Objectives

- Describe the utility of sputum GSs in patients with bacterial pneumonia.
- Discuss criteria for assessing specimen quality in the GS.
- Discuss the value of the GS smear as a reliable rapid diagnostic tool.
- Describe recognition and reporting of organisms by genera rather than organism morphology.
- Discuss the criteria for use of “mixed flora” in respiratory GS.
Gram Stain

- Rapid, inexpensive, informational
- Presumptive organism ID
  - Guide rational selection of preliminary antibiotic therapy
- Evaluation of specimen quality
  - Identify superficially contaminated specimens
  - Enhance discrimination between samples with potential pathogens vs. colonizing flora
Utility of the Gram Stain

- GS **DOES NOT** diagnose the presence of pneumonia

- Once pneumonia is diagnosed, the GS is useful in determining probable etiologic agent

- Gram stain & culture
  - GS should be used to guide selection of potential pathogens in culture that merit ID/AST
<table>
<thead>
<tr>
<th>Author (year)</th>
<th>Method</th>
<th>Minimum criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bartlett (1974)</td>
<td>Sum of PMN/LPF (10-25, 1+; &gt;25, 2+), mucus (1+); SEC (10-25, -1; &gt;25, -2)</td>
<td>Score of &gt;0</td>
</tr>
<tr>
<td>Murray and Washington (1975)</td>
<td>Enumerate SEC/LPF</td>
<td>&lt;10 SEC/LPF</td>
</tr>
<tr>
<td>Geckler et al. (1977)</td>
<td></td>
<td>&lt;25 SEC/LPF</td>
</tr>
<tr>
<td>Van Scoy (1977)</td>
<td>Enumerate PMN/LPF</td>
<td>&gt;25 PMN/LPF</td>
</tr>
<tr>
<td>Heineman and Radano (1979)</td>
<td>Ratio of PMN to SEC</td>
<td>&gt;10 PMN/SEC</td>
</tr>
<tr>
<td>Kalin et al. 1983</td>
<td></td>
<td>&gt;5 PMN/SEC</td>
</tr>
<tr>
<td>Morris et al. (1993)</td>
<td>Enumerate SEC/LPF and presence/absence of organisms/OIF</td>
<td>&lt;10 SEC/LPF and organisms present</td>
</tr>
</tbody>
</table>
Gram Stain Screening Criteria
Gram Stain Screening Criteria

GOOD
DIRECT SMEAR SUGGESTS:

No neutrophils

Many squamous cells

Not representative of lower respiratory tract secretions. Culture not performed. Please consult Microbiology if clinical considerations warrant complete processing of this specimen. (Specimen will be held 7 days).
“Mixed Flora”

- Used only with respiratory specimens
- Use of objective criteria (# of organisms present per OIF) to distinguish resident flora or colonizers from potential pathogens:
  - If low #s of organisms are seen on GS (or seen in culture and not seen in GS), they might be colonizers and not pathogens
"Respiratory Mixed Flora"

DIRECT SMEAR SUGGESTS:

Cells:
- Moderate neutrophils
- No squamous epithelial cells

Bacteria:
- Moderate Gram positive cocci/pr,ch
- Few Gram negative rods
- Many Gram negative diplococci
- Few Gram positive rods
- Few Gram negative coccobacilli
- Rare Gram positive cocci/clusters
- Few yeast
“Respiratory Mixed Flora”

**DIRECT SMEAR SUGGESTS:**

**Cells:**
- Moderate neutrophils
- No squamous epithelial cells

**Bacteria:**
- Moderate Gram positive cocci/pr,ch
- Few Gram negative rods
- Many Gram negative diplococci
- Few Gram positive rods
- Few Gram negative coccobacilli
- Rare Gram positive cocci/clusters
- Few yeast

**NO**

**DIRECT SMEAR SUGGESTS:**

**Cells:**
- Moderate neutrophils
- No squamous epithelial cells

**Bacteria:**
- Many Gram positive cocci in pairs & chains suggestive of Pneumococci
- Moderate Mixed flora

**YES**
Presumptive Organism Identification

Enteric Gram-negative rods:

