Escherichia coli and Pseudomonas.) Approximate quantitation of the number of colonies of hemolytic streptococci may be helpful to the clinician.

2. Pneumococci usually appear as very flat, smooth, translucent, grayish and sometimes mucoid colonies surrounded by a narrow zone of “green” (alpha) hemolysis.

3. Staphylococci appear as opaque, white to gold-yellow colonies with or without zones of beta hemolysis.

4. Listeria may be distinguished by their rod shape in stains, and by motility at room temperature. Small zones of beta hemolysis are produced.

5. Other organisms representing minimal flora and clinically significant isolates can also be expected to grow on this nonselective formulation.

Limitation of the Procedure
Colones of Haemophilus haemolyticus are beta-hemolytic on horse and rabbit blood agar and must be distinguished from colonies of beta-hemolytic streptococci using other criteria.6

The use of sheep blood has been suggested to obviate this problem since sheep blood is deficient in pyridine nucleotides and does not support growth of H. haemolyticus.1

References

Availability
BBL™ Blood Agar Base (Infusion Agar)
Cat. No. 211037 Dehydrated – 500 g
Cat. No. 211038 Dehydrated – 5 lb (2.3 kg)

Difco™ Blood Agar Base No. 2
Cat. No. 269620 Dehydrated – 500 g

Intended Use
Bordet Gengou Agar Base, with the addition of glycerol and sterile blood, is used in qualitative procedures for the detection and isolation of Bordetella pertussis from clinical specimens.

Summary and Explanation
Bordet Gengou Blood Agar is used in clinical laboratories as a method of diagnosing whooping cough. Bordetella pertussis, the etiologic agent of this disease, may be isolated from aspirated bronchial or nasopharyngeal secretions, perinasal swabs or, perhaps with greater difficulty due to the diversity of flora, from throat swabs.1

Bordet and Gengou introduced the medium in 1906 as a method of maintaining stock cultures.2 In 1934, Kendrick and Eldering replaced the 50% human or rabbit blood recommended in the original formulation with 15% sheep blood to make the medium more practical for laboratories to produce for routine clinical procedures.3

User Quality Control

<table>
<thead>
<tr>
<th>Identity Specifications</th>
<th>Difco™ Bordet Gengou Agar Base</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dehydrated Appearance:</td>
<td>Beige, free-flowing, homogeneous.</td>
</tr>
<tr>
<td>Solution:</td>
<td>3.0% solution, soluble upon boiling in purified water containing 1% glycerol. Solution is light to medium amber, opalescent, may have a slight precipitate.</td>
</tr>
<tr>
<td>Prepared Appearance:</td>
<td>Plain – Light to medium amber, opalescent, may have a precipitate. With 15% blood – Cherry red, opaque.</td>
</tr>
<tr>
<td>Reaction of 3.0% Solution at 25°C:</td>
<td>pH 6.7 ± 0.2</td>
</tr>
</tbody>
</table>

Cultural Response
Difco™ Bordet Gengou Agar Base
Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 48-72 hours.

<table>
<thead>
<tr>
<th>ORGANISM</th>
<th>ATCC®</th>
<th>INOCULUM CFU</th>
<th>RECOVERY WITH 15% RABBIT BLOOD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bordetella bronchiseptica</td>
<td>4617</td>
<td>30-300</td>
<td>Good</td>
</tr>
<tr>
<td>Bordetella parapertussis</td>
<td>15311</td>
<td>30-300</td>
<td>Good</td>
</tr>
<tr>
<td>Bordetella pertussis</td>
<td>8467</td>
<td>30-300</td>
<td>Good</td>
</tr>
</tbody>
</table>
Principles of the Procedure

Bordet Gengou Blood Agar contains potato infusion and glycerol to supply the nutrients necessary to support the growth of *B. pertussis*. Defibrinated animal blood supplies additional nutrients and enables the detection of hemolytic reactions, which aid in the identification of *B. pertussis*.

Formula

**Difco™ Bordet Gengou Agar Base**

Approximate Formula* Per Liter
- Potato, Infusion from 125 g ............................... 4.5 g
- Sodium Chloride ........................................................ 5.5 g
- Agar .........................................................................20.0 g

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

Directions for Preparation from Dehydrated Product

1. Suspend 30 g of the powder in 1 L of purified water containing 10 g of glycerol. 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.
4. Aseptically add 15% sterile, defibrinated blood to the medium at 45-50°C. Mix well.
5. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Use standard procedures to obtain isolated colonies from specimens. Incubate plates in an inverted position (agar side up) in a moist chamber at 35 ± 2°C for 7 days. Examine the plates daily with and without a dissecting microscope (oblique illumination) to detect the presence of *Bordetella pertussis* and spreading colonies or molds that could mask the presence of this species. Use a sterile scalpel or needle to remove the portions of the agar that contain spreading colonies or molds. Colonies of *B. pertussis* may not be visible without the aid of a microscope for 2-4 days. Plates may be discarded as negative after incubation for 7 days.

Expected Results

*Bordetella pertussis* produces small, domed, glistening colonies that resemble bisected pearls. The colonies are usually surrounded by a zone of hemolysis; however, some strains of *B. pertussis* are not hemolytic. Gram stains, biochemical tests and serological procedures should be performed to confirm findings.

Limitation of the Procedure

Some *Haemophilus* spp. will grow on *Bordetella* isolation media and cross-react with *B. pertussis* antisera. It may be prudent to rule out X and V factor dependence.

References


Availability

**Difco™ Bordet Gengou Agar Base**

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>248200</td>
<td>Dehydrated – 500 g</td>
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**Difco™ Glycerol**

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>Description</th>
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<tbody>
<tr>
<td>228210</td>
<td>Bottle – 100 g</td>
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<tr>
<td>228220</td>
<td>Bottle – 500 g</td>
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</tbody>
</table>

**BBL™ Bordet Gengou Blood Agar**

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>Description</th>
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</thead>
<tbody>
<tr>
<td>297876</td>
<td>Prepared Plates with Glycerol and 15% Sheep Blood – Pkg. of 10*</td>
</tr>
</tbody>
</table>

*Store at 2-8°C.

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**Bovine Albumin 5%**

Intended Use

Bovine Albumin 5% is used to enrich media for cultivating a large variety of microorganisms and tissue cells. Bovine albumin is also known as bovine serum albumin or BSA.¹

Summary and Explanation

Davis and Dubos² recommended the use of bovine albumin at a final concentration of 0.5% in liquid media for culturing *Mycobacterium tuberculosis*. In this study, bovine albumin neutralized the toxicity of fatty acids and permitted more luxuriant growth of *M. tuberculosis*.

Ellinghausen and McCullough³ used bovine albumin fraction V at a final concentration of 1% in liquid, semisolid and solid media for culturing leptospires. Morton et al.⁴ demonstrated that 1% bovine albumin stimulated growth of *Mycoplasma* (PPLO).

Bovine Albumin can be added to normally sterile specimens, tissues and body fluids for direct inoculation onto culture media used for isolating mycobacteria. BSA is also used as an enrichment when contaminated specimens are digested.

Bovine Albumin 5%, modified with added sodium chloride and dextrose, is available as Dubos Medium Albumin.

Principles of the Procedure

Bovine Albumin 5% is a filter sterilized solution of bovine albumin fraction V. BSA is suggested as a culture media enrichment because its buffering capacity and detoxifying effect on specimen sediment.¹ Bovine Albumin 5% also increases adhesion of the specimen to solid media.¹