Culture of Throat, Sputum and Other Respiratory Specimens

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Objectives

- List the culture media and incubation conditions used for throat, sputum and other respiratory specimens.
- Discuss which organisms are considered to be pathogens vs. normal flora in throat, sputum and other respiratory specimen cultures.
- Determine when a sputum specimen should be rejected based on the primary gram stain.

Disclosures

- Becton-Dickinson

Respiratory Tract

- Upper tract
- Lower tract
- Off tract

Anatomy of the upper respiratory tract

- Specimens
  - Oropharynx (throat)
  - Nasopharynx
  - Epiglottis
  - Nose
- Endogenous flora
  - Various aerobic and anaerobic organisms including some that are agents of respiratory tract infection

Agents of pharyngitis

- Streptococcus pyogenes
- Beta-streptococcus Group C & Group G
- Arcanobacterium haemolyticum
- (Neisseria gonorrhoeae, Corynebacterium ulcerans, C. diphtheriae, Mycoplasma pneumoniae, Yersinia enterocolitica)
**Streptococcus pyogenes**
- 15-35% of bacterial pharyngitis
- 30% in children
- 5-10% in adults
- Nonsuppurative sequelae
  - Acute rheumatic fever
  - Acute glomerulonephritis
- Suppurative sequelae
  - Peritonsillar abscess

**Direct antigen testing**
- Detects group A antigen in exudate
- Sensitivity varies with kit
- Tend to miss low numbers
- Culture follow up essential for pediatric patients
- Culture follow up at MD’s discretion for adult patients

**Culture methods**

<table>
<thead>
<tr>
<th>Medium</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sheep blood agar</td>
<td>Aerobic</td>
</tr>
<tr>
<td>with stabs</td>
<td></td>
</tr>
<tr>
<td>Sheep blood agar</td>
<td>Anaerobic</td>
</tr>
<tr>
<td>Sheep blood agar</td>
<td>5-10% CO₂ or anaerobic</td>
</tr>
<tr>
<td>with TMP/SMX</td>
<td></td>
</tr>
</tbody>
</table>

Examine for β-hemolytic colonies at 24 and 48 hr.

**Identification**
- Large-colony Group A (*S. pyogenes*) (>0.5 mm)
  - PYR positive (as is *Enterococcus*)
  - Bacitracin susceptibility (0.04U)
  - Sensitivity 99.6%, Specificity 85%, i.e., presumptive
  - Confirm with Group A antigen detection
- Large-colony Group C & G
  - MUG positive (4-methyl-umbelliferyl-β-D-glucuronide)
- Minute colony β-streptococci
  - *Streptococcus anginosus* - not agents of pharyngitis
  - Group A – PYR negative
  - Groups C, G, F, nongroupable (MUG negative)

**Molecular detection**
- Gen-Probe Group A Strep Direct Test
  - 88.6% sens 97.8% spec vs. 72 hr BAP in room air (JCM 32:1440, 1994)
  - 94.8% sens, 100% spec vs. 48 hr BAP anaerobically (Chapin et al, JCM 40:4207, 2002)
  - Also validated with Copan Dacron swabs in transport or dry
  - Probe test can be used without culture backup or to confirm antigen negative tests

**Molecular detection (cont.)**
- Real-time PCR (JCM 41:242, 2003)
  - 63 (16.4%) of 384 positive for GAS
  - 58 detected by PCR, 55 by culture (selective medium in O₂, 48 hr), 31 by Directigen (BD)
  - Can differentiate A, C, and G by melting curve
Arcanobacterium hemolyticum
- Formerly Corynebacterium hemolyticum
- Catalase negative
- Pharyngitis in teens and young adults (10 – 20 y/o)
- Rash (like scarlet fever), no RHD or AGN
- Invasive disease occurs
- May respond poorly to penicillin
- Culture
  - Best: CO₂ 48 hr, ppt, β-hemolytic, black dot in center
  - Anaerobically: slower growth
- If β-hemolytic colonies not A,C or G, Gram stain
  - If gram-positive rods, do API CORYNE

Neisseria gonorrhoeae
- NAAT tests not approved for pharyngeal samples
- Culture on selective media in CO₂

Pertussis
- Bordetella pertussis
- Nasopharyngeal specimen
- PCR>>DFA>culture (direct inoculation)
  - Some PCR detect B. pertussis/parapertussis
  - DFA and culture no longer recommended for diagnosis

