



Instructions for Use

CGB AGAR

Cat. no. G113

CGB Agar, 15x100mm Plate, 20ml

10 plates/bag

INTENDED USE

Hardy Diagnostics CGB Agar is recommended for the differentiation of *Cryptococcus neoformans* var. *neoformans* and *Cryptococcus gattii* (formerly *C. neoformans* var. *gattii*).

SUMMARY

Cryptococcosis, or torulosis, is an opportunistic and systemic infection that affects humans and mammals. It is a subacute or chronic infection most frequently involving the tissue of the central nervous system but occasionally producing lesions in the skin, bones, lungs, or other internal organs.^(1,3) Cryptococcal meningitis is extremely common in AIDS patients. Also, *Cryptococcus* spp. have been known to parasitize cats in some areas, although strains vary in virulence.⁽⁹⁻¹¹⁾ The other species of this genus are commonly considered nonpathogenic but may occasionally cause disease in severely immunosuppressed patients.

Cryptococcosis is typically caused by *Cryptococcus neoformans*, an encapsulated yeast found in soil, specifically in soil containing manure from pigeons, chickens, or turkeys. The increased pH and concentration of nitrogen allows for proliferation of this organism. The yeast cells can easily become airborne due to sweeping, cleaning and construction. *C. gattii* is also commonly associated with certain species of Eucalyptus in India; consequently, this organism has been implicated in cases of chronic cryptococcal meningitis.⁽⁸⁾

Cryptococcal cells are spherical and produce a mucoid polysaccharide capsule, which may vary in width from very thin to several times the radius of the parent cell, including buds.⁽²⁾ All species of *Cryptococcus* lack fermentative ability. Four major serotypes (A-D) exist within the capsular polysaccharide antigen, with 80-85% of isolates being either A or B. *C. neoformans* var. *neoformans* corresponds to serotypes A and D, while serotypes B and C are associated with *C. gattii*.

In 1981, Kyung J. Kwon-Chung et al. reported in the *Journal of Clinical Microbiology* that the most effective way to distinguish between the two species was to culture on a solid agar medium containing canavanine, glycine and bromothymol blue (CGB).⁽⁶⁾ This medium is a modification of CDB agar developed in 1978, which used dextrose instead of glycine, and was prone to false-negatives resulting in improper identification. According to Kwon-Chung et al., the CGB formulation effectively eliminates the false reactions and allows for a positive color change from yellow-green to cobalt blue. The blue color is the result of an alkaline pH shift produced when creatine is degraded to ammonia. All of the *C. gattii* strains tested in this study, serotypes B and C, were positive by day five. *C. neoformans*, serotypes A and D, all produced no color change at two and five days.^(6,7)

This formula contains agar for solidification, glycine as a source of carbon, and potassium phosphate, magnesium sulfate and thiamine hydrochloride as vitamins and nutrients. L-canavanine sulfate allows for the isolation of *Cryptococcus* as they are the only yeasts known to have a natural resistance.⁽⁷⁾ Bromothymol blue is added to indicate the shift in pH to 7.0 when the media appears cobalt blue.

FORMULA

Ingredients per liter of deionized water:*

Glycine	10.0gm
Potassium Phosphate	1.0gm
Magnesium Sulfate	1.0gm
Bromothymol Blue	0.08gm
L-Canavanine Sulfate	0.03gm
Thiamine Hydrochloride	0.001gm
Agar	20.0gm

Final pH 5.8 +/- 0.2 at 25°C.

* Adjusted and/or supplemented as required to meet performance criteria.

STORAGE AND SHELF LIFE

Storage: Upon receipt store at 2-8°C. away from direct light. Media should not be used if there are any signs of deterioration (shrinking, cracking, or discoloration), contamination, or if the expiration date has passed. Product is light and temperature sensitive; protect from light, excessive heat, moisture, and freezing.

The expiration dating on the product label applies to the product in its intact packaging when stored as directed. The product may be used and tested up to the expiration date on the product label and incubated for the recommended quality control incubation times.

Refer to the document "[Storage](#)" for more information.

PRECAUTIONS

This product may contain components of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not guarantee the absence of transmissible pathogenic agents. Therefore, it is recommended that these products be treated as potentially infectious, and handle observing the usual universal blood precautions. Do not ingest, inhale, or allow to come into contact with skin.

