BBL™ Sabouraud Dextrose Agar
BBL™ Sabouraud Dextrose Agar with Chloramphenicol

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QUALITY CONTROL PROCEDURES (Optional)

I INTRODUCTION

Sabouraud Dextrose Agar is a nonselective medium for the cultivation and maintenance of pathogenic and non-pathogenic fungi, particularly dermatophytes. Selectivity is achieved by the addition of chloramphenicol.

II PERFORMANCE TEST PROCEDURE

1. Liquefy Sabouraud Dextrose Agar Deeps in A tubes by boiling in a water bath.* Cool to 45–50 °C and pour into Petri dishes and allow to firm up for at least 30 min.

   ***NOTE:** Use of a microwave oven is not recommended.
2. Inoculate representative samples with the cultures listed below.
   a. Streak the agar surface with a 0.01 mL calibrated loop using fungal broth cultures (up to 7 days in age). For *Escherichia coli*, inoculate one loopful using an 18- to 24-h **Trypticase™** Soy Broth culture diluted 10^{-1}.
   b. Incubate test containers at 25–30 °C in an aerobic atmosphere. Caps should be loosened on the tubed and bottled media.
   c. Include Sabouraud Dextrose Agar slants as nonselective controls when Sabouraud Dextrose Agar with Chloramphenicol media are being tested.

      NOTE: Work with *A. brasiliensis* (ATCC® 16404) in a biological safety cabinet.
3. Examine containers for up to 7 days for growth and pigmentation and for selectivity in the medium containing chloramphenicol.
4. Expected Results

For Sabouraud Dextrose Agar

CLSI Organisms	ATCC	Recovery
**Candida albicans*	60193	Growth at 72 h
**Trichophyton mentagrophytes*	9533	Growth at 72 h

For Sabouraud Dextrose Agar with Chloramphenicol

Organisms	ATCC	Recovery
**Aspergillus brasiliensis*	16404	Growth
**Candida albicans*	10231	Growth
**Trichophyton mentagrophytes*	9533	Growth
**Escherichia coli*	25922	Inhibition (partial to complete)

*Recommended organism strain for User Quality Control.

III ADDITIONAL QUALITY CONTROL

1. Examine tubes or bottles as described under “Product Deterioration.”
2. Visually examine representative tubes or bottles 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 5.6 ± 0.2.
4. Incubate uninoculated representative tubes or bottles at 20–25 °C and 30–35 °C and examine after 7 days for microbial contamination.

PRODUCT INFORMATION

IV INTENDED USE

Sabouraud Dextrose Agar is used in qualitative procedures for cultivation of dermatophytes. The medium is rendered more selective for fungi by the addition of chloramphenicol.

V SUMMARY AND EXPLANATION

Sabouraud Dextrose Agar is a general purpose medium devised by Sabouraud for the cultivation of dermatophytes.[1] The low pH of approximately 5.6 is favorable for the growth of fungi, especially dermatophytes, and slightly inhibitory to contaminating bacteria in clinical specimens.[2-4] The addition of chloramphenicol is a modification designed to increase bacterial inhibition and enable the isolation from contaminated specimens of opportunistic fungi that cause clinical infections resembling dermatophytosis but are sensitive to the cycloheximide included in some selective fungal media.[3,4]

VI PRINCIPLES OF THE PROCEDURE

Sabouraud Dextrose Agar is a peptone medium supplemented with dextrose to support the growth of fungi. The peptones are sources of nitrogenous growth factors. Dextrose provides an energy source for the growth of microorganisms. Chloramphenicol is a broad-spectrum antibiotic which is inhibitory to a wide range of gram-negative and gram-positive bacteria when it is added to the formulation.[5]

VII REAGENTS

Sabouraud Dextrose Agar

Approximate Formula* Per Liter Purified Water

Pancreatic Digest of Casein	5.0 g
Peptic Digest of Animal Tissue	5.0 g
Dextrose	40.0 g
Agar	15.0 g

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

Sabouraud Dextrose Agar with Chloramphenicol contains 0.05 g of chloramphenicol in addition to the ingredients listed above.

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"[6-9] 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 2–8 °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 including up to 6 weeks for mycology media. 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.[10,11] Specimens should be obtained before antimicrobial agents have been administered. Provision must be made for prompt delivery to the laboratory.

