QUALITY CONTROL PROCEDURES

I INTRODUCTION
Haemophilus Isolation Agar with Bacitracin is a primary plating medium used for the selective isolation of Haemophilus species.

II PERFORMANCE TEST PROCEDURE
1. Inoculate representative samples with the cultures listed below.
   a. For S. pyogenes and N. lactamica streak inoculate 0.01 mL of a dilution containing 10^3 – 10^4 CFUs.
   b. For Haemophilus strains, inoculate 30 – 300 CFU/0.001 mL.
   c. Incubate at 35 ± 2 °C under appropriate atmospheric conditions.
   d. Include plates of a previously tested lot of Chocolate II Agar as controls for inhibited strains.
2. Examine plates at 18 – 48 h for growth and selectivity.
3. Expected Results

<table>
<thead>
<tr>
<th>Organisms</th>
<th>ATCC™</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemophilus influenzae</td>
<td>10211</td>
<td>Moderate to heavy growth</td>
</tr>
<tr>
<td>Haemophilus parainfluenza</td>
<td>51505</td>
<td>Fair to heavy growth</td>
</tr>
<tr>
<td>Neisseria lactamica</td>
<td>23970</td>
<td>Inhibition (partial to complete)</td>
</tr>
<tr>
<td>Streptococcus pyogenes</td>
<td>19615</td>
<td>Inhibition (partial to complete)</td>
</tr>
</tbody>
</table>

1Recommended 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 7.3 ± 0.3.
4. Note the firmness of plates during the inoculation procedure.
5. Incubate uninoculated representative plates at 33 – 37 °C for 72 h and examine for microbial contamination.

PRODUCT INFORMATION

IV INTENDED USE
Haemophilus Isolation Agar with Bacitracin is a primary plating medium used for the selective isolation of Haemophilus species.

V SUMMARY AND EXPLANATION
Members of the genus Haemophilus are fastidious microorganisms that require the addition of the growth factors hemin (X factor) and/or nicotinamide adenine dinucleotide (NAD or V factor). To enable the cultivation of Haemophilus species, Haemophilus Isolation Agar is formulated with Flidex Enrichment and IsoViteX™ Enrichment to supply the essential X and/or V growth factors. Horse blood provides appropriate hemolytic reactions to facilitate the differentiation of Haemophilus species.

The antimicrobial agent bacitracin is incorporated to inhibit the growth of bacteria that could mask the presence of Haemophilus species. Bacitracin is frequently utilized in enriched media as a selective agent to increase the recovery of Haemophilus species from specimens collected from the upper respiratory tract.

VI PRINCIPLES OF THE PROCEDURE
Haemophilus Isolation Agar with Bacitracin consists of Brain Heart Infusion Agar supplemented with Flidex Enrichment, IsoViteX™ Enrichment and horse blood. Flidex Enrichment is a peptic digest of sheep blood that supplies both X and V factors. IsoViteX™ Enrichment is a chemically-defined supplement that provides V factor and other nutrients, such as thiamine and cysteine, to stimulate the growth of Haemophilus species. The horse blood supplements additional nutrients and enables the detection of hemolytic reactions, which aid in the differentiation and identification of Haemophilus species. The polypeptide antibiotic bacitracin is incorporated into this medium to inhibit normal flora, including gram-positive bacteria, such as streptococci, and most species of Neisseria.

VII REAGENTS
Haemophilus Isolation Agar with Bacitracin

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>16.0 g</td>
</tr>
<tr>
<td>Brain Heart, Infusion from (Solids)</td>
<td>8.0 g</td>
</tr>
<tr>
<td>Pepic Digest of Animal Tissue</td>
<td>5.0 g</td>
</tr>
<tr>
<td>Dextrose</td>
<td>2.0 g</td>
</tr>
<tr>
<td>Sodium Chloride</td>
<td>5.0 g</td>
</tr>
<tr>
<td>Disodium Phosphate</td>
<td>2.5 g</td>
</tr>
<tr>
<td>Agar</td>
<td>13.5 g</td>
</tr>
<tr>
<td>Flidex Enrichment</td>
<td>20.0 mL</td>
</tr>
<tr>
<td>IsoViteX™ Enrichment</td>
<td>10.0 mL</td>
</tr>
<tr>
<td>Bacitracin</td>
<td>16,500 units</td>
</tr>
<tr>
<td>Horse Blood, defibrinated</td>
<td>5%</td>
</tr>
</tbody>
</table>

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

Approximate Formula* Per Liter Purified Water

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin B12</td>
<td>0.01 g</td>
</tr>
<tr>
<td>L-Glutamine</td>
<td>10.0 g</td>
</tr>
<tr>
<td>Adenine</td>
<td>1.0 g</td>
</tr>
<tr>
<td>Guanine Hydrochloride</td>
<td>0.03 g</td>
</tr>
<tr>
<td>p-Aminobenzoic Acid</td>
<td>0.013 g</td>
</tr>
<tr>
<td>Nicotinamide Adenine Dinucleotide</td>
<td>0.25 g</td>
</tr>
<tr>
<td>Ferric Nitrate</td>
<td>0.02 g</td>
</tr>
<tr>
<td>Thiamine Hydrochloride</td>
<td>0.003 g</td>
</tr>
<tr>
<td>L-Cystine</td>
<td>25.9 g</td>
</tr>
<tr>
<td>Dextrose</td>
<td>100.0 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 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" 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. 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

Refer to appropriate texts for details of specimen collection and handling procedures.¹,¹¹–¹³

Specimens should be obtained before antimicrobial agents have been administered. Provision must be made for prompt delivery to the laboratory.

IX PROCEDURE

Material Provided: Haemophilus Isolation Agar with Bacitracin

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.

Inoculate the medium as soon as possible after the specimen arrives at the laboratory. To culture a specimen from a swab, inoculate the medium by rolling the swab over a third of the agar surface, and streak the remainder to obtain isolated colonies. Material not being cultured from swabs may be streaked onto the medium with a sterilized inoculating loop. The streak plate technique is used primarily to obtain isolated colonies from specimens containing mixed flora.

Incubate the plates in an inverted position (agar side up) at 35 °C in a moist CO₂-enriched atmosphere for 24 – 48 h to obtain satisfactory growth of H. influenzae and most other Haemophilus species. H. aegyptius requires a longer incubation period, 2 – 4 days. H. ducreyi may require up to 9 days of incubation, preferably at a temperature of 33 °C.¹

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.

Haemophilus influenzae produces pale gray, smooth, glistening and slightly convex colonies. Gram staining, biochemical tests and serological procedures should be performed to confirm findings.

XI LIMITATIONS OF THE PROCEDURE

This prepared plated medium is intended for primary isolation. Some diagnostic tests may be performed with the primary plate. However, a pure culture is recommended for biochemical tests and serological procedures. Consult appropriate texts for further information.¹,¹²

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. 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
295914     BBL™ Haemophilus Isolation Agar with Bacitracin, Pkg. of 20 plates
XIII REFERENCES


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