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. 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. 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. 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.

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. 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)*

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycine</td>
<td>10.0 g</td>
</tr>
<tr>
<td>L-Canavanine Sulfate</td>
<td>30.0 mg</td>
</tr>
<tr>
<td>Thiamine HCl</td>
<td>1.0 mg</td>
</tr>
<tr>
<td>Magnesium Sulfate</td>
<td>1.0 g</td>
</tr>
<tr>
<td>Agar</td>
<td>20.0 g</td>
</tr>
<tr>
<td>Bromthymol Blue</td>
<td>0.4 g</td>
</tr>
<tr>
<td>Demineralized Water</td>
<td>1000.0 ml</td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>CONTROL</th>
<th>INCUBATION</th>
<th>RESULTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cryptococcus neoformans ATCC® 34877</td>
<td>Ambient, up to 5 days @ 25-30°C</td>
<td>Growth w/ light green-blue color</td>
</tr>
<tr>
<td>Cryptococcus neoformans B/C ATCC® 56991</td>
<td>Ambient, up to 5 days @ 25-30°C</td>
<td>Growth w/ cobalt blue color</td>
</tr>
</tbody>
</table>

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.
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.

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BIBLIOGRAPHY


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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