HAEMOPHILUS ISOLATION AGAR
(w/ Bacitracin and Horse Blood)

INTENDED USE
Remel Haemophilus Isolation Agar (w/ Bacitracin and Horse Blood) is a solid medium recommended for the use in qualitative procedures for the selective isolation of Haemophilus species from respiratory specimens.

SUMMARY AND EXPLANATION
Haemophilus influenzae, a fastidious gram-negative bacillus, is a common cause of serious diseases of the upper and lower respiratory tract. Microbiological diagnosis is frequently delayed by an inability to recover the organism on primary-plated media due to the overgrowth of commensal microbial flora. Klein and Blazevic evaluated a selective medium containing horse blood and bacitracin to determine its usefulness in recovering Haemophilus spp. from respiratory tract specimens. Horse blood and rabbit blood do not contain V factor-inactivating enzymes present in sheep blood and the use of a medium containing intact erythrocytes enables differentiation of hemolytic and nonhemolytic Haemophilus spp. The results of their study demonstrated that Haemophilus spp. were isolated more frequently on the enriched medium, selective or nonselective, which contained horse blood rather than rabbit blood.

PRINCIPLE
Beef Heart infusion and casein peptone supply the nutritional requirements for all but the very fastidious bacteria. Horse blood supplies both the X and V factors which are growth requirements for certain organisms, such as Haemophilus influenzae. Horse blood also serves to distinguish the hemolytic species of Haemophilus from those which are nonhemolytic. Bacitracin inhibits normal flora found in respiratory cultures, particularly Neisseria spp.

REAGENTS (CLASSICAL FORMULA)*

<table>
<thead>
<tr>
<th>Casein Peptone</th>
<th>Sodium Chloride</th>
<th>Yeast Extract</th>
<th>Beef Heart Infusion</th>
<th>Bacitracin</th>
<th>Horse Blood</th>
<th>Agar</th>
<th>Demineralized Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>13.0 g</td>
<td>5.0 g</td>
<td>5.0 g</td>
<td>2.0 g</td>
<td></td>
<td></td>
<td></td>
<td>1000.0 ml</td>
</tr>
</tbody>
</table>

pH 7.4 ± 0.2 @ 25°C

*Adjusted as required to meet performance standards.

PROCEDURE
1. Inoculate and streak the specimen as soon as possible after it is received in the laboratory.
2. If a swab specimen is received, roll the swab over a small area of the agar surface and streak for isolation.
3. Incubate in 5-10% CO₂ for 24-48 hours at 35-37°C.
4. Examine for typical colonial morphology and hemolysis following established laboratory procedures.

QUALITY CONTROL
All lot numbers of Haemophilus Isolation Agar (w/ Bacitracin and Horse Blood) have been tested using the following quality control organisms and have been found to be acceptable. Testing of control organisms should be performed in accordance with established laboratory quality control procedures. If aberrant quality control results are noted, patient results should not be reported.

CONTROL

<table>
<thead>
<tr>
<th>Control</th>
<th>ATCC® Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemophilus influenzae</td>
<td>10211</td>
</tr>
<tr>
<td>Haemophilus parahaemolyticus</td>
<td>10014</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>12299</td>
</tr>
<tr>
<td>Streptococcus sanguinis</td>
<td>10556</td>
</tr>
</tbody>
</table>

INCUBATION

<table>
<thead>
<tr>
<th>Control</th>
<th>CO₂</th>
<th>Aerobic</th>
<th>Growth</th>
<th>Beta hemolysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>10211</td>
<td>24-48 h @ 35-37°C</td>
<td>18-24 h @ 35-37°C</td>
<td>Growth</td>
<td>Inhibition (partial to complete)</td>
</tr>
</tbody>
</table>

RESULTS


LIMITATIONS

BIBLIOGRAPHY

Refer to the front of Remel Technical Manual of Microbiological Media for General Information regarding precautions, product storage and deterioration, specimen collection, storage and transportation, materials required, quality control, and limitations.

ATCC® is a registered trademark of American Type Culture Collection.