the test is invalid; i.e., either improperly performed or the test organisms can degrade the peptone sufficiently to produce an alkaline reaction in the absence of a specific amino acid.

The medium becomes purple to violet if the reaction is positive (alkaline). A yellow color indicates a negative test; i.e., the organism does not produce the appropriate enzyme.

Limitations of the Procedure
1. If isolated or received on a selective medium, the organism should be subcultured to Trypticase® Soy Agar with 5% Sheep Blood or other suitable culture medium before attempting to determine decarboxylase or dihydrolase activity.

2. Biochemical characteristics of the Enterobacteriaceae serve to confirm presumptive identification based on cultural, morphological, and/or serological findings. Therefore, biochemical testing should be attempted on pure culture isolates only and subsequent to differential determinations.

3. The decarboxylase reactions are part of a total biochemical profile for members of the Enterobacteriaceae and related organisms. Results obtained from these reactions, therefore, can be considered presumptively indicative of a given genus or species. However, conclusive and final identification of these organisms cannot be made solely on the basis of the decarboxylase reactions.

4. If layers of yellow and purple appear after incubation, shake the test tube gently before attempting to interpret results.

5. If a reaction is difficult to interpret, compare the tube in question to an uninoculated control tube. Any trace of purple after 24 hours of incubation is a positive test.

6. A gray color may indicate reduction of the indicator. Additional indicator may be added before the results are interpreted.

7. Salmonella gallinarum gives a delayed positive ornithine decarboxylase reaction, requiring 5-6 days incubation. Many strains of E. coli, including those that ferment adonitol, may exhibit a delayed reaction.

8. Decarboxylase Medium Base is not satisfactory for the determination of lysine decarboxylase activity with the two genera Klebsiella and Enterobacter.

9. The lysine decarboxylase activity in Salmonella is used to differentiate this group from Citrobacter freundii. Salmonella paratyphi A, however, gives an atypical negative reaction (yellow color of medium) in 24 hours when Decarboxylase Medium Base is used.

References

Availability
Difco® Decarboxylase Base Moeller

<table>
<thead>
<tr>
<th>SMWW</th>
<th>USDA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cat. No. 289020</td>
<td>Dehydrated – 500 g</td>
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</table>

BBL® Moeller Decarboxylase Broth Base and Moeller Decarboxylase Broth with Amino Acids

<table>
<thead>
<tr>
<th>SMWW</th>
<th>USDA</th>
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<tbody>
<tr>
<td>Cat. No. 211430</td>
<td>Dehydrated – 500 g*</td>
</tr>
<tr>
<td>221731</td>
<td>Prepared Tubes, 5 mL – Pkg. of 10*</td>
</tr>
<tr>
<td>221659</td>
<td>Prepared Tubes with Arginine, 5 mL – Pkg. of 10*</td>
</tr>
<tr>
<td>221660</td>
<td>Prepared Tubes with Arginine, 5 mL – Ctn. of 100*</td>
</tr>
<tr>
<td>221661</td>
<td>Prepared Tubes with Lysine, 5 mL – Pkg. of 10*</td>
</tr>
<tr>
<td>221662</td>
<td>Prepared Tubes with Lysine, 5 mL – Ctn. of 100*</td>
</tr>
<tr>
<td>221663</td>
<td>Prepared Tubes with Ornithine, 5 mL – Pkg. of 10*</td>
</tr>
<tr>
<td>221664</td>
<td>Prepared Tubes with Ornithine, 5 mL – Ctn. of 100*</td>
</tr>
</tbody>
</table>

Difco® Decarboxylase Medium Base

<table>
<thead>
<tr>
<th>BAM</th>
<th>COMPE</th>
<th>SMD</th>
<th>SMWW</th>
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</thead>
<tbody>
<tr>
<td>Cat. No. 287220</td>
<td>Dehydrated – 500 g</td>
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Difco® Lysine Decarboxylase Broth

<table>
<thead>
<tr>
<th>BAM</th>
<th>COMPE</th>
<th>SMD</th>
<th>SMWW</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cat. No. 211759</td>
<td>Dehydrated – 500 g</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Pasco® Mineral Oil

| Cat. No. 266631 | Bottle – 60 mL |

*Store at 2-8°C.

Demi-Fraser Broth Base
Fraser Broth Supplement

Intended Use
Demi-Fraser Broth Base is used with Fraser Broth Supplement in selectively and differentially enriching Listeria from foods.