- Fat Gram-negative rods which can have non-uniform sizes and can sometimes show bipolar staining report:
  - “Gram negative bacilli”; might add...
  - “...suggestive of enterics, or “...enteric-like”

- If respiratory: Report only if ≥ 10 are seen per oil immersion field; if < 10 are seen, report as part of mixed flora.
Presumptive Organism Identification

**Haemophilus:**

Gram-negative coccobacilli/pleomorphic rods that may be faint staining report:

> “Gram negative coccobacilli”; might add
> “…suggestive of *Haemophilus*” if respiratory
> “…suggestive of *Bacteroides*” if wound

If respiratory: Report only if ≥ 10 are seen per oil immersion field; if < 10 are seen, report as part of mixed flora.
Presumptive Organism Identification

**Pseudomonas:**

Thin, somewhat faint staining, uniform in shaped, sometimes in pairs end to end (“hot dogs”) report as:

- “Gram negative bacilli”; might add
- “…*Pseudomonas*-like”
- “…Nonenteric-like”

- If respiratory: Report only if ≥ 10 are seen per oil immersion field; if < 10 are seen, report as part of mixed flora.
Presumptive Organism Identification

**Moraxella/Neisseria:**

Gram-negative diplococci with flattened sides report:

> “Gram-negative diplococci”, might add
> “…suggestive of Neisseria/Moraxella”

If respiratory: Report only if $\geq 25$ are seen per oil immersion field; if $< 25$ are seen, report as part of mixed flora.
Presumptive Organism Identification

*S. pneumoniae*:

- Gram-positive cocci, usually lancet-shaped, encapsulated, report:
  - “Gram-positive cocci in pr/ch; might add
    - “…suggestive of *S. pneumoniae*”

- If respiratory: Report only if ≥ 25 pairs are seen per oil immersion field; if < 25 pairs are seen, report as part of mixed flora.

- Do not report *S. pneumoniae* morph from wounds.
Presumptive Organism Identification

*Staphylococcus*:

Gram-positive spherical Gram-positive cocci in clusters or tetrads, report:

> “Gram-positive cocci in clusters”; might add “...suggestive of *Staphylococcus*”

If respiratory: Report only if ≥ 50 are seen per oil immersion field; if < 50 are seen, report as part of mixed flora.
**Streptococcus** (not *S. pneumoniae*):

- Gram positive cocci in pairs or chains (not Pneumo-like), report:
  - “Gram positive cocci in chains”; might add
    - “…suggestive of *Streptococcus*”.

- Do not report from respiratory sources. Report as part of mixed flora.
Presumptive Organism Identification

Gram positive bacilli:

- Small Gram positive bacilli: Chinese letter appearance, report: “Small Gram positive bacilli, resembling diphtheroids/coryneforms”

- Large Gram positive bacilli: Large box-car shaped rods, report: “Large Gram positive bacilli, resembling Bacillus/Clostridium”

- Thin & long Gram positive bacilli: Sometimes in pairs or chains, report: “Gram positive bacilli” (Lactobacillus, some Clostridium species?)

- Do not report from respiratory sources. Report as part of mixed flora.
Presumptive Organism Identification

Yeast:
- Gram positive/variable yeast with/without buds, or with/without pseudohyphae, Report: “yeast” or “yeast with pseudohyphae”.

- Do not report from respiratory sources. Report as part of mixed flora.
## Respiratory Mixed Flora Criteria

<table>
<thead>
<tr>
<th>Morphology</th>
<th>Call if:</th>
</tr>
</thead>
<tbody>
<tr>
<td>GNR, GNGB</td>
<td>&gt; 10 organisms/OIF</td>
</tr>
<tr>
<td>Moraxella</td>
<td>&gt; 25 organisms/OIF</td>
</tr>
<tr>
<td>Staphylococcus</td>
<td>&gt; 50 organisms/OIF</td>
</tr>
<tr>
<td>S. pneumoniae</td>
<td>&gt; 25 organisms/OIF</td>
</tr>
</tbody>
</table>

- Streptococcus (not pneumo)
- Gram positive bacilli
- Yeast
Master the gram stain and make the most of it

- Rapid, inexpensive, informational
- Evaluation of specimen quality
- Presumptive organism ID
- Guide rational selection of preliminary antibiotic therapy