PROVIDENCE
Sacred Heart
Medical Center &
Children's Hospital

Department of Microbiology
CGB Agar Procedure
For *Cryptococcus neoformans* Differentiation

I. Purpose and Test Principle

Canavanine-glycine-bromthymol blue (CGB) agar can be used to differentiate serotypes of *Cryptococcus neoformans*. The four serotypes include *C. neoformans* var. *grubii* (serotype A), var. *neoformans* (serotype D), and var. *gattii* (serotypes B and C). *C. neoformans* is classically associated with chronic central nervous system disease in immunocompromised individuals. *C. neoformans* var. *gattii* can cause respiratory and central nervous system disease in immunocompetent individuals and more severe disease in immunocompromised people. The differentiation of *C. neoformans* var. *gattii* is primarily performed for epidemiologic purposes.

CGB agar contains canavanine, which inhibits *Cryptococcus* serotypes A and D. In addition, serotypes B and C are able to assimilate glycine as a carbon source. The alkaline end products lead to a shift in the pH of the medium causing the bromthymol blue indicator to turn from yellow to blue.

II. Specimen Information

Clinical isolates should be evaluated microscopically and physiologically for complete identification to the genus, *Cryptococcus*. Only pure isolates should be used for subculture and testing.

III. Reagents & Equipment

- CGB agar (Hardy Diagnostics)
- 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. Minimize exposure of agar to light. 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

Work with all isolates in a biologic safety cabinet. Streak yeast isolate onto the medium using a heavy inoculum.

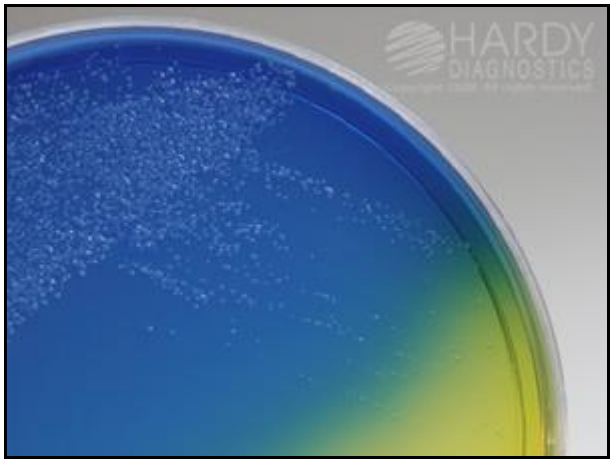
B. Incubation

Incubate at 25 - 30°C. Observe plate for growth and color change beginning on day 2. If a heavy inoculum is used, typically only 48 h is needed for a color change in the medium. Plates should be examined for color change for up to 5 days.

V. Interpretation and Reporting

A. Positive Test

A positive test result is indicated by growth and a color change from yellow-green to blue and indicates that the *Cryptococcus* isolate is the variety *gattii*.



B. Negative Test

A negative test is indicated by no color change after 5 days of incubation. Growth with no color change may be interpreted as *C. neoformans* var. *neoformans*.

VI. Quality Control

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

Control strain	Expected Results
Cryptococcus neoformans ATCC 14116	No growth or slight growth with no color change
Cryptococcus gattii ATCC MYA-4561	growth and a color change from yellow-green to blue

VII. Limitations

A. A very heavy inoculum (a heaping loopful) may produce a slight color change from yellow-green to light green with the *neoformans* variety.

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

VIII. Verification of Test Method

The CGB agar was evaluated by using clinical isolates, ATCC strains and isolates from past CAP surveys. A total of ten yeast isolates were tested on

the CGB 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, and *Candida albicans* ATCC 90028. Plates were heavily 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. The clinical *C. neoformans* var. *gattii* isolate began turning the agar from yellow to blue-green by 48 h and turned the medium blue after further incubation. All of the other *Cryptococcus* isolates failed to grow or showed very scant growth after 5 days and produced no color change. The *C. albicans* strain grew tiny colonies and did cause the agar to change to a blue-green color. This illustrates the importance of evaluating test isolates microscopically and physiologically along with performing the subculture to CGB agar.

IX. References

A. Technical Data from www.hardydiagnostics.com (061110sd).

B. The University of British Columbia website: http://www.cher.ubc.ca/cryptococcus/new/images/C_neoform_gat_CGB.jpg.

C. 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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