Lower Respiratory Tract Infection
- Clinical Diseases and etiologic agents
- Sample Collection and transport
  - Direct Smear
  (interpretation and Report)
- Culture methods
  (interpretation and Report)
“The culture of lower respiratory specimens may result in more unnecessary microbiologic effort than any other type of specimen.”

Raymond C Bartlett
Lower Respiratory Tract Infections
Epidemiology

• Pneumonia is the sixth leading cause of death in US

• Increasing numbers of patients at risk
  – Aging population
  – Increase in patients with immunocompromising conditions
Lower respiratory infections

• Overtreatment has lead to resistance
  – Multidrug resistant *Streptococcus pneumoniae*
  – Resistance among hospital acquired pathogens such as *Acinetobacter*, *Pseudomonas aeruginosa*, *E.coli*, *K.pneumonia* (ESBLs), MRSA and others
Categories of Lower Respiratory Tract Infections

• Acute bronchitis
• Community acquired pneumonia
• Hospital acquired pneumonia
• Pneumonia in the immunocompromised host
Acute Bronchitis

• Part of or preceded by an URTI
• Sputum is often clear at the onset but may become purulent
• Etiologic agents:
  - adults viral
  - Infant and preschool children: bordetella pertussis
Chronic bronchitis

Common condition affecting about 10-30% adults
Coughing up sputum on most days during at least 3 consecutive months for more than 2 successive years
Viruses are frequent causes
Non encapsulated H.influenzae/S.pneumoniae/M.catharalis
Bronchiolitis

Human metapneumovirus

RSV 40-80% cases
Pneumonia

*Children 2 month to 5 years  80%  viral  (RSV/Parainflu/Adeno/Metapneumovirus/C.pneumoniae/M.pneumoniae)

*Children 5-14 years  70 % viral and up to 80% of cases mixed infection

*Young adults<30 years
M.pneumoniae/Influ/C.pneumoniae
TABLE 1. Most common pathogens implicated in lower respiratory tract syndromes and their relative contributions\textsuperscript{a}

<table>
<thead>
<tr>
<th>Disease and pathogen</th>
<th>% of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Acute bronchitis</strong></td>
<td></td>
</tr>
<tr>
<td>Respiratory viruses\textsuperscript{b}</td>
<td>90</td>
</tr>
<tr>
<td><em>Bordetella pertussis-Bordetella parapertussis</em></td>
<td>5–10\textsuperscript{c}</td>
</tr>
<tr>
<td><em>Mycoplasma pneumoniae</em></td>
<td>5–10\textsuperscript{c}</td>
</tr>
<tr>
<td><em>Chlamydia pneumoniae</em></td>
<td>5–10\textsuperscript{c}</td>
</tr>
<tr>
<td><strong>Community-acquired pneumonia</strong></td>
<td></td>
</tr>
<tr>
<td><em>Streptococcus pneumoniae</em></td>
<td>66</td>
</tr>
<tr>
<td><em>Haemophilus influenzae</em></td>
<td>1–12</td>
</tr>
<tr>
<td><em>Legionella</em> species</td>
<td>2–15</td>
</tr>
<tr>
<td><em>Mycoplasma pneumoniae</em></td>
<td>2–14</td>
</tr>
<tr>
<td><em>Klebsiella</em> species</td>
<td>3–14</td>
</tr>
<tr>
<td>Enteric gram-negative bacilli</td>
<td>6–9</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>3–14</td>
</tr>
<tr>
<td><em>Chlamydia</em> species</td>
<td>5–15</td>
</tr>
<tr>
<td>Influenza virus</td>
<td>5–12</td>
</tr>
<tr>
<td>Hantaviruses</td>
<td>&lt;1–2</td>
</tr>
<tr>
<td>Other viruses</td>
<td>&lt;1–12</td>
</tr>
<tr>
<td><em>Mycobacterium tuberculosis</em></td>
<td>&lt;1–10</td>
</tr>
<tr>
<td><em>Moraxella catarrhalis</em></td>
<td>&lt;1–2</td>
</tr>
<tr>
<td>Unknown</td>
<td>23–49</td>
</tr>
</tbody>
</table>
Hospital Acquired Pneumonia

*5-10 % hospital in – patients develop infection during administration to ICU.