Summary and Explanation
Fraser Broth Base and Fraser Broth Supplement are based on the Fraser Broth formulation of Fraser and Sperber. The medium is used in the rapid detection of Listeria from food and environmental samples. Demi-Fraser Broth Base is a modification of Fraser Broth Base in which the nalidixic acid and acriflavine concentrations have been reduced to 10 mg/L and 12.5 mg/L respectively.
Principles of the Procedure
Peptone, beef extract and yeast extract provide carbon and nitrogen sources and the cofactors required for good growth of *Listeria*. Sodium phosphate and potassium phosphate buffer the medium. Selectivity is provided by lithium chloride, nalidixic acid and acriflavine. The high sodium chloride concentration of the medium inhibits growth of enterococci.

All *Listeria* species hydrolyze esculin, as evidenced by a blackening of the medium. This blackening results from the formation of 6,7-dihydroxycoumarin, which reacts with ferric ions.¹ Ferric ions are added to the final medium as ferric ammonium citrate in Fraser Broth Supplement.

**Formulae**

**Difco™ Demi-Fraser Broth Base**

Approximate Formula* Per Liter

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tryptose</td>
<td>10.0 g</td>
</tr>
<tr>
<td>Beef Extract</td>
<td>5.0 g</td>
</tr>
<tr>
<td>Yeast Extract</td>
<td>5.0 g</td>
</tr>
<tr>
<td>Sodium Chloride</td>
<td>20.0 g</td>
</tr>
<tr>
<td>Disodium Phosphate</td>
<td>9.6 g</td>
</tr>
<tr>
<td>Monopotassium Phosphate</td>
<td>1.35 g</td>
</tr>
<tr>
<td>Esculin</td>
<td>1.0 g</td>
</tr>
<tr>
<td>Nalidixic Acid</td>
<td>0.01 g</td>
</tr>
<tr>
<td>Acriflavine HCl</td>
<td>12.5 mg</td>
</tr>
<tr>
<td>Lithium Chloride</td>
<td>3.0 g</td>
</tr>
</tbody>
</table>

**Difco™ Fraser Broth Supplement**

Formula Per 10 mL Vial

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ferric Ammonium Citrate</td>
<td>0.5 g</td>
</tr>
</tbody>
</table>

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

Directions for Preparation from Dehydrated Product

1. Dissolve 55 g of the powder in 1 L of purified water. Mix thoroughly.
2. Autoclave at 121°C for 15 minutes. Cool to 45-50°C.
3. Aseptically add 10 mL of Fraser Broth Supplement. Mix well.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure²

1. Pre-enrich the sample in Demi-Fraser Broth. Incubate for 18-24 hours at 35 ± 2°C. Subculture onto Oxford Medium or PALCAM Medium.
2. Transfer 0.1 mL of the pre-enrichment culture into 10 mL of Fraser Broth and incubate for 48 hours at 37°C. Subculture onto Oxford Medium or PALCAM Medium after 18-24 hours and again after 42-48 hours of incubation.
3. Examine Oxford Medium or PALCAM Medium plates for the appearance of presumptive *Listeria* colonies.
4. Confirm the identity of all presumptive *Listeria* by biochemical and/or serological testing.

Expected Results

The presence of *Listeria* is presumptively indicated by the blackening of Demi-Fraser Broth after incubation for 24-48 hours at 35°C. Confirmation of the presence of *Listeria* is made

User Quality Control

**Identity Specifications**

**Difco™ Demi-Fraser Broth Base**

Dehydrated Appearance: Beige, free-flowing, homogeneous.

Solution: 5.5% solution, soluble in purified water. Solution is medium amber, clear to slightly opalescent, may have a fine precipitate.

Prepared Appearance: Medium amber, very slightly to slightly opalescent, may have a fine precipitate.

Reaction of 5.5% Solution at 25°C: pH 7.2 ± 0.2

**Difco™ Fraser Broth Supplement**

Solution Appearance: Dark brown solution.

**Cultural Response**

**Difco™ Demi-Fraser Broth Base**

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 24-48 hours.

