Appendices
APPENDIX - I

MEDIA, DIAGNOSTIC DISCS AND BIOCHEMICAL REAGENTS

1. **Gentamicin Sheep Blood Agar**
   Blood agar base + 5% defibrinated Sheep blood (citrated) 5.5 ug/ml of Gentamycin added to the autoclaved blood agar base and mixed thoroughly, before adding blood at 50°C aseptically. 20 ml/plate is poured into petridishes.

2. **Mueller-Hinton Sheep Blood Agar**
   Muller Hinton agar (Himedia) autoclaved at 121°C for 15 min. 4% sterile defibrinated sheep blood added following cooling to 50°C. 25 ml of medium is poured/plate.

3. **Chocolate agar**
   Blood agar base + 7% horse blood (citrated). Sterile blood is added to autoclaved blood agar base at 80°C and kept for 15 minutes. Cooled to 50°C and poured 20 ml/plate into petridishes.

4. **Double layered Blood Agar**
   Blood agar base + 6% horse blood agar. Double layered blood agar plate is a two-layered plate containing 10 ml of blood agar base in the bottom of the plate overlayered with 10 ml of blood agar base containing 6% (V/V) horse blood.

5. **Stuarts Transport Medium**
   Sodium thioglycollate 1 g.
   Sodium glycerophosphate 10.0 g.
   Calcium chloride 0.1 g.
   Agar 3 g.
   Demineralised water 1 litre
   Final pH 7.3 ± 0.2

6. **Hi-Sensitest Medium (for H.influenzae & Moraxella)**
   Iso-Sensitse Agar (Oxoid) + 1% Haemoglobin (Oxoid) + 1% Vitox (Isovitalex) Oxoid. Autoclaved iso-sensitse agar (at 121°C for 15 min) is added to Haemoglobin (autoclaved at 121°C for 15 min) at 56°C, 1% Vitox is added, mixed and poured into petridishes 25 ml/plate.

7. **Iso-Sensitse agar + 2% horse blood (for Pneumo * GABHS).**
   Iso-Sensitse agar is suspended in distilled water for 15 min. It is autoclaved at 121°C for 15 min, cooled, defibrinated blood 2% is added and poured 25 ml/plate into petridishes.

Todd Hewitt Broth: (For Co-A reagent)

- Beef heart infusion 3.1 gm
- Neopeptone 20 gm
- Dextrose 2.0 gm
- NaCl 2.0 gm
- Sodium carbonate 2.5 gm
- Disodium phosphate 0.4 gm
- Distilled water 1000 ml
- pH 7.8 ± 0.2
Phosphate buffered saline (for Co-A reagent)

Disodium hydrogen phosphate Na₂HPO₄ 0.1M (A).
Sodium dihydrogen phosphate NaH₂PO₄ 0.1M (B).

To 250 ml of A, 50 ml of solution B is added pH adjusted to 7.35 and volume made up to 1 litre in distilled water. 7 gm of NaCl and 1 gm sodium azide added and autoclaved at 15 lbs for 15 min. Final pH adjusted to 7.35.

Antibiotic Powders for MIC:

Chloramphenicol RM 218
Ampicillin RM 645 | Hi media
Cefotaxime RM 1193

Sulphamethoxazole 95125 IP/BP |
Trimethoprim E72683 IP/BP | Burroughs Wellcome India Ltd.

Ciprofloxacin (Oxoid) Ranbaxy Laboratories

Bacitracin disc (Oxoid) 0.02 unit
Optochin disc (Oxoid)
X-factor disc (Oxoid)
V-factor disc (Oxoid)
X-V factor disc (Oxoid)

Nucleate disc: (for 100 discs of 10 mm diameter)
Nucleate powder (Fluka) 2 gm
Distilled water 10 ml
5 M Hcl 3 drops

Nucleate powder is suspended in distilled water. With the help of a Pasteur pipette, the discs are moistened with the suspension (approximately 3 drops per disc) Discs are kept at room temperature for 30 min. They are stored at -20°C.

