PROVIDENCE
Sacred Heart
Medical Center &
Children's Hospital

Department of Microbiology
Bird Seed Agar Procedure
For *Cryptococcus neoformans* Differentiation

I. Purpose and Test Principle

Bird Seed agar is a selective and differential medium used to aid in the identification of *Cryptococcus neoformans*. The medium contains an extract from the Indian thistle plant, *Guizotia abyssinica*. The seed extract contains caffeic acid, which serves as a substrate for phenol oxidase, an enzyme present in the cell wall of *C. neoformans*. The subsequent enzymatic reaction produces the brown pigment melanin, resulting in tan to brown pigmentation of the yeast colonies. *C. neoformans* is the only species known to produce this enzyme. The medium also contains chloramphenicol to inhibit bacterial contaminants.

II. Specimen Information

Bird Seed agar may be used as a primary medium for planting specimens or as a subculture medium for suspect isolates. Clinical isolates should be evaluated microscopically and physiologically for complete identification to the genus, *Cryptococcus*.

III. Reagents & Equipment

- BBL™ Bird Seed Agar
- On receipt, store plates in the dark at 2 - 8°C in original sleeve wrapping until time of inoculation. Avoid freezing and overheating. Do not open until ready to use. Prepared plates may be inoculated up to the expiration date and incubated for the recommended incubation times. Allow the medium to warm to room temperature before inoculation.
- Inoculating loop
- Aerobic incubator set at 25 - 30°C

IV. Procedure

A. Inoculation
Inoculate the agar surface and streak for isolation.

B. Incubation
Incubate inverted plate at 25 - 30°C. Plates should be examined for tan to brown colonies for up to 7 days.

V. Interpretation and Reporting

A. Positive Test
Yeast-like organisms that produce tan to brown colonies after 4 – 7 d of incubation may be presumptively identified as *Cryptococcus neoformans*.

B. Negative Test
Yeast other than *C. neoformans* will typically produce white or non-pigmented colonies.

VI. Quality Control

Each new lot or shipment of medium should be examined for product deterioration and tested with the following control strains. Prepare a 0.5 McFarland suspension of each test strain and dilute 1:10. Use a 0.01 mL calibrated loop to inoculate the medium. Incubate plates at 25 - 30°C in an aerobic atmosphere for up to 5 days.

Control strain	Expected Results
Cryptococcus neoformans ATCC 14116	Growth. Colonies pigmented tan to brown within 5 days of incubation.
Candida albicans ATCC 90028	Growth. No brown pigment.
Staphylococcus aureus ATCC 25923	Inhibition (partial).

VII. Limitations

A. It is recommended that physiologic tests be performed on colonies from pure culture for complete identification.

VIII. Verification of Test Method

The Bird Seed agar was evaluated by using clinical isolates, ATCC strains and isolates from past CAP surveys. A total of thirteen yeast isolates were tested on the Bird Seed agar. Seven strains of *Cryptococcus neoformans* were tested, including ATCC 14116, 3 CAP proficiency test strains, and 3 clinical isolates, including a *Cryptococcus neoformans* var. *gatti* isolate. The remaining yeast included *Cryptococcus albidus* ATCC 10666, *Cryptococcus laurentii* ATCC 76483, *Candida albicans* ATCC 90028, *Candida tropicalis* ATCC 750, *Saccharomyces cerevisiae* and a *Rhodotorula* spp. Plates were inoculated with each test strain and streaked for isolation. The plates were taped and incubated at 25 - 30°C in an aerobic atmosphere. The cultures were initially examined after 48 h of incubation and then again several days later. All of the *C. neoformans* isolates produced tan colonies within 3 days of incubation. The colonies continued to darken to brown with further incubation. Except for the *Rhodotorula* spp., which failed to grow, the other yeast isolates produced white colonies that did not turn tan or brown after 7 days of incubation.

IX. References

A. Package insert: BD BBL™ Bird Seed Agar, 88-0175-1, January 1999.
B. Clinical Microbiology Procedures Handbook, 3rd ed. and 2007 update, Vol. 2. Garcia, L.S., editor in chief. ASM Press, Washington, D.C.

X. Document Control

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