BD™ Bordet Gengou Agar with 15% Sheep Blood •
BD Bordetella Agar with Charcoal and 7% Horse Blood

INTENDED USE
BD Bordet Gengou Agar with 15% Sheep Blood and BD Bordetella Agar with Charcoal and 7% Horse Blood are selective media for the isolation of Bordetella pertussis and B. parapertussis.

PRINCIPLES AND EXPLANATION OF THE PROCEDURE
Microbiological method.
All Bordetella species are respiratory pathogens, residing in ciliated epithelial cells of the respiratory tract. B. pertussis and B. parapertussis are uniquely human pathogens. B. pertussis is the major cause of whooping cough. B. parapertussis is associated with a milder form of this disease. B. bronchiseptica is primarily an animal pathogen, but may produce bronchitis, pneumonia and extra-respiratory tract infections in humans.

Bordet Gengou Agar Base is a modification of the medium originally described by Bordet and Gengou for the cultivation of Bordetella pertussis. BD Bordet Gengou Agar with 15% Sheep Blood is prepared according to the modified formula recommended by the American Public Health Association. In this medium, infusion from potato provides nitrogen and vitamins. Many compounds may inhibit growth of B. pertussis, including fatty acids present in nasal secretions or cotton from the collection swab. Starch, present from the potato infusion, absorbs fatty acids. Glycerol is a carbon source. Sodium chloride maintains the osmotic balance of the medium. The addition of 15% sheep blood provides essential complex growth factors.

BD Bordetella Agar with Charcoal and 7% Horse Blood is based on the formula of Mishulow et al. who found it to be an efficient substitute for Bordet-Gengou Agar. Later, this medium was further developed and became known as Regan-Lowe Charcoal Agar. In this medium, beef extract and gelatin peptone provide nutrients. Starch and charcoal act as adsorbents of toxic fatty acids, radicals and peroxides, replacing the potato infusion in Bordet Gengou Agar. Niacin is a vitamin that promotes growth. Horse blood provides complex growth factors and further reduces the toxic effect of peroxides.

In BD Bordet Gengou Agar with 15% Sheep Blood and BD Bordetella Agar with Charcoal and 7% Horse Blood cefalexin is added as a selective agent to partially suppress normal respiratory tract flora.

REAGENTS
Formulas* Per Liter Purified Water

<table>
<thead>
<tr>
<th>BD Bordet Gengou Agar with 15% Sheep Blood</th>
<th>BD Bordetella Agar with Charcoal and 7% Horse Blood</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potato, Infusion from Solids</td>
<td>4.5 g</td>
</tr>
<tr>
<td>Pancreatic Digest of Casein</td>
<td>5.0</td>
</tr>
<tr>
<td>Peptic Digest of Animal Tissue</td>
<td>5.0</td>
</tr>
<tr>
<td>Sodium Chloride</td>
<td>5.5</td>
</tr>
<tr>
<td>Agar</td>
<td>20.0</td>
</tr>
<tr>
<td>Cefalexin</td>
<td>0.04</td>
</tr>
<tr>
<td>Glycerol</td>
<td>10.0 ml</td>
</tr>
<tr>
<td>Sheep Blood, defibrinated</td>
<td>15%</td>
</tr>
<tr>
<td>pH 7.2 +/- 0.2</td>
<td></td>
</tr>
<tr>
<td>pH 7.4 +/- 0.2</td>
<td></td>
</tr>
</tbody>
</table>

*Adjusted and/or supplemented as required to meet performance criteria.
PRECAUTIONS
For professional use only.
Do not use plates if they show evidence of microbial contamination, discoloration, drying, cracking or other signs of deterioration.
Consult GENERAL INSTRUCTIONS FOR USE document for aseptic handling procedures, biohazards, and disposal of used product.

STORAGE AND SHELF LIFE
On receipt, store plates in the dark at 2 to 8° C, in their original sleeve wrapping until just prior to use. Avoid freezing and overheating. The plates may be inoculated up to the expiration date (see package label) and incubated for the recommended incubation times.
Plates from opened stacks of 10 plates can be used for one week when stored in a clean area at 2 to 8° C.

USER QUALITY CONTROL
Inoculate representative samples with the following strains (for details, see GENERAL INSTRUCTIONS FOR USE document). Incubate aerobically in a moist chamber for 5 to 7 days at 35 to 37° C. Do not incubate in an aerobic atmosphere enriched with carbon dioxide.

