Work up of Respiratory & Wound Cultures:
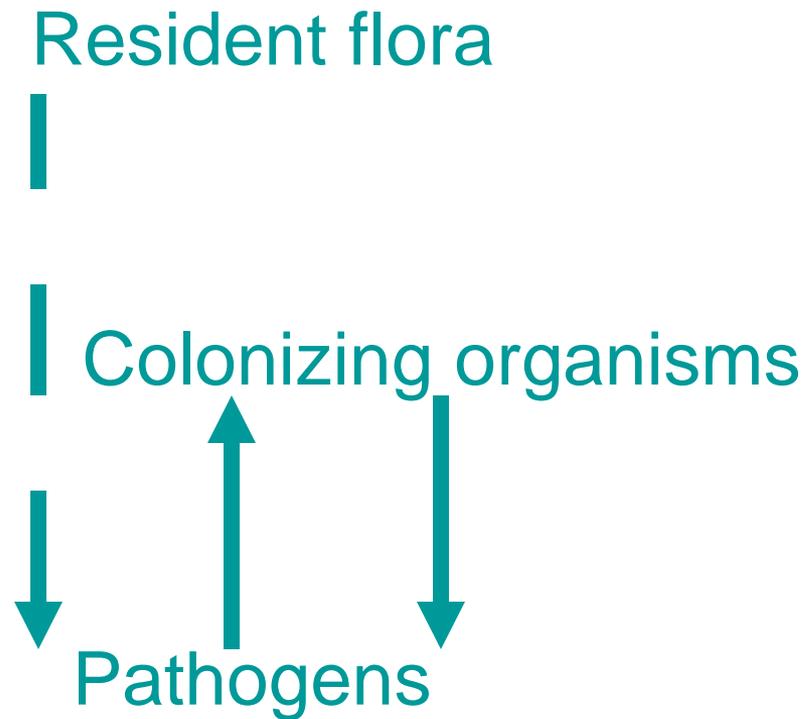
- Culture work up
  - 2 Systematic approaches

Work up of Respiratory & Wound Cultures

Resident flora

Colonizing organisms

Pathogens

Work up of Respiratory & Wound Cultures:
Clinical Relevance

➤ **There are no clear guidelines for working up bacterial cultures.**
  ➤ **literature**
  ➤ **colleagues**

➤ **There seems to be a need for some consistency when performing culture work up.**
  ➤ **uniformity in work up and reporting of bacterial isolates**
  ➤ **agreement in AST testing**

MIC.21530 Direct Gram Stains

STAINS

MIC.21530 Direct Gram Stain Procedures - (Phase I)

- **The laboratory has policies 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 policies for the interpretation of the Gram stain reaction of the organism, morphology of the organism, and the quantification of organisms and cells. The policy should address correlation of direct gram stain results with final culture results.

This does not mean that interpretation of the Gram stain morphology suggesting a specific organism identification (e.g. gram positive diplococci morphologically suggestive of pneumococcus) is required.

Evidence of Compliance:

- ✓ Written procedure for gram stain (laboratories may use the correlation of Gram stain results with the final culture results as a component of QC program).

Work up of Respiratory & Wound Cultures:
Specimen Quality

- **Premise:**
  - ◆ **PMN are an indication of infection or inflammation**

  - ◆ **SEC indicate superficial contamination = If a specimen contains a large amount of SEC, superficial contamination is likely**
    - ☞ **the specimen should "ideally" be recollected (resp)**
    - ☞ **alternatively, bacteria isolated from such specimens should be minimally worked up (wounds)**

  - ◆ **Extensive testing on heavily mixed cultures should not routinely be performed.**

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Work up of Respiratory & Wound Cultures:

Two approaches*

- **Q-Score System**

- **Q/234 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)

Squamous cells (-)

"Q-SCORE" =
# of potential pathogens (PP) to work up

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

Neutrophils (+)

	0	-1	-2	-3
0	3	0	0	0
+1	3	0	0	0
+2	3	1	0	0
+3	3	2	1	0

Q0 = no cult
Q1 = 1PP
Q2 = 2PP
Q3 = 3PP

Work up of Respiratory & Wound Cultures: Q-Score System

"Q-Score" system

Up to 3 organisms can be considered potential pathogens (PP) and be worked up (ID/AST) if from a good quality specimen (Q3).

