room temperature (25 ± 2°C) for up to 3 d in the dark. Examine daily for growth.
- E. Examine by placing the plate, without its lid, on the microscope stage and using the low power (X 100) and high-dry (X 400) objective. The most characteristic morphology is often found along the edge of the coverslip.

V. Interpretation

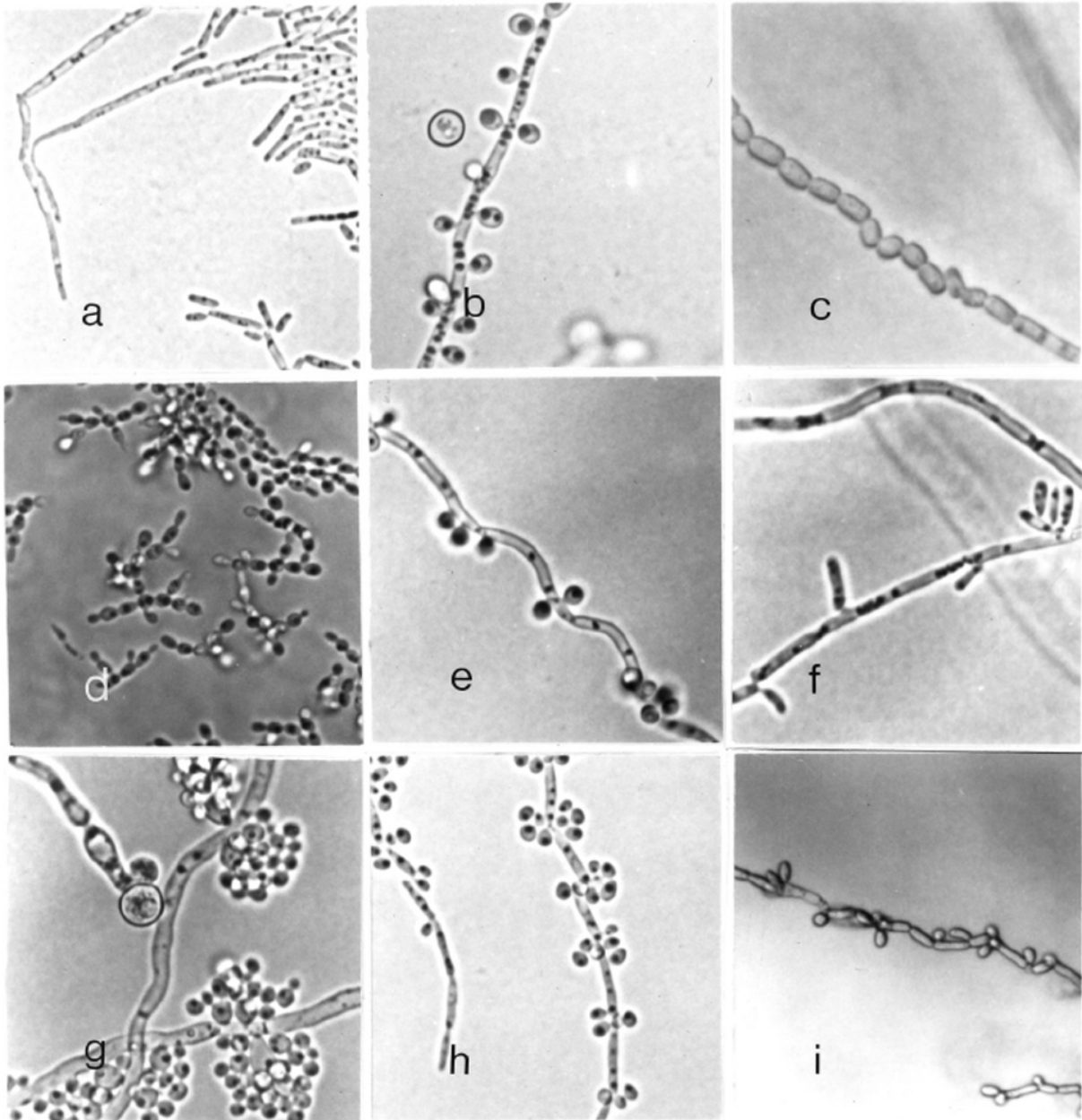
TABLE 1 Microscopic appearance of yeasts on morphology agar.