<table>
<thead>
<tr>
<th>ORGANISM</th>
<th>ATCC*</th>
<th>INOCULUM CFU</th>
<th>RECOVERY/ APPEARANCE</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Enterococcus faecalis</em></td>
<td>29212</td>
<td>10^1-2 x 10^5</td>
<td>Partial to complete inhibition</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>25922</td>
<td>10^1-2 x 10^5</td>
<td>Inhibition</td>
</tr>
<tr>
<td><em>Listeria monocytogenes</em></td>
<td>19114</td>
<td>10^1-10^1</td>
<td>Good/blackening of the medium</td>
</tr>
<tr>
<td><em>Listeria monocytogenes</em></td>
<td>19115</td>
<td>10^1-10^1</td>
<td>Good/blackening of the medium</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>25923</td>
<td>10^1-2 x 10^5</td>
<td>Inhibition</td>
</tr>
</tbody>
</table>

Uninoculated Tube  
Listeria monocytogenes ATCC™ 19114
following subculture onto appropriate media and biochemical/serological identification.

References

Availability
Difco™ Demi-Fraser Broth Base
Cat. No. 265320 Dehydrated – 500 g
265310 Dehydrated – 10 kg

Difco™ Fraser Broth Supplement
Cat. No. 211742 Tube – 6 x 10 mL*
*Store at 2-8°C.

Dermatophyte Test Medium Base • Dermatophyte Test Medium, Modified with Chloramphenicol

Intended Use
Dermatophyte Test Medium (DTM) is a selective and differential medium used for the detection and presumptive identification of dermatophytes from clinical and veterinary specimens.1 Because of the unavailability of one of the inhibitory agents, chlorotetracycline, Dermatophyte Test Medium (DTM), Modified with Chloramphenicol is recommended as a substitute for the original DTM formation.

Summary and Explanation
Dermatophytes cause cutaneous fungal infections of the hair, skin and nails generally referred to as tinea or ringworm.2-4 Members of the genera Trichophyton, Microsporum and Epidermophyton are the most common etiologic agents of these infections.

User Quality Control

Identity Specifications
BBL™ Dermatophyte Test Medium Base
Dehydrated Appearance: Fine, homogeneous, free of extraneous material.
Solution: 4.05% solution, soluble in purified water upon boiling. Solution is light to medium, yellow orange, clear to slightly hazy.
Prepared Appearance: Light to medium, yellow orange, clear to slightly hazy.
Reaction of 4.05% Solution at 25°C: pH 5.5 ± 0.2

Cultural Response
BBL™ Dermatophyte Test Medium Base
Prepare the medium per label directions with added gentamicin sulfate-chloramphenicol solution. Inoculate with fresh cultures and incubate at 25 ± 2°C for 7 days.

<table>
<thead>
<tr>
<th>ORGANISM</th>
<th>ATCC**</th>
<th>RECOVERY</th>
<th>REACTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspergillus niger</td>
<td>16404</td>
<td>Partial</td>
<td>–</td>
</tr>
<tr>
<td>Microsporum audouinii</td>
<td>9079</td>
<td>Fair to good</td>
<td>Alkaline (red)</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>10145</td>
<td>Partial to complete inhibition</td>
<td>–</td>
</tr>
<tr>
<td>Trichophyton mentagrophytes</td>
<td>9533</td>
<td>Fair to good</td>
<td>Alkaline (red)</td>
</tr>
</tbody>
</table>

Taplin et al. developed DTM as a screening medium for the selective isolation and detection of dermatophytes from clinical specimens.2 A combination of three antimicrobial agents (cycloheximide, chlorotetracycline and gentamicin) inhibited bacteria and saprophytic yeasts and molds. Lack of availability of chlorotetracycline in late 1992 resulted in the substitution of chloramphenicol for chlorotetracycline.

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.3,5 Taplin et al. reported the medium (with chlorotetracycline) to be 97 to 100% accurate for identifying dermatophytes.5

Principles of the Procedure
The soy peptone provides nitrogenous and carbonaceous substances essential for microbial growth. Dextrose is a source of energy for metabolism. The pH indicator, phenol red, is used to detect acid production.

Cycloheximide inhibits most saprophytic molds. The additives, gentamicin and chloramphenicol, aid in the selectivity of the medium. Gentamicin inhibits gram-negative bacteria including Pseudomonas species. Chloramphenicol is a broad-spectrum antibiotic that inhibits a wide range of gram-positive and gram-negative bacteria.

Formula

BBL™ Dermatophyte Test Medium Base
Approximate Formula* Per Liter
Papaic Digest of Soybean Meal ................................ 10.0 g
Dextrose .................................................................... 10.0 g
Phenol Red ............................................................. 0.2 g
Cycloheximide ......................................................... 0.5 g
Agar ........................................................................ 20.0 g

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

Directions for Preparation from Dehydrated Product
1. Suspend 40.5 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.