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

$\qquad$ **# PP in culture $\leq$ Q-score: work up PP with ID/AST**
$\qquad$ **(2PP)** $\qquad$ **(Q3)**

$\qquad$ **# PP in culture > Q-score: Look to Gram stain**
$\qquad$ **(3PP)** $\qquad$ **(Q2)**

$\qquad$ **-Work up PP that were seen in Gram stain with ID/AST**
$\qquad$ **-If all PP in the culture are seen in Gram stain**
$\qquad$ **= do not work up; morphological identify (MID) them**

Work up of Respiratory & Wound Cultures: Q/234 System

"Q/2-3-4" system:

- **Gram stain Quality Check: PMN & SEC**

    Reject any sputum for culture according to normal protocol.

Culture work up is based on number of PP present:

2PP = Work up ($\leq$ 2 PP)

4PP = MID

3PP = 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 mixed flora > PPs = MID PPs.

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Example 1: sputum

GS: my PMN (+3), few SEC (-1), my enteric-like gnb
CULT: mod. *P.aeruginosa*, mod. *E.coli*, mod. *Proteus* sp, few diphtheroids

WORK UP:
   Q-Score (Q2=2PP):

   Q/2-3-4 (3PP):

Example 1: sputum

GS: my PMN (+3), few SEC (-1), my enteric-like gnb
CULT: mod. *P.aeruginosa*, mod. *E.coli*, mod. *Proteus* sp,
few diphtheroids

WORK UP:
   Q-Score (Q2=2PP): > Work up *E.coli* & *Proteus* sp;
                                     > MID PSA & report mixed flora

   Q/2-3-4 (3PP):

Example 1: sputum

GS: my PMN (+3), few SEC (-1), my enteric-like gnb
CULT: mod. *P.aeruginosa*, mod. *E.coli*, mod. *Proteus* sp, few diphtheroids

WORK UP:
Q-Score (Q2=2PP):   > Work up *E.coli* & *Proteus* sp;
                    > MID PSA & report mixed flora

Q/2-3-4 (3PP):   > Work up *E.coli* & *Proteus* sp;
                    > MID PSA & report mixed flora

Example 1a: sputum

GS: my PMN (+3), few SEC (-1), my ~~enteric-like~~ gnb
CULT: mod. *P.aeruginosa*, mod. *E.coli*, mod. *Proteus* sp,
few diphtheroids

WORK UP:
  Q-Score (Q2=2PP):

  Q/2-3-4 (3PP):

Example 1a: sputum

GS: my PMN (+3), few SEC (-1), my ~~enteric-like~~ gnb
CULT: mod. *P.aeruginosa*, mod. *E.coli*, mod. *Proteus* sp, few diphtheroids

WORK UP:
　　Q-Score (Q2=2PP):　> MID *E.coli, Proteus* sp, PSA
　　　　　　　　　　　　> report mixed flora
　　Q/2-3-4 (3PP):

Example 1a: sputum

GS: my PMN (+3), few SEC (-1), my ~~enteric-like~~ gnb
CULT: mod. *P.aeruginosa*, mod. *E.coli*, mod. *Proteus* sp,
few diphtheroids

WORK UP:
  Q-Score (Q2=2PP):   > MID *E.coli*, *Proteus* sp, PSA
                      > report mixed flora

  Q/2-3-4 (3PP):   > MID *E.coli*, *Proteus* sp, PSA
                      > report mixed flora

Example 2: wound

- **GS: mod. PMN (+2), few SEC (-1), my gpc/clusters (staph), my gpc/chains (strep)**

- **CULT: my *S.aureus*, mod. β-strep, mod. CoNS, few diphtheroids**

- **WORK UP:**
  - ◆ **Q-Score (Q1= 1PP):**

  - ◆ **Q/2-3-4 (2 PP):**

Example 2: wound

- **GS: mod. PMN (+2), few SEC (-1), my gpc/clusters (staph), my gpc/chains (strep)**

- **CULT: my *S.aureus*, mod. β-strep, mod. CoNS, few diphtheroids**

- **WORK UP:**

  ◆ **Q-Score (Q1= 1PP): > MID SAU, β-Strep**

  　　　　　　　　　> report mixed flora
  _____

  ◆ **Q/2-3-4 (2 PP):**

Example 2: wound

- **GS: mod. PMN (+2), few SEC (-1), my gpc/clusters (staph), my gpc/chains (strep)**