*80% of hospital associated pneumonia is linked with mechanical ventilation (VAP)

*10-30% of critical care patients develop VAP

*VAP is the most common and fatal infection of ICU

*VAP may account for up to 60% of all HAI
<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hospital-acquired pneumonia</td>
<td></td>
</tr>
<tr>
<td><strong>Gram-negative bacilli</strong></td>
<td></td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>16</td>
</tr>
<tr>
<td><em>Enterobacter</em> species</td>
<td>11</td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em></td>
<td>7</td>
</tr>
<tr>
<td>Other enteric gram-negative bacilli</td>
<td>9</td>
</tr>
<tr>
<td><em>Acinetobacter</em></td>
<td>3</td>
</tr>
<tr>
<td><em>Legionella</em> species</td>
<td>0–2</td>
</tr>
<tr>
<td><em>Haemophilus influenzae</em></td>
<td>0–2</td>
</tr>
<tr>
<td>Other</td>
<td>0–10</td>
</tr>
<tr>
<td><strong>Gram-positive cocci</strong></td>
<td></td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>17</td>
</tr>
<tr>
<td><em>Streptococcus pneumoniae</em></td>
<td>2–20</td>
</tr>
<tr>
<td>Other</td>
<td>2–5</td>
</tr>
<tr>
<td>Anaerobes</td>
<td>10–20</td>
</tr>
<tr>
<td>Fungi</td>
<td>0–10</td>
</tr>
<tr>
<td>Mixed</td>
<td>13–54</td>
</tr>
</tbody>
</table>
Organism isolated from tracheal tube aspirates in Milad Hospital

<table>
<thead>
<tr>
<th>organism</th>
<th>No(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>K. pneumoniae</td>
<td>67(20)</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>62(18.5)</td>
</tr>
<tr>
<td>A. baumannii</td>
<td>60(18)</td>
</tr>
<tr>
<td>S. aureus</td>
<td>51(15.5)</td>
</tr>
<tr>
<td>S. marsescense</td>
<td>32(9.5)</td>
</tr>
<tr>
<td>E. coli</td>
<td>22(6.6)</td>
</tr>
<tr>
<td>Enterobacter spp</td>
<td>10(3)</td>
</tr>
<tr>
<td>Candida spp</td>
<td>5(1.5)</td>
</tr>
<tr>
<td>Others</td>
<td>26(7.7)</td>
</tr>
</tbody>
</table>

Rhbar and Hajia accepted for publication in ICHE
Aspiration pneumonia

Anaerob

S.aureus

Enterobacteriaceae

Pseudomonas

H.influenzae

Legionella

Acinetobacter

Moraxella
Chronic LRTI

*M. tuberculosis
*M. avium intracellular
*Actinomyces/Nocardia
*Fungi
Cystic Fibrosis

* Mucoid Pseudomona
* S. aureus
* H. influ
* Burkholderia cepacia
* RSV/Flu/Aspergylus
Immunocompromised patients

*Neoplasm
*Transplant Recipients
*HIV
Community Acquired Pneumonia Diagnosis

Available Test Methodologies

• Sputum Gram stain and culture
• Blood cultures
• Serologic studies
• Antigen detection tests
• Nucleic acid amplification tests
Specimen collection
Sputum Collection

• Proper patient instruction
  – Food should not have been ingested for 1-2 h prior to expectoration
  – The mouth should be rinsed with saline or water
  – Avoid using mouth wash solution
  – Patient should breathe and cough deeply
  – Patient should expectorate into a sterile container

• Transport container immediately to lab

• Perform Gram stain and plant specimen as soon as possible
Sputum collection

• Sputum of less than 2ml should not be processed unless obviously purulent
• Only 1 sputum per 24hr submitted
• Transportation in <2 hr is recommended with refrigeration if delays anticipated.
• Handle all samples using universal precautions.
• Some scoring system should be used to reject specimen that re oral contamination.
Induced sputum

*Patients who are unable to produce sputum may be assisted by respiratory therapy technician. Aerosol induced specimen are collected by allowing the patient to breath aerosolized droplets of a solution of 15% sodium chloride and 10% glycerin for approximately 10 minute. Obtaining such specimen may avoid the need for a more invasive procedures, such as bronchoscopy or needle aspiration, in many cases.