This product is for *in vitro* diagnostic use only. It is to be used only by adequately trained and qualified laboratory personnel. Observe approved biohazard precautions and aseptic techniques. All laboratory specimens should be considered infectious and handled according to "standard precautions." The "Guidelines for Isolation Precautions" is available from the Centers for Disease Control and Prevention at www.cdc.gov/ncidod/dhqp/gl_isolation.html.

For additional information regarding specific precautions for the prevention of the transmission of all infectious agents from laboratory instruments and materials, and for recommendations for the management of exposure to infectious disease, refer to CLSI document M-29: *Protection of Laboratory Workers from Occupationally Acquired Infections: Approved Guideline*.

Sterilize all biohazard waste before disposal.

Refer to the document "[Precautions When Using Media](#)" for more information.

Refer to the document [SDS Search](#) instructions on the Hardy Diagnostics' website for more information.

PROCEDURE

Inoculate the medium with an isolate of *Cryptococcus* (see note under limitations) and incubate between 25 and 30°C. for up to five days.⁽⁶⁾

INTERPRETATION OF RESULTS

Growth and a color change to medium blue indicates *C. gattii*; no growth or minimal growth that is medium yellow or green may be interpreted as *C. neoformans* var. *neoformans*.^(2,5,6)

LIMITATIONS

It is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on colonies from pure culture for complete identification.

A very heavy inoculum (a heaping loopful) may produce a slight color change from yellow-green to light green with the *C. neoformans* variety.⁽⁶⁾

Note : Some yeast isolates other than *C. gattii* may be CGB Agar positive.⁽¹²⁾ Therefore, suspected cryptococcal isolates should be initially identified by a positive urease test using Urea Agar (Cat. no. R42 or L65) or Urea Disks (Cat. no. DU7525) and by a positive phenoloxidase test when grown on Caffeic Acid Agar (Cat. no. G213 or L13) or through the use of Caffeic Acid Disks (Cat. no. Z118). Cryptococci are urease positive and most other fungi, except *Trichosporon* spp. and some *Candida* spp., are urease negative.⁽¹²⁾ *C. neoformans* and *C. gattii* may be differentiated from other Cryptococci by observing colonies for melanin production in the presence of caffeic acid.⁽¹²⁾

Refer to the document "[Limitations of Procedures and Warranty](#)" for more information.

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as loops, swabs, applicator sticks, other culture media such as Caffeic Acid Agar (Cat. no. G213 or L13) or Urea Agar (Cat. no. R42 or L65), incinerators, and incubators, etc., as well as serological and biochemical reagents such as Urea Disks (Cat. no. DU7525) or Caffeic Acid Disks (Cat. no. Z118), are not provided.

QUALITY CONTROL

Hardy Diagnostics tests each lot of commercially manufactured media using appropriate quality control microorganisms and quality specifications as outlined on the Certificates of Analysis (CofA). The following organisms are routinely used for testing at Hardy Diagnostics:

Test Organisms	Inoculation Method*	Incubation			Results
		Time	Temperature	Atmosphere	
<i>Cryptococcus gattii</i> ATCC® MYA-4560**	B	up to 5 days	15-30°C	Aerobic	Growth; deep blue color change
<i>Cryptococcus neoformans</i> var. <i>neoformans</i> ATCC® 204092**	B	up to 5 days	15-30°C	Aerobic	Growth minimal; if present medium yellow or green

* Refer to the document "[Inoculation Procedures for Media QC](#)" for more information.

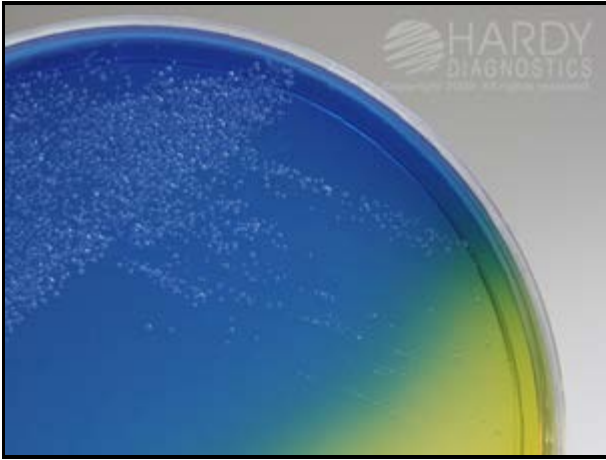
** Recommended QC strains for User Quality Control according to the CLSI document M22 when applicable.

