Wound & Sputum Cultures
Not as difficult as you might think!

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Objectives

- Review a CAP requirement for utilization of the Gram stain
- Discuss use of the Gram stain
  - Screening tool for specimen quality
  - Guide for the work up of wound and respiratory cultures
- Discuss the tools and advantages of a systematic approach
- Review two different approaches for cost-effective and clinically relevant wound and respiratory culture work-up.
Introduction

- Wound and sputum cultures are frequently contaminated with resident flora → difficult to tell which organisms are potential pathogen.

- The work up of heavily mixed or superficially contaminated cultures can lead to misleading results and inappropriate therapy.

- Healthcare resources and technologist time can be wasted working up cultures of little clinical value.
What’s a Microbiologist to do? Look to the Gram stain

- Screening tool to assess specimen quality
  - PMNs indicative of inflammation or infection
  - Squamous epithelial cells suggestive of superficial contamination
- Guides interpretation of culture results → extent of culture work-up
  - Enhance discrimination between samples with colonizing flora and potential pathogens
Gram stain is a useful tool
Keep it sharp!

To be useful as a tool, the Gram stain must be properly prepared and performed.

- **Preparation and staining** - Targeted selection of mucopurulent portions of the specimen and appropriate staining of the smear is necessary.

- **Interpretation** - There must be standardized screening criteria provided for interpretation.
Gram stain is a useful tool
Keep it clear!

Training and competency programs must assess technical ability but should also reinforce the following:

- Consistent interpretation and reporting among technologists
- Culture results are always correlated with the Gram stain.
- Gram stain results guide the interpretation and work-up of the culture.
Gram stain is a useful tool
Make it meaningful!

Oral flora – $10^{10}$-10$^{12}$ CFU/mL
- Corynebacterium
- Coagulase negative staphylococci
- Staphylococcus aureus
- Streptococcus pneumoniae
- Moraxella catarrhalis
- Neisseria spp.
- Gram negative bacilli
Gram stain is a useful tool
Make it meaningful!

<table>
<thead>
<tr>
<th>Morphology</th>
<th>Call if:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram negative bacilli</td>
<td>≥ 10 organisms/OIF</td>
</tr>
<tr>
<td>Gram negative diplococci</td>
<td>≥ 25 organisms/OIF</td>
</tr>
<tr>
<td>Gram positive cocci in clusters</td>
<td>≥ 50 organisms/OIF</td>
</tr>
<tr>
<td>Gram positive diplococci</td>
<td>≥ 25 pairs/OIF</td>
</tr>
</tbody>
</table>

- Used with respiratory (not wound specimens).
- Can also be used for objective criteria (# of organisms present/OIF) to distinguish resident flora/colonizers from PP

Bartlett 1982 *JAMA*; Wright et al. 1990 *Am J Med*; Normandin et al. 1997 *ASM C-91*
1) DIRECT SMEAR SUGGESTS:

Cells:
- Moderate neutrophils
- No squamous cells

Bacteria:
- Few Gram negative rods
- Many Gram positive diplococci
- Moderate Gram negative diplococci
- Moderate Gram positive rods
  - Few Gram positive cocci in clusters
  - Few yeast

2) DIRECT SMEAR SUGGESTS:

Cells:
- Moderate neutrophils
- No squamous cells

Bacteria:
- Many Gram positive diplococci (suggestive of *S. pneumoniae*)
  - Moderate mixed flora
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**NEW** 07/11/2011

**Revised** 07/31/2012

MIC.21530 Direct Gram Stains
MIC.21530 Direct Gram Stains (Phase I)

New 07/11/2011
Revised 7/31/2012

- The laboratory has protocols in place to use Gram stain results to provide a preliminary identification of organisms, evaluate specimen quality when appropriate, and to guide work-up of cultures.

NOTE: The laboratory should have guidelines for the interpretation of the gram stain reaction of the organism, morphology of the organism, and the quantification of organisms and cells. The protocol should address correlation of direct gram stain results with final culture results.

