

BIRDSEED AGAR

INTENDED USE

Remel Birdseed Agar is a solid medium recommended for use in qualitative procedures for selective and differential isolation of *Cryptococcus neoformans* from other yeasts, including other *Cryptococcus* species.

SUMMARY AND EXPLANATION

The etiological agents of cryptococcosis are presumptively identified by their ability to produce dark, melanin-like pigments with various phenolic compounds as substrates. In 1962, Staib formulated a medium containing *Guizotia abyssinica* seeds which are frequently found in birdseed mixtures.¹ Birdseed Agar became the generic name for Staib's agar medium. In 1966, Shields and Ajello modified Birdseed Agar by adding an antibiotic to make the medium selective.²

PRINCIPLE

The extract of *Guizotia abyssinica* seeds contains caffeic acid which serves as a substrate for detection of phenoloxidase, an enzyme produced by *C. neoformans*.³ The action of phenoloxidase on caffeic acid results in the production of melanin which is absorbed by the yeast cell wall forming a tan to brown pigment. Dextrose is the energy source in this medium. Chloramphenicol is an antibiotic added to inhibit bacteria and fast-growing fungi. Creatinine enhances melanization of some strains of *C. neoformans*.^{4,5}

REAGENTS (CLASSICAL FORMULA)*

<i>Guizotia abyssinica</i> Seeds	50.0 g	Monopotassium Phosphate	1.0 g
Creatinine	1.0 g	Chloramphenicol	0.05 g
Dextrose	1.0 g	Agar	15.0 g
		DeminerIALIZED Water	1000.0 ml

pH 6.7 ± 0.2 @ 25°C

*Adjusted as required to meet performance standards.

PROCEDURE

1. Inoculate Birdseed Agar with a heavy inoculum removed from a pure, 3 day culture of the test isolate.
2. Streak the slant or plate surface.
3. Incubate aerobically at 25-30°C for up to 2 weeks.
4. Examine for growth and pigmentation twice daily.

Pour Tube: Melt the pour tube in a boiling water bath and cool to 45-50°C. Mix and dispense into a sterile petri dish and proceed with the instructions above.

INTERPRETATION OF THE TEST

Positive Test - Growth with tan to golden-brown pigmented colonies

Negative Test - No pigmentation of colonies

QUALITY CONTROL

All lot numbers of Birdseed Agar have been tested using the following quality control organisms and have been found to be acceptable. Testing of control organisms should be performed in accordance with established laboratory quality control procedures. If aberrant quality control results are noted, patient results should not be reported.

CONTROL

Cryptococcus neoformans ATCC® 34877
Candida albicans ATCC® 10231
Staphylococcus aureus ATCC® 25923

INCUBATION

Aerobic, up to 7 days @ 25-30°C
Aerobic, up to 7 days @ 25-30°C
Aerobic, 72 h @ 25-30°C

RESULTS

Positive (dark-brown colonies)
Negative (white colonies)
Inhibition (partial to complete)

LIMITATIONS

1. Studies by Nurudeen and Ahearn reported that no single medium supported pigmentation of all *C. neoformans* strains within a 72-hour incubation period.⁵ False-negative reactions may occur.
2. Organisms that produce pigmented colonies on Birdseed Agar should be compared with growth of the same organism on another medium, such as Sabouraud dextrose agar, to determine if the organism is naturally pigmented.⁶

BIBLIOGRAPHY

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Refer to the front of Remel *Technical Manual of Microbiological Media* for **General Information** regarding precautions, product storage and deterioration, specimen collection, storage and transportation, materials required, quality control, and limitations.

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