Organism	Pseudo-hyphae	Blasto-conidia	Arthro-conidia	Annello-conidia	Chlamydo-spores	Asco-spores
<i>B. capitatus</i>	X	X		X		
<i>C. albicans</i> / <i>C. dubliniensis</i>	X	X			X	
Other <i>Candida</i>	X ^{var}	X				X ^{var}
<i>Cryptococcus</i>		X				
<i>Geotrichum</i>			X			
<i>Pichia</i>	X ^{var}	X				X
<i>Rhodotorula</i>		X				
<i>Saccharomyces</i>	X ^{var}	X				X
<i>Trichosporon</i>	X	X	X			

var = strain variation

FIGURE 1

Morphological features of some yeast and yeast-like organisms on cornmeal agar at 48 h and ambient temperature.



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- a. *Candida krusei*: extremely elongated, rarely branched pseudohyphae; few blastoconidia.
- b. *Candida tropicalis*: blastoconidia formed at septa and between septa.
- c. *Geotrichum candidum*: arthroconidia.
- d. *Candida guilliermondii*: chains of blastoconidia forming sparse pseudohyphae in a young culture.
- e. *Candida lusitanae*: short distinctly curved pseudohyphae with blastoconidia formed at, and occasionally between, septa.
- f. *Blastoschizomyces capitatus*: true hyphae and annelloconidia resembling arthroconidia.
- g. *Candida albicans*: blastoconidia, chlamydoconidia, true hyphae, and pseudohyphae.
- h. *Candida parapsilosis*: elongated, delicately curved pseudohyphae with blastoconidia at septa.
- i. *Trichosporon* spp.: blastoconidia formed at the corners of arthroconidia. Magnification, x370.

VI. Quality Control

Each new lot or shipment of media should be examined for product deterioration and tested with the following control strains. Media should not be used if there are any signs of deterioration (shrinking, cracking, or discoloration), contamination, or if the expiration date has passed. One plate may be divided and used for testing both control strains. Incubate plate at $25 \pm 2^{\circ}\text{C}$ in an aerobic atmosphere.

Control strain	Expected Results
<i>C. albicans</i> ATCC 90028	Growth; hyphae, budding cells, and chlamydo spores seen
<i>C. glabrata</i> ATCC 15126	Growth; no chlamydo spores seen

VII. Limitations

- A. Chlamydo spore formation is inhibited at $30\text{-}37^{\circ}\text{C}$.⁽¹²⁾ A temperature of 25 degrees C. is recommended for the best results.
- B. Morphology alone cannot be relied upon for identification. Refer to the yeast isolate identification procedures.
- C. Repeated subculturing of some *Candida* strains result in a loss of their ability to produce chlamydo spores.

VIII. References

- A. [Hardy Diagnostics Corn Meal Agar Tween 80 Instructions for Use](#)
- B. Larone, D.H. 2011. Medically Important Fungi: A Guide to Identification, 5th ed., ASM Press, Washington, D.C.
- C. Fig. 1 & Table 1: Versalovic, J, K. C. Carroll, G. Funke, J. H. Jorgensen, M. L. Landry, D. W. Warnock. 2011. Manual of Clinical Microbiology, 10th ed., Vol. 1, ASM Press, Washington, D.C.
- D. *Quality Control for Commercially Prepared Microbiological Culture Media*; M22-A3, Vol. 24, No. 19. 2004. Clinical Laboratory Standards Institute (CLSI - formerly NCCLS).