Evidence of Compliance:
- ✓ Written procedure for gram stain
### Screening For Specimen Quality

<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</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>
Screening for Specimen Quality

Respiratory Rejection criteria = MCM 10th edition - 2011:

Specimen Quality (Gram stain assessment)

- **Sputum**
  - > 10 SEC or > 25 SEC / 10x

- **Endotracheal aspirate**
  - > 10 SEC / 10x with no bacteria 20 fields

- **BAL**
  - > 1% of cells present are SEC
Screening for Specimen Quality
Wound and Sputum Cultures
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Work up of Respiratory & Wound Cultures

Resident flora

Colonizing organisms

Pathogens
Work up of Respiratory & Wound Cultures:

Clinical Relevance

- There are no established guidelines for working up respiratory and wound cultures.
  - literature
  - colleagues

- Systematic approach is needed

- Tools for systematic approach
  - Direct Gram stain (SEC / PMN)
  - Site of collection / resident flora
  - Predominance of the organism (culture & Gram stain)
  - Patient / culture history
Work up of Respiratory & Wound Cultures:

Two approaches*

- Q-Score System
- Q234 System

* 2004: ASM Cumitech 7B: Lower Respiratory Tract Infections (can also be used for wounds)
**Work up of Respiratory & Wound Cultures:**

**Q-Score System** (RC Bartlett, 1974)

<table>
<thead>
<tr>
<th>Squamous cells (−)</th>
<th>0</th>
<th>-1</th>
<th>-2</th>
<th>-3</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>+1</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>+2</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>+3</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Neutrophils (+)</th>
<th>Q0</th>
<th>Q1</th>
<th>Q2</th>
<th>Q3</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>no cult</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1PP</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>2PP</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>3PP</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**“Q-SCORE”** = # of potential pathogens (PP) to work up

**key:**

- 0 = no cells
- 1 = 1-9/lpf
- 2 = 10-24/lpf
- 3 = ≥25/lpf

Q0 = no cult
Q1 = 1PP
Q2 = 2PP
Q3 = 3PP
Work up of Respiratory & Wound Cultures: Q-Score System

“Q-Score” system

Q3 - Specimens without SEC are good quality specimens regardless of the number of PMNs. The Q score = 3 and up to 3 potential pathogens (PP) can be worked up (ID/AST).

The lower quality of the specimen (e.g., the more SEC present) the fewer the organisms worked up (Q2, Q1).
Work up of Respiratory & Wound Cultures: Q-Score System

“Q-Score” system

# PP in culture \(\leq\) Q-score: work up PP with ID/AST

\( (2PP) \) \( (Q3) \)

# PP in culture \(>\) Q-score: Look to Gram stain

\( (3PP) \) \( (Q2) \)

- Work up PP that were seen in Gram stain with ID/AST
- If all PP in the culture are seen in Gram stain
  = do not work up; morphological identify (MID) them
Work up of Respiratory & Wound Cultures: Q234 System

“Q 2-3-4” system:
• Gram stain Quality Check: PMN & SEC
  Reject any sputum for culture according to your laboratory’s protocol.

Culture work up is based on number of PP present:
  \( \leq 2\text{PP} = \text{Work up with ID/AST} \)
  \( 4\text{PP} = \text{MID} \)
  \( 3\text{PP} = \text{Look to Gram stain*} \)

*Work up to 2 PP if they are seen in the GS.
If all 3 PP are seen in the GS, MID all 3.

NOTE: If quantity of PP is < quantity of mixed flora MID PPs.
Example 1: sputum

GS: many PMN (+3), no SEC (-0), many GNB

WORK UP:
Example 1: sputum

GS: my PMN (+3), few SEC (-0), my gnb

WORK UP:

Q-Score (Q3=3PP): > work up *P. aeruginosa*, *E. coli* & *Proteus* species
Example 1: sputum

GS: my PMN (+3), few SEC (-0), my gnb  

WORK UP:

Q-Score (Q3=3PP): > work up *P. aeruginosa*, *E. coli* & *Proteus* species

Q 2-3-4 (3PP): > MID all 3
Example 2: wound

GS: mod. PMN (+2), few SEC (-1), many gpc/clusters, many gpc/chains

CULT: many \textit{S.aureus}, mod. \textit{\beta}-strep, mod. coag - staph, few diphtheroids

WORK UP:

- Q-Score (Q1= 1PP): > MID SAU, \textit{\beta}-Strep, & report mixed flora
- Q/2 - 3 - 4 (2 PP): > work up SAU & \textit{\beta}-Strep, & report mixed flora

Example 2: wound
Example 2: wound

GS:  mod. PMN (+2), few SEC (-1), many gpc/clusters, many gpc/chains
CULT: many *S. aureus*, mod. β-strep, mod. coag - staph, few diphtheroids

WORK UP:

- Q-Score (Q1= 1PP):  > MID SAU, β-Strep, & report mixed flora
Example 2: wound

GS: mod. PMN (+2), few SEC (-1), many gpc/clusters, many gpc/chains
CULT: many *S.aureus*, mod. β-strep, mod. coag - staph, few diphtheroids

