



## QUALITY CONTROL PROCEDURES

### I INTRODUCTION

Brain Heart CC Agar is a selective medium for the isolation of pathogenic fungi.

### II PERFORMANCE TEST PROCEDURE

- Inoculate representative samples by the streak method using a 0.01 mL calibrated loop.
  - Use fresh fungal cultures of the fungi up to 1 month in age as inocula. Use a  $10^{-1}$  dilution of a 5-h **Trypticase™** Soy Broth culture of *Escherichia coli*.
  - Incubate bottles with loosened caps at  $25 \pm 2^{\circ}\text{C}$  in an aerobic atmosphere.
  - Include Brain Heart Infusion Agar Slants as nonselective controls for all organisms.
- Examine bottles at intervals up to 7 days for growth and selectivity.
- Expected Results

Organisms	ATCC™	Recovery
* <i>Candida albicans</i>	10231	Growth
* <i>Trichophyton mentagrophytes</i>	9533	Growth
<i>Blastomyces dermatitidis</i>	56218	Growth (fair to moderate)
* <i>Escherichia coli</i>	25922	Inhibition (complete)
* <i>Aspergillus niger</i>	16404	Inhibition (partial to complete)

\*Recommended organism strain for User Quality Control.

### III ADDITIONAL QUALITY CONTROL

- Examine bottles as described under "Product Deterioration."
- Visually examine representative bottles to assure that any existing physical defects will not interfere with use.
- Determine the pH potentiometrically at room temperature for adherence to the specification of  $7.4 \pm 0.2$ .
- Incubate uninoculated representative bottles at  $20\text{--}25^{\circ}\text{C}$  and  $30\text{--}35^{\circ}\text{C}$  and examine after 7 days for microbial contamination.

## PRODUCT INFORMATION

### IV INTENDED USE

Brain Heart CC Agar is a selective medium used for the isolation of pathogenic fungi from specimens heavily contaminated with bacteria and saprophytic fungi.

### V SUMMARY AND EXPLANATION

BHI Agar is recommended as a general medium for aerobic bacteriology and for the primary recovery of fungi from clinical specimens.<sup>1</sup> The presence of the antimicrobial agents, cycloheximide and chloramphenicol, inhibits the growth of a wide variety of bacteria and fungi and enhances the isolation of pathogenic fungal species.

### VI PRINCIPLES OF THE PROCEDURE

BHI Agar derives its nutrients from the brain heart infusion, peptone and dextrose components. The peptones and infusion are sources of organic nitrogen, carbon, sulfur, vitamins and trace substances. Dextrose is the carbohydrate source that microorganisms utilize by fermentative action. The medium is buffered through the use of disodium phosphate. Chloramphenicol is a broad-spectrum antibiotic which inhibits a wide range of gram-positive and gram-negative bacteria. Cycloheximide inhibits most saprophytic molds.

### VII REAGENTS

#### Brain Heart CC Agar

Approximate Formula\* Per Liter Purified Water

Brain Heart, Infusion from (solids) .....	8.0 g	Disodium Phosphate .....	2.5 g
Peptic Digest of Animal Tissue .....	5.0 g	Cycloheximide .....	0.5 g
Pancreatic Digest of Casein .....	16.0 g	Chloramphenicol .....	0.05 g
Sodium Chloride .....	5.0 g	Agar .....	13.5 g
Dextrose .....	2.0 g		

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

#### Warnings and Precautions: For *in vitro* Diagnostic Use.

Tubes with tight caps should be opened carefully to avoid injury due to breakage of glass.

Pathogenic microorganisms, including hepatitis viruses and Human Immunodeficiency Virus, may be present in clinical specimens. "Standard Precautions"<sup>2-5</sup> and institutional guidelines should be followed in handling all items contaminated with blood and other body fluids. After use, prepared tubes, specimen containers and other contaminated materials must be sterilized by autoclaving before discarding.

**Storage Instructions:** On receipt, store tubes in the dark at  $2\text{--}8^{\circ}\text{C}$ . Avoid freezing and overheating. Do not open until ready to use. Minimize exposure to light. Tubed media stored as labeled until just prior to use 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.

**Product Deterioration:** Do not use tubes if they show evidence of microbial contamination, discoloration, drying or other signs of deterioration.

## VIII SPECIMEN COLLECTION AND HANDLING

Specimens suitable for culture may be handled using various techniques. For detailed information, consult appropriate texts.<sup>6,7</sup> Specimens should be obtained before antimicrobial therapy has been administered. Provision must be made for prompt delivery to the laboratory.

## IX PROCEDURE

**Material Provided:** Brain Heart CC Agar **Mycoflask™** Bottles

**Materials Required But Not Provided:** Ancillary culture media, reagents, quality control organisms and laboratory equipment as required.