*Specimen resemble saliva
*Should be accepted in the laboratory without prescreening
*High diagnostic value in pneumocystis, mycobacterium and fungi
Gastric aspiration

- The gastric aspiration is used exclusively for isolation of acid-fast bacilli and may be collected from patients who are unable to produce sputum, particularly young children. The relative resistance of mycobacteria allows them to remain viable for a short period. Gastric lavage must be delivered to the lab immediately so that the acidity can be neutralized. Specimen can be first neutralized and then transported if immediate delivery is not possible.
IDSA Practice Guidelines
Diagnostic Tests for CAP

• Outpatients
  – Empiric therapy with a macrolide, doxycycline, or a fluoroquinolone

• Hospitalized patients with CAP
  – Gram stain and culture of sputum
  – 2 pretreatment blood cultures
  – Studies for Mtb, Legionella in select patients

ATS Guidelines
Diagnostic Tests for CAP

• Empiric therapy for outpatients
  – Macrolide or tetracycline

• Hospitalized patients with CAP
  – 2 sets of pre-treatment blood cultures
  – Pleural fluid Gram stain/culture when appropriate
  – Studies for Legionella, Mtb, fungi in select patients
  – Sputum Gram stain/culture only if resistant or unusual pathogen is suspected
  – Avoid extensive testing

Hospital Acquired Pneumonia Diagnosis

- American College of Chest Physicians: Clinical findings are not sufficient for definitive diagnosis
- Qualitative culture or endotracheal sputum has poor predictive value
- Endotracheal aspiration or suction samples should be treated as sputum by laboratory (GNB clonization)
- Bronchoscopy is recommended by many pulmonologists
  - Bronchial washes (contamination with URT flora)
  - Protected specimen brushing (Aspiration pneumonia)
  - Bronchoalveolar lavage specimens (BAL)
  - Transbronchial biopsy
Respiratory Specimens

- Protected Brush Specimen
  - To procure uncontaminated lower airway secretions
  - Brush within 2 catheters
Respiratory Specimens

• Bronchoalveolar Lavage (BAL)
  – Samples large area of the lung
  – Performed using a bronchoscope
  – 100 to 250 ml of saline injected
  – Injected saline along with secretions is collected by aspiration

• Transthoracic Aspiration
  – Involves percutaneous introduction of a needle directly into the infiltrate
Direct Gram Smear
Prescreening for adequacy

Specimen Acceptability Criteria
Gram Stain Smear
*Expectorated sputum
*Endotracheal aspiration or suction
Sputum Gram Stain

Good quality specimens

- Quantify number and types of inflammatory cells
- Note presence of bronchial epithelial cells
- Concentrate on areas with WBCs when looking for organisms
Table 2. Screening criteria for microscopic examination of sputum samples in the diagnosis of lower respiratory tract infections.

<table>
<thead>
<tr>
<th>Author</th>
<th>Method</th>
<th>Criteria for acceptance of sample (score)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bartlett</td>
<td>SEC, PMN</td>
<td>Positive numerical score</td>
</tr>
<tr>
<td>Murray</td>
<td>SEC/LPF</td>
<td>&lt;10</td>
</tr>
<tr>
<td>Geckler</td>
<td>SEC/LPF</td>
<td>&lt;25</td>
</tr>
<tr>
<td>Van Scoy</td>
<td>PMN/LPF</td>
<td>&gt;25</td>
</tr>
<tr>
<td>Barry</td>
<td>SEC, PMN, mucus</td>
<td>Positive numerical score</td>
</tr>
<tr>
<td>Heineman</td>
<td>PMN:SEC</td>
<td>&gt;10</td>
</tr>
</tbody>
</table>

NOTE. LPF = low-power field; PMN = polymorphonuclear leukocytes; SEC = squamous epithelial cells.
<table>
<thead>
<tr>
<th>Author (reference)</th>
<th>Method*</th>
<th>Criteria for acceptability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bartlett (3)</td>
<td>Assign + and − values; +2 if &gt;25 WBC; +1 if 10–25 WBC; +1 if mucus seen; −2 if &gt;25 EPI; −1 if 10–25 EPI</td>
<td>Any positive score (sum of + and − values assigned)</td>
</tr>
<tr>
<td>Murray and Washington (7)</td>
<td>Avg no. of EPI/LPF</td>
<td>&lt;10 EPI/LPF</td>
</tr>
<tr>
<td>Geckler et al. (4)</td>
<td>Avg no. of EPI/LPF</td>
<td>&lt;25 EPI/LPF</td>
</tr>
<tr>
<td>Van Scoy (8)</td>
<td>Avg no. of WBC/LPF</td>
<td>&gt;25 WBC/LPF</td>
</tr>
<tr>
<td>Barry (1)</td>
<td>Assign + and − values: +3 if &gt;150 WBC; +2 if 76–150 WBC; +1 if 1–75 WBC; −3 if &gt;25 EPI; −2 if 16–25 EPI; −1 if 5–15 EPI</td>
<td>Any positive score (sum of + and − values assigned)</td>
</tr>
<tr>
<td>Heineman and Radano (6)</td>
<td>Avg ratio of WBC to EPI</td>
<td>&gt;10 WBC/EPI</td>
</tr>
</tbody>
</table>