Biochemical reagents:

Oxidase:

Moistened filter paper with reagent. Used for testing Moraxella catarrhalis.

Nitrocefin solution(Oxoid): Whatman No.1 filter paper is moistened and used immediately for detection of beta-lactamase.

Carbohydrate peptone broth (for conformation of Moraxella)

Test carbohydrates - glucose, sucrose, maltose, lactose.

Peptone 10 gm
Sodium chloride 5 gm
Test carbohydrate 10 gm
Distilled water 1 litre
Andrade indicator 10 ml

Ingredients are dissolved and pH adjusted to 7.4 to 7.5. Autoclaved at 121°C for 15 min. Dispensed into tubes and used.
APPENDIX - II

COMMERCIAL KITS, ANTISERA AND EQUIPMENTS

1. **Visuwell Strep.A11 TF 032**
   ADI Diagnostics Inc.
   Mississauga, Ontario.

2. **ASO TEST**
   Rashmi Diagnostics Pvt. Ltd.
   Bangalore-560078, India.

3. **Directigen 02.1664-7**
   Individual Tests
   Haemophilus influenzae type b
   Becton Dickinson Microbiology Systems
   250 Schilling Circle
   Cockeysville, Maryland 21030 USA.

4. **Haemophilus influenzae Antiserum:**
   BACTO H.influenzae antiserum
   Difco Laboratories
   Detroit, Michigan 48232-7058
   U.S.A.

5. **Streptococcal Grouping Antisera:**
   LAT TEST. Strep Test
   Rashmi Diagnostic Pvt. Ltd.
   Bangalore-560078, India.

6. **Pneumococcal Omniserum:**
   Statens Serum Institute,
   Copenhagen, Denmark.

Swabs:

**Ray Swabs: Type 4**
Rayen tipped
Spectrum Laboratories Inc.
Houston, Texas, USA.

Gas Pak:

**BBL Gas Pak 70304**
Anaerobic System
Becton Dickinson Microbiology Systems
250 Schilling Circle
Cockeysville, Md. 21030 USA.
### PROFORMA - I

**STREPTOCOCCAL SCHOOL SURVEY**

<table>
<thead>
<tr>
<th>Date:</th>
<th>Name:</th>
<th>Case Number:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age:</td>
<td>Sex:</td>
<td>School:</td>
</tr>
<tr>
<td>Father's Name:</td>
<td>Class:</td>
<td>Area:</td>
</tr>
<tr>
<td>Section:</td>
<td>Examined by:</td>
<td></td>
</tr>
</tbody>
</table>

**CLINICAL DATA:**

- Sore throat
- Fever
- Cough
- Nasal Discharge

**DIAGNOSIS:**

- Tonsillitis
- Upper respiratory tract infection (Pharyngitis)
- Lower respiratory tract infection

**Recommended sample collection:**

- 1. Throat swab:
  - Filter paper:
  - Direct inoculation:
- 2. Throat swab for direct antigen detection:

**LABORATORY FINDINGS:**

<table>
<thead>
<tr>
<th>Throat swab culture:</th>
<th>1st day:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2nd day:</td>
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<tr>
<td></td>
<td>Identification:</td>
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<table>
<thead>
<tr>
<th>Antibiotic sensitivity:</th>
<th>Zone diameter</th>
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<tbody>
<tr>
<td>Erythromycin:</td>
<td>MIC</td>
</tr>
<tr>
<td>Tetracycline:</td>
<td></td>
</tr>
<tr>
<td>Cotrimoxazole:</td>
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</tr>
<tr>
<td>Clindamycin:</td>
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</table>

<table>
<thead>
<tr>
<th>Antigen detection:</th>
<th>ASLO titre:</th>
</tr>
</thead>
</table>
Date:  
Place:  
Urban □ Rural □

COLLECTED BY:

NAME OF PATIENT:  
AGE:  
SEX:  