Epiglottitis
- Inflammation & edema of epiglottis
- Medical emergency
- Usually due to Haemophilus influenzae type b
  - Blood cultures
  - Swab epiglottis for culture only after artificial airway established
  - Include chocolate agar

Anatomy of the lower respiratory tract
- Pneumonia
  - #1 infectious cause of death
  - #6 overall cause of death
  - Symptoms
    - Fever, chills, cough, chest pain
  - Routes of acquisition
    - Inhaled
    - Upper airway spread
    - Aspiration
    - Hematogenous


Common Agents of Pneumonia
<table>
<thead>
<tr>
<th>Community Acquired</th>
<th>Healthcare Associated</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. pneumoniae</td>
<td>S. aureus</td>
</tr>
<tr>
<td>S. aureus</td>
<td>S. pneumoniae</td>
</tr>
<tr>
<td>H. influenzae</td>
<td>Enterobacteriaceae</td>
</tr>
<tr>
<td>K. pneumoniae</td>
<td>P. aeruginosa</td>
</tr>
<tr>
<td>Mixed anaerobes</td>
<td>Ascinetobacter baumanii</td>
</tr>
<tr>
<td>Legionella</td>
<td>Stenotrophomonas maltophilia</td>
</tr>
<tr>
<td>Mycoplasma, Chlamydia</td>
<td>Other NF gram-neg rods</td>
</tr>
<tr>
<td>BT agents</td>
<td></td>
</tr>
<tr>
<td>Mycobacterium tuberculosis</td>
<td></td>
</tr>
</tbody>
</table>
Specimen Collection - increasing invasiveness
- Expectorated sputum
- Induced sputum
- Suctioned sputum/endotracheal aspirate
- Tracheal aspirate/ventilated patient
- Bronchoalveolar lavage/protected brush
- (Percutaneous biopsy, FNA)
- (Open lung biopsy)
- (Pleural fluid/paracentesis)

Gram stain functions
- Specimen quality
- Quantity and type of WBC
- Morphology and quantity of organisms
- Quality Assurance

Screening for appropriateness: rejection criteria
- Expectorated or induced sputum and endotracheal aspirate
  - If >10 squamous epithelial cells (SEC)/LPF → “Culture request canceled. Culture results on specimens with >10 squamous epithelial cells reflect oral flora and are generally clinically insignificant.”
  - UNLESS WBC > SEC and predominance of a single pathogen → culture and ID/AST only pathogen seen on GS

Contaminated with oral secretions

Quality specimen
- Report the quantity and morphotypes of organisms detected

Culture of quality specimens
- BAP
- EMB/MAC
- Chocolate
- 35°C, 24-48 hr, CO₂
Screening for appropriateness: rejection criteria (cont.)

- Tracheal Aspirates (from ventilated patients)
  - If >10 SEC/LPF
  - UNLESS WBC > SEC and predominance of a single pathogen → culture and ID/AST only
  - OR no organisms seen (other than yeast resembling Candida) → “Culture request canceled. The negative predictive value of a Gram stain with no organisms is 95%.”

No organisms seen

Candida (in blood)

Cryptococcus (in blood)

Blastomyces dermatitidis

Possible aspiration

- <10 SEC, >>25 WBC
- Mixed oral flora including anaerobic morphotypes → “Culture request canceled. Gram stain suggestive of aspiration.”
Summary table

<table>
<thead>
<tr>
<th>SEC</th>
<th>WBC</th>
<th>Organisms</th>
<th>Culture</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;10</td>
<td>NA</td>
<td>None or only candida</td>
<td>No</td>
<td>&quot;Culture request canceled. The negative predictive value of a Gram stain with no organisms is 99%.&quot;</td>
</tr>
<tr>
<td>&lt;10</td>
<td>NA</td>
<td>Bacteria</td>
<td>Yes</td>
<td>ID/AST only pathogen seen on GS</td>
</tr>
<tr>
<td>&lt;10</td>
<td>&gt;25</td>
<td>Mixed flora w/anerobes</td>
<td>No</td>
<td>&quot;Culture request canceled. Gram stain suggestive of aspiration.&quot;</td>
</tr>
<tr>
<td>&gt;10</td>
<td>&gt;SEC</td>
<td>Potential pathogen</td>
<td>Yes</td>
<td>ID/AST only pathogen seen on GS</td>
</tr>
<tr>
<td>&gt;10</td>
<td>SEC</td>
<td>No predominant pathogen</td>
<td>No</td>
<td>&quot;Culture request canceled. Culture results on specimens with &gt;10 squamous epithelial cells reflect oral flora and are usually clinically insignificant.&quot;</td>
</tr>
</tbody>
</table>

What to work up?

Expectorated or induced and <10 SEC/LPF

<table>
<thead>
<tr>
<th>EF</th>
<th>Pathogen</th>
<th>Do</th>
</tr>
</thead>
<tbody>
<tr>
<td>Little or none</td>
<td>&gt;10 colonies</td>
<td>ID/AST</td>
</tr>
<tr>
<td>Little or none</td>
<td>&lt;10 colonies</td>
<td>Include in endogenous flora</td>
</tr>
<tr>
<td>&gt;Moderate</td>
<td>&lt;Moderate</td>
<td>Prelim. ID</td>
</tr>
<tr>
<td>&gt;Moderate</td>
<td>&gt;Moderate</td>
<td>ID/AST</td>
</tr>
</tbody>
</table>

What to work up (Part II)

Expectorated or induced and >10 SEC/LPF

AND

Tracheal aspirates (from ventilated patients) regardless of SEC:

- Work up only the predominant pathogens seen on Gram stain, regardless of the amount of endogenous flora.
- If > moderate pathogen not seen on original Gram stain, review Gram stain.

NOTE: Filamentous fungi, Cryptococcus neoformans, Rhodococcus, Nocardia, Mycobacterium are significant in any amount.

Correlate Gram stain with culture

- If predominant pathogen on GS not recovered ➔
  "Some morphotypes not recovered on culture. Please call Dr. Spiegel at xxx-xxxx for consult if further clarification is needed."

- If morphotype present that we will not recover on routine culture ➔
  Call and suggest add-on cultures.

Protected Brush and BAL

- Procedures
  - Protected brush
  - Bronchoscopic BAL
  - Nonbronchoscopic (blind) BAL
- Advantage
  - Collect specimen directly from lower airway
- Disadvantage
  - Invasive ➔ bleeding possible
- More expensive

Protected Brush

- Place brush in 1 mL PBS or TSB
- Vortex 30-60 sec
- Plate 0.1 mL (100 µL) on each plate (label 10⁻¹)
- Plate 0.01 mL (10 µL) on each plate (label 10⁻²)
- BAP, EMB/MAC, chocolate
- Spread over entire plate for quantitation
- 35°C, 24-48 hr, CO₂
- ≥10³ CFU/mL is considered significant
Bronchoalviolar lavage (BAL)
- Label 2 plates each BAP, EMB/MAC, chocolate
- Vortex 30-60 sec
- Gram stain 1 drop of unspun sample
- Transfer 0.1 mL (100 µL) BAL into 0.9 mL TSB
- Vortex to mix
- Plate 0.01 mL (10 µL) on each plate (label 10⁻³)
- Plate 0.001 mL (1 µL) on each plate (label 10⁻⁴)
- Spread over entirety of all plates for quantitation
- >10⁴ CFU/mL is considered significant
- ID/AST pathogens
- "Mixed oral flora" few = 1x10³, mod = 1x10⁴, and many = 1x10⁵

Nose
- Only for S. aureus or MRSA carriage
- BAP, CNA/PEA
- Chromogenic media for MRSA
- More sensitive and specific than mannitol salt agar w/oxacillin
- Molecular methods
  - MRSA
  - Staph S/R

Sinus Infections
- Acute – H. influenzae, S. pneumoniae > S. pyogenes, Moraxella catarrhalis, zygomycetes
- Chronic – as above plus anaerobes, P. aeruginosa, moulds
- Specimens
  - Sinus drainage or washings not acceptable
  - Needle aspirate or "window" acceptable
- BAP, EMB/MAC, chocolate

Ear Infections
- EF: sparse in external ear
- Otitis externa
  - Acute - S. aureus, S. pyogenes, P. aeruginosa, (Aspergillus)
  - Chronic – P. aeruginosa, anaerobes
- Otitis media
  - Acute – S. pneumoniae, H. influenzae, S. pyogenes
  - Chronic – anaerobes > S. aureus, P. aeruginosa, Proteus spp.
Ear Infections (cont)

- Otitis external
  - Swab
- Otitis media
  - Treatment usually empiric
  - Tympanocentesis
- Media
  - BAP, EMB/MAC, chocolate, anaerobic blood agar, LKV, PEA

Thank you for your attention!