Respiratory Cultures – Sputum Culture Setups

CLS 418/CLS 419
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Selection of Culture Media

• Sputum is cultured for the isolation of common respiratory infection pathogens
• Selection of media based on suspected pathogens (common etiologic agents)
  – Primary pathogens
  – Possible pathogens

Common Etiologic Agents of Respiratory Infections

<table>
<thead>
<tr>
<th>Pathogens</th>
<th>Media to use</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strep. pneumoniae</td>
<td>5% Sheep blood agar (BAP)</td>
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<tr>
<td>Strep. pyogenes (Grp A)</td>
<td></td>
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<tr>
<td>Staphylococcus aureus</td>
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<tr>
<td>Other beta-Strep.</td>
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<tr>
<td>Moraxella catarrhalis</td>
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<tr>
<td>Haemophilus influenzae</td>
<td>Chocolate agar (CHOC)</td>
</tr>
</tbody>
</table>

Common Etiologic Agents of Respiratory Infections

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<th>Pathogens</th>
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<tbody>
<tr>
<td>Enterics</td>
<td>MacConkey (MAC), or EMB agar</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td></td>
</tr>
<tr>
<td>Bordetella pertussis</td>
<td>Specialty media (special orders given to culture for these pathogens)</td>
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<tr>
<td>Mycobacterium tuberculosis</td>
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<tr>
<td>Legionella pneumophila</td>
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</tbody>
</table>

Selection of Culture Media

• Most laboratories will routinely setup BAP, CHOC and MAC
  – Nutrient, enriched and selective media
  – Please refer to procedure manual for specifics on what media your clinical site sets up
• Keep in mind contaminants / commensal flora of the upper respiratory tract will also grow on these media

Why is a chocolate agar plate used to setup a sputum culture?

Some of the common potential pathogens (Neisseria and Haemophilus) will only grow on Chocolate agar and not on the BAP or MAC.
**Evaluation of Specimen**

- Use a swab to prepare direct smear using representative areas of the specimen
  - Sample areas that are purulent or bloody
  - Avoid areas that look like saliva
  - Roll swab onto a slide. Allow to air dry.
  - Gram stain and evaluate specimen for adequacy

**Evaluation of Specimen**

- Specimen adequacy (Sputum)
  - CRITICAL step!
  - Must evaluate if specimen is adequate for culture
  - Specimens grossly contaminated with oral respiratory flora are difficult to interpret
  - Specimen from deep cough (limited oral contamination)

**Evaluation of Specimen**

- Specimen adequacy (Sputum)
  - Evaluate slide under low power for squamous epithelial cells
  - Large numbers of squamous epithelial cells indicates oral contamination from collection process

**Evaluation of Specimen**

- Specimen adequacy (Sputum)
  - >25 epithelial cells/LPF indicates oral contamination
    - Contact caregiver and request new specimen
  - <25 epithelial cells/LPF
    - Proceed with culture set up
    - See clinical site procedures for sputum rejection criteria

**Evaluation of Specimen**

- Good sputum sample
  - A good sputum sample with contain many PMN’s (in a person with a normal immune system) and relatively few squamous epithelial cells
- Poorly collected sample
  - A poorly collected sample will contain increased amounts of squamous epithelial cells

**Inoculation of Media**

- Use a swab to inoculate plates using representative areas of the specimen
  - Sample areas that are purulent or bloody
  - Avoid areas that look like saliva
  - Make primary streak with swab
  - Streak for isolation using sterile loop
  - Non inhibitory media (BAP and CHOC) should always be inoculated first
### Inoculation of Media

- **Streak for Isolation**
  - Why not set up a sputum culture with the colony count method?
  - Sputum not a sterile specimen, non-invasive collection techniques
  - High numbers of normal flora organisms present in respiratory tract
  - Under normal conditions, balance of organisms is maintained
  - Culture to identify predominance or large quantity of primary or possible pathogens in relation of normal flora

### Incubation of Media

- **BAP and CHOC**
  - Incubate at 35ºC in a CO2 incubator
  - Full 48 hours
  - First plate read at 24 hours

- **MAC**
  - Incubate at 35ºC in ambient air
  - Full 48 hours
  - First plate read at 24 hours

### Review

- **Select culture media**
  - BAP, CHOC and MAC
  - Common etiologic agents of respiratory infections

- **Inoculation of media**
  - Streak for isolation

- **Incubation of media**
  - Increased CO2

### Reference: