IFU

Instructions for Use

CAFFEIC ACID AGAR - "BIRD SEED AGAR" FOR THE IDENTIFICATION OF CRYPTOCOCCUS NEOFORMANS

Cat. no. G213	Caffeic Acid Agar, 15x100mm Plate, 18ml	10 plates/bag

INTENDED USE

Hardy Diagnostics Caffeic Acid Agar is recommended for the selective isolation and differentiation of *Cryptococcus neoformans* and *Cryptococcus gattii*.

SUMMARY

Cryptococcus neoformans is an encapsulated yeast that produces the enzyme phenoloxidase, an enzyme necessary in melanin synthesis. When in the presence of caffeic acid, the enzyme attacks the acid resulting in the production of melanin. Subsequently, melanin is absorbed by the cell wall of the yeast producing tan to brown pigmented colonies.

The brown pigmented colonies of *Cryptococcus neoformans* were observed by Staib in 1962 when he grew cultures of the yeast on media containing *Guizotia abyssinica* seeds.[6] It was later determined that the seeds contain caffeic acid, which served as the melanin-producing substrate.

In 1966, Shields and Ajello modified Staib's Birdseed Agar by making the medium selective with an antimicrobic additive.[7] Hardy Diagnostics Caffeic Acid Agar is a modification of the latter formula.

FORMULA

Ingredients per liter of deionized water:*

Ammonium Sulfate	5.0gm
Glucose	5.0gm
Yeast Extract	2.0gm
Dipotassium Phosphate	0.8gm
Magnesium Sulfate	0.7gm
Caffeic Acid	0.18gm
Chloramphenicol	0.05gm
Ferric Citrate Solution	4.0ml
Agar	15.0gm

Final pH 6.5 +/- 0.3 at 25ºC.

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

STORAGE AND SHELF LIFE

Storage: Upon receipt store at 2-8ºC. 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 and freezing.

The expiration date 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 incubation times as stated below.

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 Precautions for blood. 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." Refer to the document "Guidelines for Isolation Precautions" from the Centers for Disease Control and Prevention.

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 M29: *Protection of Laboratory Workers from Occupationally Acquired Infections.*

Sterilize all biohazard waste before disposal.

Refer to the document "Precautions When Using Media" for more information.

PROCEDURE

Specimen Collection: Infectious material should be submitted directly to the laboratory without delay and protected from excessive heat and cold. Consult listed references for information on specimen collection.[2-5]

Method of Use:

1. Prior to use, allow the medium to equilibrate to room temperature.

2. Using aseptic technique, inoculate sample to medium.

Note: Sputa specimens can be inoculated directly to the agar surface with a sterile loop or rayon-tipped swab. Urine samples should be centrifuged for 15 minutes at 1,500Xg and the sediment recovered with a sterile transfer pipette. Evenly distribute sediment over agar surface.

3. Incubate at room temperature (15-30ºC.) for 3-5 days.

4. Observe daily for the production of brown to black pigmented colonies.

INTERPRETATION OF RESULTS

Development of brown to black pigmented colonies is a positive presumptive identification for *C. neoformans*.

LIMITATIONS

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

Yeasts other than *C. neoformans* may rarely produce brown pigmentation on media containing caffeic acid.

A Sabouraud Dextrose Agar (Cat. no. W70) control should be inoculated in parallel to the Caffeic Acid Agar to ensure that a dark pigment is not naturally produced by the colonies. *Aureobasidium*, *Sporothrix*, *Wangiella*, and *Phialophora* may produce dark brown colonies, but the pigment will not be a result of enzymatic activity which is made evident by pigmentation developing in colonies on all media.

Rare strains of *C. neoformans* may not produce pigmented colonies.

Specimens heavily contaminated with bacteria may obscure growth and/or pigmentation of *C. neoformans*.

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, other culture media, swabs, applicator sticks, incinerators, and incubators, etc., as well as serological and biochemical reagents, 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 Certificate of Analysis (CofA) and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media*. The following microorganisms are routinely used for testing at Hardy Diagnostics:

Test Organisms	Inoculation Method**	Incubation			Results
		Time	Temperature	Atmosphere	
Cryptococcus neoformans ATCC® 32045*	A	72-96hr	15-30°C	Aerobic	Growth; brown to black pigmented colonies
Escherichia coli ATCC® 25922*	B	24hr	35°C	Aerobic	Partial to complete inhibition

* 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 certificate of analysis (CofA) available from Hardy Diagnostics Certificate of Analysis website. Also refer to the document "Finished Product Quality Control Procedures," and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media* for more information on the appropriate QC procedures. See the references below.

PHYSICAL APPEARANCE

Caffeic Acid Agar should appear slightly opaque, with precipitate, and light gray in color.

REFERENCES

1. Anderson, N.L., et al. *Cumitech 3B; Quality Systems in the Clinical Microbiology Laboratory*, Coordinating ed., A.S. Weissfeld. American Society for Microbiology, Washington, D.C.

2. Jorgensen., et al. *Manual of Clinical Microbiology*, American Society for Microbiology, Washington, D.C.

3. Tille, P., et al. *Bailey and Scott's Diagnostic Microbiology*, C.V. Mosby Company, St. Louis, MO.

4. Isenberg, H.D. *Clinical Microbiology Procedures Handbook*, Vol. I, II & III. American Society for Microbiology, Washington, D.C.

5. Koneman, E.W., et al. *Color Atlas and Textbook of Diagnostic Microbiology*, J.B. Lippincott Company, Philadelphia, PA.

6. Staib, F. 1962. *Hyg. Infektionskr. Med. Mikeobiol. Immunol. Virol.* ; 148:466-475.

7. Shields, A.B. and Ajello, L. 1966. Medium for Selective Isolation of *Cryptococcus neoformans*, Service; 151:208-209.

8. Denning, D.W., et al. 1990. *Journal of Clinical Microbiology*; Vol. 28, No. 11, p. 2565-2567.

9. La Rocco, Mark, Ph.D. 1992. *Clinical Microbiology Newsletter*; Vol. 14, No. 23, p. 177-181.

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1430 West McCoy Lane, Santa Maria, CA 93455, USA
Phone: (805) 346-2766 ext. 5658
Fax: (805) 346-2760
Website: HardyDiagnostics.com
Email: TechnicalServices@HardyDiagnostics.com
Ordering Information

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

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