BBL™ Sabouraud Brain Heart Infusion Agar with Chloramphenicol and Cycloheximide (Deep Fill)

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QUALITY CONTROL PROCEDURES

I INTRODUCTION

Sabouraud Brain Heart Infusion Agar with Chloramphenicol and Cycloheximide is a selective medium for use in the cultivation of pathogenic and nonpathogenic fungi from a variety of clinical and nonclinical sources.

II PERFORMANCE TEST PROCEDURE

1. Inoculate representative samples with the cultures listed below.
   a. Inoculate organisms directly from stock plate using fresh fungal cultures (up to three weeks in age). For *E. coli* and *C. albicans*, prepare suspensions equivalent to a 0.5 McFarland standard and inoculate 0.01 mL of 10^{-1} and 10^{-2} dilutions, respectively.
   b. Incubate plates at 25 ± 2 °C in an aerobic atmosphere.
   c. Include Sabouraud Dextrose Agar plates as nonselective controls for all organisms.
2. Examine plates for up to 7 days for amount of growth, pigmentation and selectivity.
3. Expected Results

Organisms	ATCC™	Recovery
**Candida albicans*	10231, 60193	Moderate to heavy growth
**Trichophyton mentagrophytes*	9533	Moderate to heavy growth
**Escherichia coli*	25922	Inhibition (partial to complete)
**Aspergillus brasiliensis*	16404	Inhibition (partial to complete)
Blastomyces dermatitidis	56218	Fair to heavy growth. Colonies are white
Microsporum audouinii	9079	Fair to heavy growth. Colonies are slow-growing, cottony, white to tan surface with white to brown under-surface.
**Penicillium roquefortii*	9295	Inhibition (partial to complete). Colonies powdery, green to blue.

*Recommended organism strain for User Quality Control.

III ADDITIONAL QUALITY CONTROL

1. Examine plates as described under “Product Deterioration.”
2. Visually examine representative plates to assure that any existing physical defects will not interfere with use.
3. Determine the pH potentiometrically at room temperature for adherence to the specification of 6.8 ± 0.2.
4. Note the firmness of plates during the inoculation procedure.
5. Incubate uninoculated representative plates aerobically at 25 ± 2 °C for 72 h and examine for microbial contamination.

PRODUCT INFORMATION

IV INTENDED USE

Sabouraud Brain Heart Infusion Agar with Chloramphenicol and Cycloheximide is a selective medium used in qualitative procedures for cultivation of pathogenic and nonpathogenic fungi from clinical and nonclinical specimens. The plates are deep-filled to reduce the effects of drying during prolonged incubation.

V SUMMARY AND EXPLANATION

Sabouraud Dextrose Agar is a general purpose medium devised by Sabouraud for the cultivation of dermatophytes.[1] Brain Heart Infusion (BHI) Agar has proven to be effective in the cultivation of a wide variety of microorganisms and is recommended for the primary recovery of fungi from clinical specimens.[2] Sabouraud Brain Heart Infusion Agar combines the ingredients of these two formulations to provide a medium which was found to yield greater recovery of pathogenic fungi than either medium individually.[3] It is recommended for the recovery of fungi from clinical specimens.[4] Selectivity is achieved by the addition of Chloramphenicol and Cycloheximide to the Sabouraud Brain Heart Infusion Agar formulation.

VI PRINCIPLES OF THE PROCEDURE

Sabouraud Brain Heart Infusion Agar with Chloramphenicol and Cycloheximide contains two peptones and brain heart infusion solids as sources of amino acids, nitrogen, sulfur, carbon and trace ingredients. Dextrose is an energy source for the metabolism of microorganisms. Sodium chloride provides essential electrolytes. Chloramphenicol is a broad spectrum antibiotic which inhibits a wide range of gram-positive and gram-negative bacteria. Cycloheximide inhibits most saprophytic molds but is not active against yeasts and dermatophytes.

VII REAGENTS

Sabouraud Brain Heart Infusion Agar with Chloramphenicol and Cycloheximide

Approximate Formula* Per Liter Purified Water

Brain Heart, Infusion from (solids)	4.0 g	Disodium Phosphate	1.25 g
Peptic Digest of Animal Tissue	5.0 g	Agar	15.0 g
Pancreatic Digest of Casein	10.5 g	Chloramphenicol	0.05 g
Dextrose	21.0 g	Cycloheximide	0.5 g
Sodium Chloride	2.5 g		

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

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

If excessive moisture is observed, invert the bottom over an off-set lid and allow to air dry in order to prevent formation of a seal between the top and bottom of the plate during incubation.

Pathogenic microorganisms, including hepatitis viruses and Human Immunodeficiency Virus, may be present in clinical specimens. "Standard Precautions"[5-8] and institutional guidelines should be followed in handling all items contaminated with blood and other body fluids. After use, prepared plates, specimen containers and other contaminated materials must be sterilized by autoclaving before discarding.

Storage Instructions: On receipt, store plates in the dark at 2 – 8 °C. Avoid freezing and overheating. Do not open until ready to use. Minimize exposure to light. Prepared plates stored in their original sleeve wrapping at 2 – 8 °C until just prior to use may be inoculated up to the expiration date and incubated for recommended incubation times including up to 6 weeks for mycology media. Allow the medium to warm to room temperature before inoculation.

Product Deterioration: Do not use plates if they show evidence of microbial contamination, discoloration, drying, cracking 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.[9,10] Specimens should be obtained before antimicrobial therapy has been administered. Provision must be made for prompt delivery to the laboratory.

IX PROCEDURE

Material Provided: Sabouraud Brain Heart Infusion Agar with Chloramphenicol and Cycloheximide

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

Test Procedure: Observe aseptic techniques.

The agar surface should be smooth and moist, but without excessive moisture.

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

For isolation of fungi from potentially contaminated specimens, a nonselective medium should be inoculated along with the selective medium. Incubate the plates aerobically at 25 – 30 °C in an inverted position (agar side up) with increased humidity. For isolation of fungi causing systemic mycoses, two sets of media should be inoculated, with one set incubated aerobically at 25 – 30 °C and a duplicate set aerobically 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 guidance and CLIA regulations for appropriate Quality Control practices.

X RESULTS

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

Examine plates 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.[2]

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.[9-13]

A single medium is rarely adequate for detecting all organisms of potential significance in a specimen. It should be recognized that organisms generally susceptible to the antimicrobial agent in a selective medium may be completely or only partially inhibited depending upon the concentration of the agent, the characteristics of the microbial strain and the number of organisms in the inoculum. Organisms that are generally resistant to the antimicrobial agent should not be inhibited. Cultures of specimens grown on selective media should, therefore, be compared with specimens cultured on nonselective media to obtain additional information and help ensure recovery of potential pathogens.

XII AVAILABILITY

Cat. No.	Description
297803	**BBL™** Sabouraud Brain Heart Infusion Agar with Chloramphenicol and Cycloheximide (Deep Fill), Pkg. of 10 plates

XIII REFERENCES

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Technical Information: In the United States contact BD Technical Services and Support at 800-638-8663 or www.bd.com/ds.

Becton, Dickinson and Company
7 Loveton Circle
Sparks, MD 21152 USA