CLINICAL DATA:

SYMPTOMS:  
Sore throat □  Fever □  Cough □  Nasal discharge □

DIAGNOSIS:  
Tonsillitis
Upper respiratory tract infection
Lower respiratory tract infection

RECOMMENDED
SAMPLE COLLECTION:  
Throat swab
Nasopharyngeal swab

ANTIBIOTICS
DURING LAST WEEK  
Yes □ No □

ORGANISM:  
H.influenzae
S.pneumoniae
M.catarrhalis
GABHS

ANTIBIOTIC SENSITIVITY

Penicillin
Erythromycin
Chloramphenicol
Cotrimoxazole
Ampicillin
Cefotaxime
PROFORMA - III
BACTERIAL STUDY OF ALRI IN HOSPITALISED CHILDREN
CASE HISTORY

<table>
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<tr>
<th>Name:</th>
<th>Study No.:</th>
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<tbody>
<tr>
<td>Hospital No.:</td>
<td>Date of Birth:</td>
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<tr>
<td>Mother's Name:</td>
<td>Date of discharge:</td>
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<tr>
<td>Father's Name:</td>
<td></td>
</tr>
<tr>
<td>Date of admission:</td>
<td></td>
</tr>
<tr>
<td>Duration of Hospital stay(days):</td>
<td></td>
</tr>
<tr>
<td>History of previous antibiotics:</td>
<td>Yes/No</td>
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1. **SYMPTOM** | Duration(days) |
<table>
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<tbody>
<tr>
<td>Cough</td>
<td>Y N</td>
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<tr>
<td>Fever</td>
<td>Y N</td>
</tr>
<tr>
<td>Rapid breathing</td>
<td>Y N</td>
</tr>
<tr>
<td>Runny nose</td>
<td>Y N</td>
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<tr>
<td>Decreased appetite</td>
<td>Y N</td>
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<tr>
<td>Vomiting</td>
<td>Y N</td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>Y N</td>
</tr>
<tr>
<td>Red eyes</td>
<td>Y N</td>
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LABORATORY RESULTS

Bacteriology:

A. Bacteria isolated from

1. Blood
   Tick (√) the box
   Serotype
   1 Haemophilus influenzae
   2 Streptococcus pneumoniae
   3 Staphylococcus aureus
   4 Enterobacteriaeae
   5 Pseudomonas aeruginosa

B. Antigen detection

in [ ] serum:
   [1] Directigent
   [2] Hib
   [3] Pnc

ANTIBIOTIC SENSITIVITY ORGANISM(1):

<table>
<thead>
<tr>
<th></th>
<th>ZONE DIAMETER</th>
<th>MIC(ug/ml)</th>
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<tbody>
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<td>Penicillin</td>
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<tr>
<td>Erythromycin</td>
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<td>Chloramphenicol</td>
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<td>Cotrimoxazole</td>
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<tr>
<td>Ampicillin</td>
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<td></td>
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<tr>
<td>Cefotaxime</td>
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ANTIBIOTIC SENSITIVITY ORGANISM (2):

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<td>Cefotaxime</td>
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### SPECIMEN COLLECTION RECORD

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<th>Date &amp; Time collected</th>
<th>Collected by</th>
<th>Date &amp; Time received in Lab</th>
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<tbody>
<tr>
<td>Blood culture</td>
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<td>Nasopharyngeal/Throat swab</td>
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<tr>
<td>Urine</td>
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<tr>
<td>Lung aspirate</td>
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<td>Blood for Antigen detection</td>
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#### LABORATORY RESULTS

**Bacteriology:**

A. Bacteria isolated from

1. Blood

Serotype
INDEX CASE PHYSICAL FINDINGS

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<th>Day Post-Admission</th>
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<td>Pulse per minute/H.R.</td>
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<td>Temperature*</td>
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<td>Resp. rate**</td>
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<td>Chest indrawing (Y/N)</td>
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* Highest temperature of the day
** Highest rate recorded