BBL™ Dermatophyte Test Medium (DTM), Modified with Chloramphenicol

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QUALITY CONTROL PROCEDURES

I INTRODUCTION

Dermatophyte Test Medium (DTM), Modified with Chloramphenicol is a selective and differential medium used for the detection and presumptive identification of dermatophytes from clinical and veterinary specimens.

II PERFORMANCE TEST PROCEDURE

1. Inoculate representative samples with the cultures listed below.
   a. For *E. coli* and *P. aeruginosa*, streak inoculate 1 µL (0.001 mL) from a 4 – 5 h culture of **Trypticase™** Soy Broth diluted to yield 10^6 – 10^7 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* and *P. aeruginosa*; use plates of a previously tested lot of Sabouraud Dextrose Agar as controls for remaining organisms.
2. Examine plates at intervals up to 7 days for growth and selectivity.
3. Expected Results

Organisms	ATCC®	Recovery	Medium Coloration
**Aspergillus brasiliensis*	16404	Inhibition (partial to complete)	N/A
Escherichia coli	25922	Inhibition (partial to complete)	N/A
**Microsporum audouinii*	9079	Moderate to heavy growth	Pink to red
**Pseudomonas aeruginosa*	10145	Inhibition (partial to complete)	N/A
**Trichophyton mentagrophytes*	9533	Moderate to heavy growth	Pink to red

*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 5.5 ± 0.2.
4. Note the firmness of the agar beds during the inoculation procedure.
5. Incubate uninoculated representative plates at 33 – 37 °C and 20 – 25 °C for 72 h and examine for microbial contamination.

PRODUCT INFORMATION

IV INTENDED USE

Dermatophyte Test Medium (DTM) is a selective and differential medium used for the detection and presumptive identification of dermatophytes from clinical specimens. Because of the unavailability of one of the inhibitory agents, chlortetracycline, Dermatophyte Test Medium (DTM), Modified with Chloramphenicol is recommended as a substitute for the original DTM formulation.

V SUMMARY AND EXPLANATION

Dermatophytes cause cutaneous fungal infections of the hair, skin and nails generally referred to as tinea or ringworm.[1-3] Members of the genera *Trichophyton*, *Microsporum* and *Epidermophyton* are the most common etiologic agents of these infections.

Taplin et al. developed DTM as a screening medium for the selective isolation and detection of dermatophytes from clinicalspecimens.[4] A combination of three antimicrobial agents (cycloheximide, chlortetracycline and gentamicin) inhibited bacteria and saprophytic yeasts and molds. Lack of availability of chlortetracycline in late 1992 resulted in the substitution of chloramphenicol for chlortetracycline.

Dermatophytes are presumptively identified based on gross morphology and the production of alkaline metabolites, which raise the pH and cause the phenol red indicator to change the color of the medium from yellow to pink to red.[2-4] Taplin et al. reported the medium (with chlortetracycline) to be 97 to 100% accurate for identifying dermatophytes.[4]

VI PRINCIPLES OF THE PROCEDURE

Papaic digest of soybean meal provides the amino acids and other nitrogenous substances necessary to support fungal growth. Dextrose is a source of energy. Phenol red, a colorimetric indicator, is included to visualize the rise in pH of the medium.

Antimicrobial agents improve the recovery of dermatophytes by inhibiting bacteria and saprophytic fungi. Cycloheximide is an antifungal agent active against saprophytic yeasts and molds, but inactive against dermatophytes. Chloramphenicol is a broad-spectrum antibiotic that inhibits a wide range of gram-positive and gram-negative bacteria. Gentamicin is an aminoglycoside antibiotic that inhibits gram-negative and some gram-positive bacteria, including staphylococci.

VII REAGENTS

Dermatophyte Test Medium, Modified with Chloramphenicol

Approximate Formula* Per Liter Purified Water

Papaic Digest of Soybean Meal	10.0 g	Cycloheximide	0.5 g
Dextrose	10.0 g	Gentamicin	0.1 g
Agar	24.0 g	Chloramphenicol	0.1 g
Phenol Red	0.2 g		

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

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

Tubes and bottles 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"[5-8] and institutional guidelines should be followed in handling all items contaminated with blood and other body fluids. Prior to discarding, sterilize prepared plates, bottles, specimen containers and other contaminated materials by autoclaving.

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 recommended incubation times. Allow the medium to warm to room temperature before inoculation.

Product Deterioration: Do not use medium if it shows evidence of microbial contamination, discoloration, drying or other signs of deterioration.

VIII SPECIMEN COLLECTION AND HANDLING

Refer to appropriate texts for details of specimen collection and handling procedures.[1-3,9]

IX PROCEDURE

Material Provided: Dermatophyte Test Medium (DTM), Modified with Chloramphenicol

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

Test Procedure: Observe aseptic techniques.

Inoculate the medium as soon as possible after the specimen arrives at the laboratory. Place the specimen in the center of the agar surface and press it lightly into the agar to ensure firm contact with the medium.

Replace the cap loosely to allow air to circulate and incubate at 22 – 25 °C for up to 14 days.

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

Dermatophytes produce typical morphology and a pink to red color in the medium around the colony within 10 – 14 days of incubation. Disregard color changes after the fourteenth day of incubation because they may be caused by contaminating fungi.[4]

XI LIMITATIONS OF THE PROCEDURE

A single medium is rarely adequate for detecting all organisms of potential significance in a specimen. The agents in selective media may inhibit some strains of the desired species or permit growth of a species they were designed to inhibit, especially if the species is present in large numbers in the specimen. Specimens cultured on selective media should, therefore, also be cultured on nonselective media to obtain additional information and help ensure recovery of potential pathogens.

For identification, organisms must be in pure culture. Morphological, biochemical and/or serological tests should be performed for final identification. Consult appropriate texts for information.[1-3,9-11]

XII PERFORMANCE CHARACTERISTICS

Prior to release, all lots of Dermatophyte Test Medium (DTM) Modified with Chloramphenicol are tested for performance characteristics. Representative samples of the lot are inoculated directly with fresh cultures of *Trichophyton mentagrophytes* ATCC 9533, *Microsporum audouinii* ATCC 9079 and *Aspergillus brasiliensi*s ATCC 16404, grown on **BBL** Sabouraud Dextrose Agar plates. Containers are incubated at 20 – 27 °C for up to seven days in an aerobic atmosphere. Moderate to heavy growth and pink/red color development in the medium is observed with *T. mentagrophytes* and *M. audouinii*. *A. brasiliensi*s is partially to completely inhibited.

XIII AVAILABILITY

Cat. No.	Description
299701	**BD BBL™** Dermatophyte Test Medium, Modified with Chloramphenicol Slants, Pkg. of 10 Size C Tubes

XIV REFERENCES

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