QUALITY CONTROL PROCEDURES (Optional)

I INTRODUCTION
Sabouraud Dextrose Agar with Chloramphenicol and Sabouraud Dextrose Agar with Chloramphenicol and Gentamicin are used in qualitative procedures for cultivation of dermatophytes. The addition of antimicrobial agents renders these media more selective than Sabouraud Dextrose Agar.

II PERFORMANCE TEST PROCEDURE

A. Sabouraud Dextrose Agar with Chloramphenicol
1. Inoculate representative samples with the cultures listed below.
   a. Streak the plates for isolation using fresh fungal broth cultures (up to one month in age) of the fungi and a 10⁻¹ dilution of an 18- to 24-h BD Trypticase™ Soy Broth culture of the Escherichia strain.
   b. Incubate plates at 25 ± 2 °C in an aerobic atmosphere.
   c. Include Sabouraud Dextrose Agar plates as nonselective controls for all strains.
2. Examine plates at intervals up to 7 days for amount of growth, pigmentation and selectivity.
3. Expected Results

<table>
<thead>
<tr>
<th>Organisms</th>
<th>ATCC®</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>*Candida albicans</td>
<td>10231</td>
<td>Growth</td>
</tr>
<tr>
<td>*Trichophyton mentagrophytes</td>
<td>9533</td>
<td>Growth</td>
</tr>
<tr>
<td>*Escherichia coli</td>
<td>25922</td>
<td>Inhibition (complete)</td>
</tr>
<tr>
<td>Aspergillus brasiliensis</td>
<td>16404</td>
<td>Fair to heavy growth</td>
</tr>
<tr>
<td>Blastomyces dermatitidis</td>
<td>56218</td>
<td>Fair to heavy growth. Colonies are white and cottony.</td>
</tr>
<tr>
<td>Microsporum audouinii</td>
<td>9079</td>
<td>Fair to heavy growth. Colonies are slow-growing, cottony, white to tan surface with white to brown under-surface.</td>
</tr>
<tr>
<td>Penicillium roquefortii</td>
<td>9295</td>
<td>Moderate to heavy growth. Early colonies (by 3 days) are powdery and white. Older colonies (by 5 to 7 days) are green to blue.</td>
</tr>
</tbody>
</table>

*Recommended organism strain for User Quality Control.

B. Sabouraud Dextrose Agar with Chloramphenicol and Gentamicin
1. Inoculate representative samples with the cultures listed below.
   a. For *E. coli*, streak inoculate 1 µL (0.001 mL) from a 4 – 5 h culture of BD Trypticase™ Soy Broth diluted to yield 10⁶ – 10⁷ CFU/mL.
   b. For fungal organisms, inoculate directly from stock plate using fresh fungal cultures (up to one month in age).
   c. Incubate at 25 ± 2 °C in an aerobic atmosphere.
   d. Include plates of a previously tested lot of TSA with 5% Sheep Blood as controls for *E. coli*; use plates of a previously tested lot of SDA as controls for remaining organisms.
2. Examine plates at intervals up to 7 days for amount of growth, and selectivity.
3. Expected Results

<table>
<thead>
<tr>
<th>CLSI Organisms</th>
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<tr>
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</tr>
<tr>
<td>*Escherichia coli</td>
<td>25922</td>
<td>Inhibition (partial to complete)</td>
</tr>
</tbody>
</table>

*Recommended organism strain for User Quality Control.

NOTE: This medium is exempt from User QC testing according to CLSI M22-A3.

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 5.6 ± 0.2.
4. Note the firmness of plates during the inoculation procedure.
5. For Sabouraud Dextrose Agar with Chloramphenicol incubate uninoculated representative plates at 25 ± 2 °C for 72 h and examine for microbial contamination. For Sabouraud Dextrose Agar with Chloramphenicol and Gentamicin incubate uninoculated plates at 35 ± 2 °C and 25 ± 2 °C for 72 h and examine for microbial contamination.

PRODUCT INFORMATION

IV INTENDED USE
These media are used in qualitative procedures for cultivation of dermatophytes. 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. 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. The addition of antibiotics is a modification designed to increase bacterial inhibition and improve the isolation from contaminated specimens of opportunistic fungi that cause clinical infections resembling dermatophytosis but are sensitive to cycloheximide.

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. Gentamicin is an aminoglycoside antibiotic that inhibits the growth of gram-negative bacteria.

VII REAGENTS

**Sabouraud Dextrose Agar with Chloramphenicol**

Approximate Formula * Per Liter Purified Water

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pancreatic Digest of Casein</td>
<td>5.0 g</td>
</tr>
<tr>
<td>Peptic Digest of Animal Tissue</td>
<td>5.0 g</td>
</tr>
<tr>
<td>Dextrose</td>
<td>40.0 g</td>
</tr>
<tr>
<td>Agar</td>
<td>15.0 g</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>0.05 g</td>
</tr>
</tbody>
</table>

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

**Sabouraud Dextrose Agar with Chloramphenicol and Gentamicin**

Approximate Formula * Per Liter Purified Water

<table>
<thead>
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<td>15.0 g</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>0.05 g</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>0.05 g</td>
</tr>
</tbody>
</table>

*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 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" 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. 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 Dextrose Agar with Chloramphenicol (Deep Fill) or Sabouraud Dextrose Agar with Chloramphenicol and Gentamicin (Deep Fill)

**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 using a sterile inoculating loop to obtain isolated colonies. Consult appropriate references for information about the processing and inoculation of specimens.

For isolation from contaminated specimens of opportunistic fungi that cause clinical infections resembling dermatophytosis but are sensitive to cycloheximide.

**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.

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 these media.³
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.¹⁰-¹⁴
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

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>221851</td>
<td>BD BBL™ Sabouraud Dextrose Agar with Chloramphenicol (Deep Fill), Pkg. of 20 plates</td>
</tr>
<tr>
<td>296359</td>
<td>BD BBL™ Sabouraud Dextrose Agar with Chloramphenicol and Gentamicin (Deep Fill), Pkg. of 20 plates</td>
</tr>
</tbody>
</table>

XIII REFERENCES

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

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Sparks, MD 21152 USA

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