* Stained smears were examined under 100× magnification, and the number of squamous epithelial cells (EPI) or white blood cells (WBC) per LPF was determined.
WBC

Number of WBC may not be relevant because
*many patients are severely neutropenic from
disease or treatment
*Defect in effective inflammatory response such
as Immunocompromised patients (bone
marrow implant)
*Legionella pneumonia sputum
*Foreign body (endotracheal catheter)
Sputum Gram Stain
Poor Quality
Sputum Gram Stain
Good Quality
Bronchoalveolar Lavage (BAL) Specimen Acceptability

- Microscopic examination of Gram-stained smear
  - Acceptable
    - <1% of cells present are squamous epithelial cells
  - Unacceptable
    - >1% of cells present are squamous epithelial cells
    - Presence of ciliated columnar bronchial epithelial cells/goblet cells or pulmonary macrophage in specimen obtained by bronchoscopy or BAL indicates a specimen from LRT
Gram Stain Smear

Interpretation and Report
- No squamous epithelial cell seen
- Few squamous epithelial cell seen
- Abundant (many) squamous epithelial cell seen indicating oropharyngeal contamination
- Contamination with saliva please submit another specimen
- No WBC Seen
Smears with predominant EP and without WBC

No need to culture and report of organisms

Smears with EP and WBC

-Predominant organism
Gram negative bacilli predominant organism seen on Gram smear

-Presence of other organisms
Mixed bacterial morphotypes also present on Gram smear

-Without predominant organism
Mixed bacterial morphotypes seen on Gram smear

-Without organism
No organisms seen

-One morphotype
Report morphotype as few/moderate and many
Gram Stain Reports

• Be as descriptive as possible
  – Moderate neutrophils
  – Moderate Gram positive diplococci suggestive of *Streptococcus pneumoniae*
  – Few bacteria suggestive of oral flora

• Keep report short—avoid line listing of all morphotypes present
Culture
Routine culture

- Blood Agar
- Chocolate Agar
- Mac Conkey
- Thioglycolate (for aspirated specimen biopsy)
Routine culture

- Because of contaminating oral flora, sputum specimens, specimens obtained by bronchial washing, and lavage trachestomy, or endotracheal tube aspirates are not inoculated to enriched broth or incubated anaerobically.

- Only specimens obtained by percutaneous aspiration (including transtracheal aspiration) and by protected bronchial brush are suitable for anaerobic culture.
Culture

- Transtracheal and percutaneous lung aspiration material may be inoculated to enriched thioglycollate, as well as to solid media. For suspected cases of legionella disease, buffered charcoal yeast extract (BCYE) agar and selective BCYE are inoculated.
Culture

- Sputum specimens from patients known to have cystic fibrosis should be inoculated to selective agar, such as manitol salt agar for recovery of *S. aureus* and selective horse blood-bacitracin, incubated anaerobically and aerobically, for recovery of *H. influenzae* that may be obscured by the mucoid *P. aeruginosa* on routine media.
Bronchoscopy Specimens Processing

- **Bronchoscopy brush protected**
  - Aerobic bacterial culture and Gram stain
  - Anaerobic bacterial culture
  - Limited volume

- **Bronchoscopy brush, unprotected**
  - No anaerobic culture
  - Limited volume

- **Bronchial washings**
  - Useful only for pneumonia caused by strict pathogens
  - Reasonable requests: Mtb, Fungi, *Legionella, Pneumocystis*

- **Bronchoalveolar lavage**
  - No anaerobe culture
Bronchoscopy Samples
Quantitative Methods

PSB or BAL


vortex 30-60s

Final dilutions

Plate 0.1 ml

Chocolate, blood 1:10

Dilute

Plate 0.1 ml

Chocolate 1:1000

Dilute 0.1 ml to 9.9 ml saline

Plate 0.1 ml

Chocolate 1:100,000

Dilute 0.1 ml to 9.9 ml saline

Dilute

Plate 0.1 ml

Chocolate, blood

Dilute

Plate 0.1 ml

Chocolate, blood
Bronchoscopy Samples
Quantitative Methods

Calibrated loop method


- PSB
- vortex 30-60 s
- BAL

Plate 0.1 ml
Plate 0.01 ml
Plate 0.001 ml

Chocolate
Chocolate
Chocolate

**Final Dilutions**

1:10
1:100
1:1000
Interpretation of Quantitative PSB/BAL

• Dilution Method
  – Quantify each morphotype present and express as CFU/ml

• Calibrated Loop Method
  – Quantify each morphotype present and express as $\log_{10}$ colony count ranges

