

CGB AGAR

INTENDED USE

Remel CGB Agar is a solid medium recommended for use in qualitative procedures for selective and differential isolation of *Cryptococcus gattii* (i.e., *Cryptococcus* serotypes B and C) from other *Cryptococcus* spp.

SUMMARY AND EXPLANATION

Cryptococcus gattii (formerly *Cryptococcus neoformans* var. *gattii*) has emerged in recent years as a cause of serious infections of the pulmonary and central nervous systems in individuals with normal immunity, as well as in immunocompromised individuals.¹ The disease presentation in non-immunocompromised individuals is often represented by large multifocal lesions (cryptococcomas) in the lung and brain and has been reported to mimic lung cancer.² Previously known to be endemic in tropical areas of the world, a recent outbreak (1997-2002) occurred on Vancouver Island, British Columbia, a region with a temperate climate. The outbreak affected 59 mostly immunocompetent individuals.³ *C. gattii* is now considered endemic in the Pacific Northwest, a region which includes the states of Washington and Oregon in the U.S. The Centers for Disease Control and Prevention, in a recent issue of *Morbidity and Mortality Weekly Report*, has recommended implementation of systematic surveillance to track *C. gattii* infections and increase awareness among healthcare providers and public health practitioners.⁴

Cryptococcus is a round to somewhat oval yeast-like fungus with single budding and a polysaccharide capsule. *Cryptococcus* species are divided into serotypes, A, B, C, and D, based on their capsular antigens; *C. gattii* isolates are serotype B or C. Most clinical microbiology laboratories do not identify the serotypes of *Cryptococcus* isolates and the biochemical methods currently available do not differentiate *C. gattii* from *C. neoformans*. In 1982, Kwon-Chung et al. developed a medium to separate *Cryptococcus* serotypes A and D from serotypes B and C.⁵ The medium, described as canavanine-glycine-bromthymol blue agar (CGB Agar), differentiates *C. gattii* from other *Cryptococcus* spp., based on the inherent resistance of *C. gattii* to L-canavanine. *C. gattii* can grow in the presence of L-canavanine and utilize glycine as a sole carbon source, thereby triggering a bromthymol blue color reaction in the medium.

PRINCIPLE

Glycine serves as a sole source of carbon. Thiamine, magnesium sulfate, and potassium phosphate supply vitamins and other nutrients necessary for the growth of *Cryptococcus*. Bromthymol blue serves as the indicator which changes the color of the medium from yellow-green to cobalt blue as growth occurs resulting in an alkaline pH. Agar is a solidifying agent.

REAGENTS (CLASSICAL FORMULA)*

Glycine	10.0 g	L.-Canavanine Sulfate	30.0 mg
Potassium Phosphate	1.0 g	Thiamine HCl	1.0 mg
Magnesium Sulfate	1.0 g	Agar	20.0 g
Bromthymol Blue	0.4 g	Deminerlized Water	1000.0 ml

pH 5.8 ± 0.1 @ 25°C

*Adjusted as required to meet performance standards.

PROCEDURE

1. Inoculate CGB Agar from a pure culture of the yeast isolate.
2. Using an inoculating needle, make a single streak on the surface of the medium.
3. Incubate in ambient air at 25-30°C for up to 5 days.
4. Examine for growth and color change in the medium from yellow-green to cobalt blue.

INTERPRETATION OF THE TEST:

Positive Test - Growth with cobalt blue color change
Negative Test - Growth with light green-blue color change

QUALITY CONTROL

All lot numbers of CGB 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
Cryptococcus neoformans B/C ATCC® 56991

INCUBATION

Ambient, up to 5 days @ 25-30°C
Ambient, up to 5 days @ 25-30°C

RESULTS

Growth w/ light green-blue color
Growth w/ cobalt blue color

LIMITATIONS

1. This test is only part of the overall scheme for identification of *C. gattii*. Further biochemical and/or serological testing is required for definitive identification. Consult appropriate references for further instructions.^{5,6}
2. *Cryptococcus* spp. other than *C. gattii* may grow on CGB Agar and cause a color change of the medium if a heavy inoculum is used.⁷

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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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