**Test Procedure:** Observe aseptic techniques.

Streak the specimen as soon as possible after it is received in the laboratory using a sterile inoculating loop to obtain isolated colonies.

Media may be inoculated up to the expiration date and incubated for up to 6 weeks.

For isolation of fungi from potentially contaminated specimens, a nonselective medium should be inoculated along with the selective medium. Incubate the bottles at 25–30°C with increased humidity. For isolation of fungi causing systemic mycoses, two sets of media should be inoculated, with one set incubated at 25–30°C and a duplicate set at 35 ± 2°C.

All cultures should be examined at least weekly for fungal growth and should be held for 4–6 weeks before being reported as negative.

**User Quality Control:** See "Quality Control Procedures."

Quality Control requirements must be performed in accordance with applicable local, state and/or federal regulations or accreditation requirements and your laboratory's standard Quality Control procedures. It is recommended that the user refer to pertinent CLSI (formerly NCCLS) guidance and CLIA regulations for appropriate Quality Control practices.

A single electrode of sufficiently small size to fit into the tubes should be used to determine the pH potentiometrically of tubed, bottled and **Mycoflask** brand media. The tip of the electrode should be positioned in the central portion of the agar mass in semisolid or solid media.

## X RESULTS

After sufficient incubation, the bottles should show isolated colonies in streaked areas and confluent growth in areas of heavy inoculation.

Examine bottles for fungal colonies exhibiting typical color and morphology. Biochemical tests and serological procedures should be performed to confirm findings.

## XI LIMITATIONS OF THE PROCEDURE

Some fungi may be inhibited by the antibiotics in this medium.<sup>8</sup>

For identification, organisms must be in pure culture. Morphological, biochemical and/or serological tests should be performed for final identification. Consult appropriate texts for detailed information and recommended procedures.<sup>6,7,9-11</sup>

## XII AVAILABILITY

Cat. No.	Description
221834	<b>BBL™</b> BHI CC Agar (with Chloramphenicol and Cycloheximide), Pkg. of 10 <b>Mycoflask™</b> bottles.

## XIII REFERENCES

1. Chapin, K.C., and P.R. Murray. 1999. Media, p. 1687-1707. In P.R. Murray, E.J. Baron, M.A. Pfaller, F.C. Tenover, and R.H. Tenover (ed.), *Manual of clinical microbiology*, 7th ed. American Society for Microbiology, Washington, D.C.
2. National Committee for Clinical Laboratory Standards. 2001. Approved Guideline M29-A2. Protection of laboratory workers from occupationally acquired infections, 2nd ed. NCCLS, Wayne, PA.
3. Garner, J.S. 1996. Hospital Infection Control Practices Advisory Committee, U.S. Department of Health and Human Services, Centers for Disease Control and Prevention. Guideline for isolation precautions in hospitals. *Infect. Control Hospital Epidemiol.* 17:53-80.
4. U.S. Department of Health and Human Services. 1999. Biosafety in microbiological and biomedical laboratories, HHS Publication (CDC), 4th ed. U.S. Government Printing Office, Washington, D.C.
5. Directive 2000/54/EC of the European Parliament and of the Council of 18 September 2000 on the protection of workers from risks related to exposure to biological agents at work (seventh individual directive within the meaning of Article 16(1) of Directive 89/391/EEC). *Official Journal L262*, 17/10/2000, p. 0021-0045.
6. Murray, P.R., E.J. Baron, J.H. Tenover, M.A. Pfaller, and R. H. Tenover (ed.). 2003. *Manual of clinical microbiology*, 8th ed. American Society for Microbiology, Washington, D.C.
7. Forbes, B.A., D.F. Sahm, and A.S. Weissfeld. 2002. *Bailey and Scott's diagnostic microbiology*, 11th ed. Mosby, Inc., St. Louis.
8. Ajello, J., L.K. Georg, W. Kaplan, and L. Kaufman. 1963. *CDC laboratory manual for medical mycology*. PHS Publication No. 994. U.S. Government Printing Office, Washington, D.C.
9. Holt, J.G., N.R. Krieg, P.H.A. Sneath, J.T. Staley, and S.T. Williams (ed.). 1994. *Bergey's Manual™ of determinative bacteriology*, 9th ed. Williams & Wilkins, Baltimore.
10. Koneman, E.W., S.D. Allen, W.M. Janda, P.C. Schreckenberger, and W.C. Winn, Jr. 1997. *Color atlas and textbook of diagnostic microbiology*, 5th ed. Lippincott-Raven, Philadelphia.
11. Isenberg, H.D. (ed.). 2004. *Clinical microbiology procedures handbook*, vol. 1, 2 and 3, 2nd ed. American Society for Microbiology, Washington, D.C.

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