IX PROCEDURE

Material Provided: Sabouraud Dextrose Agar or Sabouraud Dextrose Agar with Chloramphenicol

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

Test Procedure: Observe aseptic techniques.

Liquefy the agar contained in A tubes by boiling in a water bath*, cool to 45–50 °C and pour into Petri dishes. Allow to solidify for at least 30 min.

With plates and bottles, streak the specimen as soon as possible after it is received in the laboratory, using a sterile inoculating loop to obtain isolated colonies. Consult appropriate references for information about the processing and inoculation of specimens.[3,4]

Prepared tubed slants primarily are intended for use with pure cultures for maintenance or other purposes.

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 selective medium should be inoculated along with the nonselective medium. Incubate the containers at 25–30 °C with increased humidity. All cultures should be examined at least weekly for fungal growth and should be held for 4 – 6 weeks before being reported as negative.

***NOTE:** Use of a microwave oven is not recommended.

User Quality Control: See "Quality Control Procedures."

Each lot of media has been tested using appropriate quality control organisms and this testing meets product specifications and CLSI standards, where relevant. As always, QC testing should be performed in accordance with applicable local, state, federal or country regulations, accreditation requirements, and/or your laboratory's standard quality control procedures.

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 containers should show isolated colonies in streaked areas and confluent growth in areas of heavy inoculation.

Transfer of growth from slants to plated media may be required in order to obtain pure cultures of fungi.

Examine containers for fungal colonies exhibiting typical microscopic and colonial morphology.[4,12] Biochemical tests and serological procedures should be performed to confirm findings.

XI LIMITATIONS OF THE PROCEDURE

Some fungi (e.g., *Blastomyces dermatitidis*) may not be recovered on this medium due to the high carbohydrate content.[13]

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.[10,11]

XII PERFORMANCE CHARACTERISTICS

Sabouraud Dextrose Agar

Prior to release, all lots of Sabouraud Dextrose Agar containers are tested for performance characteristics. Using a 0.01 mL calibrated loop, representative samples of the lot are streak-inoculated with fresh fungal broth cultures of *Trichophyton mentagrophytes* (ATCC 9533) and *Candida albicans* (ATCC 60193). The containers are read for growth and colony pigmentation after 2, 5 and 7 days incubation at 25–30 °C. *C. albicans* demonstrates fair to heavy growth with white to cream colonies. *T. mentagrophytes* demonstrates fair to heavy growth with white to cream to tan colonies.

Sabouraud Dextrose Agar with Chloramphenicol

Prior to release, all lots of Sabouraud Dextrose Agar with Chloramphenicol containers are tested for performance characteristics. Using a 0.01 mL calibrated loop, representative samples of the lot are streak-inoculated with fresh fungal broth cultures of *Trichophyton mentagrophytes* (ATCC 9533), *Candida albicans* (ATCC 60193), *Aspergillus brasiliensis* (ATCC 16404) and a **Trypticase** Soy Broth culture of *Escherichia coli* (ATCC 25922). The containers are read for growth and colony pigmentation after 2, 5 and 7 days incubation at 25–30 °C. *C. albicans* demonstrates fair to heavy growth with white to cream colonies. *T. mentagrophytes* demonstrates fair to heavy growth with white colonies. *A. brasiliensis* exhibits fair to heavy growth with brown to black colonies. Growth of *E. coli* is either light or completely inhibited.

XIII AVAILABILITY

Cat. No.	Description
221012	**BD BBL™** Sabouraud Dextrose Agar Slants, Pkg. of 10 size A tubes
221013	**BD BBL™** Sabouraud Dextrose Agar Slants, Ctn. of 100 size A tubes
296182	**BD BBL™** Sabouraud Dextrose Agar Deeps (Pour Tubes), 20 mL, Ctn. of 100 size A tubes
221136	**BD BBL™** Sabouraud Dextrose Agar, **Mycoflask™** Bottles, Pkg. of 10
221825	**BD BBL™** Sabouraud Dextrose Agar with Chloramphenicol Slants, Ctn. of 100 size A tubes
221314	**BD BBL™** Sabouraud Dextrose Agar with Chloramphenicol, **Mycoflask™** Bottles, Pkg. of 10

XIV REFERENCES

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