<table>
<thead>
<tr>
<th>Strains</th>
<th>BD Bordet Gengou Agar with 15% Sheep Blood</th>
<th>BD Bordetella Agar + Charcoal + 7% Horse Blood</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Bordetella pertussis</em></td>
<td>Growth fair to excellent</td>
<td>Growth good to excellent</td>
</tr>
<tr>
<td>ATCC™ 9797</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Bordetella parapertussis</em></td>
<td>Growth fair to excellent</td>
<td>Growth good to excellent</td>
</tr>
<tr>
<td>ATCC 15311</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Streptococcus pneumoniae</em></td>
<td>Inhibition partial to complete</td>
<td>Inhibition partial to complete</td>
</tr>
<tr>
<td>ATCC 6305</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Streptococcus pyogenes</em></td>
<td>Inhibition partial to complete</td>
<td>Inhibition partial to complete</td>
</tr>
<tr>
<td>ATCC 19615</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uninoculated</td>
<td>Dark red with brownish hue, opaque</td>
<td>Shiny black with a reddish hue, opaque</td>
</tr>
</tbody>
</table>

PROCEDURE
Materials Provided
*BD Bordet Gengou Agar with 15% Sheep Blood* or *BD Bordetella Agar with Charcoal and 7% Horse Blood*, both provided in 90 mm Stacker™ plates. Microbiologically controlled.

Materials Not Provided
Ancillary culture media, reagents and laboratory equipment as required.

Specimen Collection and Specimen Types
The “cough plate” method for the diagnosis of whooping cough is no longer recommended.
Secretions from the posterior nasopharynx, nasopharyngeal lavage, or a nasopharyngeal swab (calcium alginate on a wire handle) should be collected within the first week of paroxysmal coughing. Do not use cotton swabs! The collection of secretions by vacuum suction is recommended. Specimens from the throat should not be used. Special transport media, based on charcoal, have been described. It is mandatory that the specimens are transported to the laboratory as fast as possible, even if transport media are used. Transport times of > 24 hours will reduce the viability of *Bordetella* significantly. For details, consult the references.

Test Procedure
Inoculate the plates as soon as possible after the arrival of the specimens in the laboratory and streak for dilution. Incubate the plates in ambient air in a moist chamber (e.g., a BD GasPak™ jar containing some water in a beaker) for 7 to 10 days, and inspect daily, using a magnifying lens. Do not incubate in an aerobic atmosphere enriched with carbon dioxide since the gas
may suppress the organism. The optimal incubation temperature is 34 to 36° C. Higher temperature may reduce the growth of *Bordetella*.

**Results**

After sufficient incubation, *B. pertussis* colonies on **BD Bordet Gengou Agar with 15% Sheep Blood** and on **BD Bordetella Agar with Charcoal and 7% Horse Blood** will be small, white, domed and glistening, resembling bisected pearls. On **BD Bordet Gengou Agar with 15% Sheep Blood**, colonies of *B. pertussis* and *B. parapertussis* may be surrounded by a weak beta-hemolysis.

**PERFORMANCE CHARACTERISTICS AND LIMITATIONS OF THE PROCEDURE**

**BD Bordet Gengou Agar with 15% Sheep Blood** and **BD Bordetella Agar with Charcoal and 7% Horse Blood** are approved selective isolation media for *Bordetella* species, including *B. pertussis* and *B. parapertussis*. Most recent data support the use of the charcoal based medium Cefalexin is included into these media as an inhibitor of many Gram positive and certain Gram negative bacteria present in the normal throat flora, but is not completely inhibitory to all organisms. *Bordetella bronchiseptica* may grow on these media. It is an opportunistic pathogen which may also produce respiratory infections. Further tests are necessary for the complete identification of *Bordetella pertussis* and *B. parapertussis*. The direct fluorescent antibody (DFA) test which can also be used along with culture for the direct detection of the agents from the specimens, can also be used for confirmation of the isolates obtained on these media.

**REFERENCES**


**PACKAGING/AVAILABILITY**

**BD Bordet Gengou Agar with 15% Sheep Blood**

Cat. No. 254400 Ready-to-use Plated Media, cpu 20
BD Bordetella Agar with Charcoal and 7 % Horse Blood
Cat. No. 256054 Ready-to-use Plated Media, cpu 20

FURTHER INFORMATION
For further information please contact your local BD representative.

BD Diagnostic Systems
Tullastrasse 8 – 12
D-69126 Heidelberg/Germany
Phone: +49-62 21-30 50 Fax: +49-62 21-30 52 16
Reception_Germany@europe.bd.com

BD Diagnostic Systems Europe
Becton Dickinson France SA
11 rue Aristide Bergès
38800 Le Pont de Claix/France
Tel: +33-476 68 3636 Fax: +33-476 68 3292 http://www.bd.com

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