INTENDED USE
Remel Regan-Lowe Semisolid Transport Medium is recommended for use in qualitative procedures for the selective isolation of *Bordetella pertussis* and *Bordetella parapertussis*.

SUMMARY AND EXPLANATION
The first recorded appearance of whooping cough was an outbreak of the disease in Paris, France in 1578.1 The causative agents of whooping cough, *B. pertussis* and *B. parapertussis*, are among the most fastidious bacteria known and require special culturing techniques.

Ploom showed that nicotinic acid was an essential growth factor for the *Bordetellae*.2 Ensminger, Culbertson, and Powell used a charcoal medium for the growth of *B. pertussis* in vaccine production.3 Mishulow, Sharpe, and Cohen found that charcoal agar could replace the Bordet Gengou "cough plate technique" for *B. pertussis* cultivation.4 Sutcliffe and Abbott added 40 µg/ml of cephalexin to suppress unwanted nasopharyngeal flora and compared its inhibitory effect with that of penicillin.5 Stauffer, Brown, and Sandstrom compared growth of *Bordetella* with added cephalexin to that with penicillin and methicillin.6 Both groups of researchers found that media supplemented with cephalexin was superior to media supplemented with penicillin and/or methicillin for the selective recovery of *B. pertussis*. Regan and Lowe demonstrated that charcoal agar supplemented with 40 µg/ml cephalexin and 10% defibrinated horse blood was an excellent enrichment and transport medium.7 A study by Hoppe reported that horse blood was superior to sheep blood or human blood for recovery of *Bordetella* spp. and incubation in ambient air results in more luxuriant growth than incubation in 5-10% CO₂.8 As a transport medium, Remel Regan-Lowe Semisolid Transport Medium is superior to Jones-Kendrick Transport Medium which has a similar composition but is blood-free.9

PRINCIPLE
This medium consists of a charcoal agar base with 10% lysed horse blood which provides an enriched medium. Charcoal and starch absorb toxic fatty acids and peroxides that are inhibitory to *B. pertussis*. Nicotinic acid is added as an essential nutrient for *Bordetella*. Horse blood is both a growth factor for *B. pertussis* and a neutralizer of inhibiting substances.10 The addition of cephalexin, which is inhibitory to penicillin-resistant staphylococci, and some coliforms, as well as to strains of *Haemophilus influenzae*, allows for the selective isolation of *Bordetella* from respiratory specimens. Amphotericin B inhibits yeasts and fungi.

REAGENTS (CLASSICAL FORMULA)*

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beef Extract</td>
<td>5.0 g</td>
</tr>
<tr>
<td>Peptone</td>
<td>5.0 g</td>
</tr>
<tr>
<td>Starch</td>
<td>5.0 g</td>
</tr>
<tr>
<td>Sodium Chloride</td>
<td>2.5 g</td>
</tr>
<tr>
<td>Charcoal</td>
<td>2.0 g</td>
</tr>
<tr>
<td>Amphotericin B</td>
<td>50.0 mg</td>
</tr>
<tr>
<td>Cephalexin</td>
<td>40.0 mg</td>
</tr>
<tr>
<td>Nicotinic Acid</td>
<td>0.05 mg</td>
</tr>
<tr>
<td>Lysed Horse Blood</td>
<td>100.0 ml</td>
</tr>
<tr>
<td>Agar</td>
<td>6.0 g</td>
</tr>
<tr>
<td>Demineralized Water</td>
<td>1000.0 m</td>
</tr>
</tbody>
</table>

pH 7.4 +/- 0.2 @ 25°C

*Adjusted as required to meet performance standards.

PROCEDURE
All suspected cases of pertussis should have a nasopharyngeal (NP) aspirate and/or NP swab obtained for culture. Specimens should be collected from the posterior nasopharynx up to 4 weeks after onset of symptoms. NP aspirates are the preferred method of specimen collection because they have a higher recovery rate of *Bordetella* spp. than NP swabs. If swabs are used, only calcium alginate or polyester swabs are acceptable. Cotton swabs may contain inhibitors that decrease isolation rates. Consult appropriate references for detailed specimen collection guidelines.10-12

Optimally, media should be directly inoculated at the time of specimen collection. If a delay cannot be avoided, the specimen should be placed in a transport system, such as Regan-Lowe Semisolid Transport Medium (REF R064141). The CDC Pertussis Laboratory recommends shipping clinical specimens for recovery of *Bordetella* spp. at 4°C. Incubation of transport medium prior to shipment to the laboratory has been shown to result in overgrowth of other flora and decreased yield of *B. pertussis*.11

1. Inoculate specimen onto plated media, such as Charcoal Blood Agar (REF R01298), as soon as possible after receipt in the laboratory and streak for isolation. Both selective and nonselective media should be inoculated.
2. Incubate plates in ambient air at 35-36°C for at least 7 days; incubation up to 12 days may increase yield.10
   **Note:** *B. pertussis* will not grow at 37°C; incubation at 35°C is optimal.10 The CDC Pertussis Laboratory recommends incubation at 35-36°C.11
3. To avoid drying, place plates in a plastic bag or moist chamber with a sterile moistened filter paper; drying reduces the recovery rate of *B. pertussis*.10
4. If specimen is received in Regan-Lowe Semisolid Transport Medium, it should be incubated along with the plates and subcultured after 48 hours to selective and nonselective media.12
5. Examine plates daily for growth. *Bordetella* colonies are tiny, smooth, transparent, glistening, and domed.
QUALITY CONTROL
All lot numbers of Regan-Low e Semisolid Transport Medium have been tested using the following quality control organism and have been found to be acceptable. This quality control testing conforms with or exceeds CLSI standards. 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
*Bordetella pertussis ATCC® 12742

INCUBATION
Aerobic, 48 h @ Room Temperature

RESULTS
Growth recovered on subculture

*CLSI recommended organism.

LIMITATIONS
1. Specimens collected prior to the administration of antibiotics have higher recovery rates for Bordetella.10,12

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.

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