• Thresholds for significance
  – PSB > $10^3$ CFU/ml
  – BAL > $10^4$ CFU/ml

Special notes

Specimen without EP or few EP
α hemolytic Streptococci

Heavy or moderate (predominant) growth or colony resemble *S. pneumoniae*

Diagnostic tests

*S. pneumoniae*  
α hemolytic Streptococci as normal microbial isolated
S. pneumoniae

S. pneumoniae mucoid colony
S. pseudopneumoniae  
S. pneumoniae
S. pneumoniae

Ambient atmosphere

S. pseudopneumoniae

5% CO2
It is not soluble in bile

S. pseudopneumoniae  S. pneumoniae
There is no pneumococcal capsule (and is therefore not typable)

S. pneumoniae  S. pseudopneumoniae
**β hemolytic Streptococci**

Mixed with other colony or few colony

Isolation and subculture(stabing)

Good growth

Diagnosis tests

Group A Streptococci Isolated

Serotyping for other
Hemophilus

Predominant culture GNB resemble Hemophilus

Isolation on choc

Diagnosis test
Nieserria and Moraxella

Predominant culture GNDB or GNDB in Gram smear

Diagnosis test
### Other Gram Negative

<table>
<thead>
<tr>
<th>No. of: Colony types</th>
<th>Colonies</th>
<th>Normal microbiota</th>
<th>Action*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-2</td>
<td>Rare to few</td>
<td>Absent to few, or if gram-negative bacilli predominant on Gram stain</td>
<td>ID and AST</td>
</tr>
<tr>
<td>1-2</td>
<td>Moderate to abundant</td>
<td></td>
<td>ID and AST only if requested</td>
</tr>
<tr>
<td>&gt; 2</td>
<td>Few to abundant</td>
<td>Present or absent</td>
<td>ID and AST only if requested</td>
</tr>
</tbody>
</table>

* ID, identify; AST, perform antimicrobial susceptibility testing.
Staphylocoocci

<table>
<thead>
<tr>
<th>No. of colonies</th>
<th>Colony morphotypes</th>
<th>Normal microbiota</th>
<th>Action</th>
<th>Report</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any no.</td>
<td>CNS</td>
<td>Moderate to abundant</td>
<td>None</td>
<td>Normal microbiota</td>
</tr>
<tr>
<td>Moderate, abundant, or predominant</td>
<td>CNS</td>
<td>Absent to few</td>
<td>ID</td>
<td>CNS as normal microbiota</td>
</tr>
<tr>
<td>Rare to few</td>
<td><em>S. aureus</em></td>
<td>Moderate to abundant</td>
<td>None</td>
<td>Normal microbiota</td>
</tr>
<tr>
<td>Rare to few</td>
<td><em>S. aureus</em></td>
<td>Absent to few or gram-positive cocci predominant on Gram stain</td>
<td>ID, AST</td>
<td><em>S. aureus</em></td>
</tr>
<tr>
<td>Moderate to abundant, or predominant</td>
<td><em>S. aureus</em></td>
<td>Present or absent</td>
<td>ID, AST</td>
<td><em>S. aureus</em></td>
</tr>
</tbody>
</table>
Yeast

- Rare to moderate of yeast
- Moderate to abundant growth of normal flora

Normal microbiota

- Rare to moderate of yeast
- No growth of normal flora or predominant yeast cell in Gram smear

ID

Predominant culture

ID
Special notes

Specimen with many EP and WBC
predominant organisms in gram stain not seen

No need to additional diagnosis and complementary culture

One organism seen in gram stain

- Growth on culture  ID / AST
- Normal flora  no need to ID
- Predominant GNB in gram smear and isolation of one type colony or predominant colony  ID / AST
- Two or more colony type of GNB without predominant type

Multiple species of gram negative bacilli, no predominant organism isolated

Culture without predominant organisms

Mixed bacterial morphotypes
College of American Pathologist Recommendation

*Examine all sputum specimen microscopically
  *Report microscopic finding quantitatively
  *Use microscopic criteria for acceptability
  *Compare Gram stain and culture results
*Organisms seen in the smear don’t grow in culture
*Organism that grow in moderate quantity or less are not seen in the Gram smear
*List both the Gram smear and culture results together in the same report