WORK UP:

- Q-Score (Q1= 1PP): > MID SAU, β-Strep, & report mixed flora
- Q 2-3-4 (2 PP): > work up SAU & β-Strep, & report mixed flora
Example 3: sputum

GS:  many PMN (+3), few SEC (-1), many gpc in clusters and many mixed flora (w/ few gnb-enterics)
CULT:  mod. CN-staph, mod. diphths, few E.coli, rare S.aureus

WORK UP:

- 
- 
-
Example 3: sputum

GS: many PMN (+3), few SEC (-1), many gpc in clusters and many mixed flora (w/ few gnb-enterics)
CULT: mod. CN-staph, mod. diphths, few *E.coli*, rare *S.aureus*

WORK UP:

- **Q-Score (Q2=2PP):** > Work up *E.coli* & *S.aureus*, > Report mixed flora
Example 3: sputum

GS: many PMN (+3), few SEC (-1), many gpc in clusters and many mixed flora (w/ few gnb-enterics)

CULT: mod. CN-staph, mod. diphths, few E.coli, rare S.aureus

WORK UP:

- **Q-Score (Q2=2PP):** > Work up E.coli & S.aureus,
  > Report mixed flora

- **Q 2-3-4 (2PP):** > Report mixed flora,
  > MID E.coli & S.aureus**

**NOTE:** If mixed flora > PP = MID PP
Example 4: wound

GS: many PMN (+3), no SEC (0), many GNB, many GNCB

CULT: mod. Kleb sp., mod. *Bacteroides* sp., few *Enterococcus*

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Example 4: wound

GS: many PMN (+3), no SEC (0), many GNB, many GNCB

CULT: mod. Kleb sp., mod. *Bacteroides* sp., few *Enterococcus*

WORK UP:
- Q-Score (Q3=3PP): > work up *Klebsiella*, *Bacteroides* & *Enterococcus*
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Example 4: wound

GS: many PMN (+3), no SEC (0), many GNB, many GNCB

CULT: mod. Kleb sp., mod. Bacteroides sp., few Enterococcus

WORK UP:

- Q-Score (Q3=3PP): > work up Klebsiella, Bacteroides & Enterococcus

- Q 2-3-4 (3PP): > work up Klebs & Bacteroides > MID Enterococcus
Premise for “Q” systems

- That quality of the Gram stain and consistency in reporting are key to the use of either system.

- The quality of specimen is important in determining acceptability for culture and extent of culture work up;

- The more superficially contaminated the specimen, the higher the # of colonizing organisms present;

- If organisms seen in smear, greater chance they are associated with an infective process.
Advantages of “Q” systems

1. Offers a consistent approach for interpreting cultures:
   - Based on specimen quality (primarily SECs).
   - Based on organisms seen in Gram stain
   - Limits # of organisms worked up from mixed cultures, so that the reporting of misleading information can be minimized.
   - Provide a consistent structure for training new technologists to Gram stain and culture work-up.
Advantages of “Q” systems

2. No Potential Pathogen is ever ignored:

- All PP reported; but they may not be fully worked up with ID/AST
- The pathogens that some believe should always be worked up are always indicated on the report.
- Q systems can be modified
  - screening for MRSA, VRE, ESBLs, CRE, etc.
  - ↑number of PP worked up from sterile/significant sites
Advantages of “Q” systems

3. Compliance with CAP.21530
   ▪ Screen for quality
   ▪ Guide work-up of the culture

4. Cost Effective:
   ▪ Eliminates unnecessary work-up
   ▪ Reagent cost savings → compared to some traditional work-ups
   ▪ Factor in technologist time → savings would be even greater

Advantages of “Q” systems

5. Guidelines:
   ▪ The Q-Systems offer “Guidelines” for a systematic culture interpretation approach.
   ▪ Allow technologists to make independent & consistent decisions about the significance of organisms in culture.
   ▪ These Guidelines are just that = Guidelines! Exceptions can always be made.
   ▪ Clinicians can consult with microbiology to have further work performed whenever clinically indicated.
Q SYSTEMS

Consistent approach for work up of the culture.

Tool for training new employees

flexible

Clinically relevant results

Cost effective

CAP Compliance
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P’s and Q’s:

- work hard
- be honest
- be kind
- pick up your room
- no singing at the table
- taste everything on your plate
- share

Your best
P’s and Q’s:

- Mind your Ps
- Select a Q

Report cost effective and clinically relevant culture results!
Thank you