USER QUALITY CONTROL

End users of commercially prepared culture media should perform QC testing in accordance with applicable government regulatory agencies, and in compliance with accreditation requirements. Hardy Diagnostics recommends end users check for signs of contamination and deterioration and, if dictated by laboratory quality control procedures or regulation, perform quality control testing to demonstrate growth or a positive reaction and to demonstrate inhibition or a negative reaction, if applicable. Hardy Diagnostics quality control testing is documented on the certificates of analysis (CofA) available from Hardy Diagnostics [Certificates of Analysis](#) website. In addition, refer to the following document "[Finished Product Quality Control Procedures](#)," for more information on QC or see reference(s) for more specific information.

PHYSICAL APPEARANCE

CGB Agar should appear clear, and bright yellow-green in color.



Cryptococcus gattii (ATCC® MYA-4560) colonies growing on CGB Agar (Cat. no. G113). Incubated aerobically for 72 hours at 30°C.

REFERENCES

1. Hensyl, William R., et al. 1990. *Stedman's Medical Dictionary*, 25th ed. Williams & Wilkins, Baltimore, MD.
2. St. Germain, Guy, et al. 1996. *Identifying Filamentous Fungi*. Star Publishing Company, Belmont, CA.
3. Larone, D.H. *Medically Important Fungi: A Guide to Identification*, American Society for Microbiology. Washington, D.C.
4. *The Oxoid Vade-Mecum of Microbiology*. 1993. Unipath Ltd., Basingstoke, UK.
5. Koneman, E.W., et al. *Color Atlas and Textbook of Diagnostic Microbiology*, J.B. Lippincott Company, Philadelphia, PA.
6. Kwon-Chung, K. J., I. Polacheck, and J.E. Bennett. 1982. Improved Diagnostic Medium for Separation of *Cryptococcus neoformans* var. *neoformans* (serotypes A and D) and *Cryptococcus neoformans* var. *gattii* (serotypes B and C). *Journal of Clinical Microbiology* ; 15:525-537.
7. Polacheck, I. and K. J. Kwon-Chung. 1985. Canavanine Resistance in *Cryptococcus neoformans*. *Antimicrobial Agents and Chemotherapy*; 29.3:468-473.
8. Chakrabarti, Arunaloke, et al. 1997. Isolation of *Cryptococcus neoformans* var. *gattii* from *Eucalyptus camaldulensis* in India. *Journal of Clinical Microbiology*; 35.12:3340-3342.

9. Gionfriddo, J. R. 2000. Feline Systemic Fungal Infections. *Vet Clin North Am Small Anim Pract.* 30(5):1029-50.
10. Gerds-Grogan S. and B. Dayrell-Hart B. 1997. Feline Cryptococcosis: A Retrospective Evaluation. *J. Am. Anim. Hosp. Assoc.* 33(2):118-22.
11. Malik R., D.I. Wigney, D.B. Muir, D.J. Gregory and D.N. Love. 1992. Cryptococcosis in Cats: Clinical and Mycological Assessment of 29 Cases and Evaluation of Treatment Using Orally Administered Fluconazole. *J. Med. Vet. Mycol.* 30(2):133-44.
12. Klein, K.R., L. Hall, S.M. Deml, J.M. Rysavy, S.L. Wohlfiel and N.L. Wengenack. 2009. Identification of *Cryptococcus gattii* by Use of L-Canavanine Glycine Bromothymol Blue Medium and DNA Sequencing. *J. Clin. Micro.* 47(11):3669-3672.

ATCC is a registered trademark of the American Type Culture Collection.

IFU-10100[B]



1430 West McCoy Lane, Santa Maria, CA 93455, USA

Phone: (805) 346-2766 ext. 5658

Fax: (805) 346-2760

Website: www.HardyDiagnostics.com

Email: TechService@HardyDiagnostics.com

[Ordering Information](#)

Distribution Centers:

California · Washington · Utah · Arizona · Texas · Ohio · New York · Florida · North Carolina

The Hardy Diagnostics manufacturing facility and quality management system is certified to ISO 13485.

Copyright© 2020 by Hardy Diagnostics. All rights reserved.

HDQA 2207F [C]