- **CULT: my *S.aureus*, mod. β-strep, mod. CoNS, 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: wound

- **GS: my PMN (+3), no SEC (0), my gnr (enterics), my gncb**
- **CULT: mod. Kleb sp., mod. *Bacteroides* sp., few *Enterococcus***

- **WORK UP:**
  - ◆ **Q-Score (Q3=3PP):**

  - ◆ **Q/2-3-4 (3PP):**

Example 3: wound

- GS: my PMN (+3), no SEC (0), my gnr (enterics), my gncb
- CULT: mod. Kleb sp., mod. *Bacteroides* sp., few *Enterococcus*

- WORK UP:
  - ◆ Q-Score (Q3=3PP): > Work up Kleb, *Enterococcus* & *Bacteroides* sp.
  - ◆ Q/2-3-4 (3PP):

Example 3: wound

- **GS: my PMN (+3), no SEC (0), my gnr (enterics), my gncb**

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

- **WORK UP:**

  - ◆ **Q-Score (Q3=3PP): > Work up Kleb, *Enterococcus* & *Bacteroides* sp.**

  - ◆ **Q/2-3-4 (3PP):   > Work up Kleb & *Bacteroides* sp.**
    > MID *Enterococcus*

Example 4: sputum

- **GS: mod PMN (+3), few SEC (-1), many gpc-staph, many mixed flora**
- **CULT: mod. CoNS, mod. diphths, few *E.coli*, rare *S.aureus***

- **WORK UP:**
  - ◆ **Q-Score (Q2=2PP):**

  - ◆ **Q/2-3-4 (2PP):**

Example 4: sputum

- **GS: mod PMN (+3), few SEC (-1), many gpc-staph, many mixed flora**

- **CULT: mod. CoNS, 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):**

Example 4: sputum

- **GS: mod PMN (+3), few SEC (-1), many gpc-staph, many mixed flora**

- **CULT: mod. CoNS, 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*

Example 4: sputum

- **GS: mod PMN (+3), few SEC (-1), many gpc-staph, many mixed flora**

- **CULT: mod. CoNS, 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*****

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Premise for "Q" systems

- Based on published prevalence of potential pathogen colonization of the oropharynx;

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

- Quality of specimen is important in determining acceptability of specimen and extent of culture work up;

- If organisms seen in smear, greater chance they are associated with an infective process.

Advantages for "Q" systems

- 1. **Offers a consistent approach for interpreting cultures:**
    - ☞ Based on specimen quality (primarily SECs).

    - ☞ Based on organisms seen in Gram stain (if see organism on smear, should be in a significant number in the specimen, $\geq 10^5$/ml).

    - ☞ Limits # of organisms worked up from mixed cultures, so that the reporting of misleading information can be minimized.

Advantages for "Q" systems

- **2. No Potential Pathogen is ever ignored:**

  ☞ **All PP listed out; but may not be fully identified or have full AST performed.**

  ☞ **The pathogens that some believe should "ALWAYS BE WORKED UP", such as _S. aureus,_ β-strep, _P. aeruginosa_ are identified and always indicated on the report.**

  ☞ **Can be modified to include screening for MRSA, VRE, etc.**

Advantages for "Q" systems

- **3. Guidelines:**
  - ☞ **The Q-Systems offer "Guidelines" for a systematic culture interpretation approach**

  - ☞ **These Guidelines are just that = Guidelines! Exceptions can be made if necessary.**

  - ☞ **Any concerned physician can consult with microbiology to have further work performed on any culture if clinically indicated.**

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Q-Reference:

C Matkoski, S E Sharp, and D L Kiska. Evaluation of the Q Score and Q234 Systems for Cost-Effective and Clinically Relevant Interpretation of Wound Cultures. 2006. *J. Clin. Microbiol*. 44: 1869-1872.

Matkoski, et. al. Conclusions

- **The utilization of one of the Q-systems can help to:**
  - ◆ **minimize the likelihood of reporting out misleading information to clinicians that might result in inappropriate antibiotic therapy**

  - ◆ **limit unnecessary work-up of mixed specimens that have little clinical relevance**

- **One must keep in mind that exceptions to these protocols exist, and a physician may have a valid reason for deviation from these recommendations.**
  - ◆ **The laboratory needs to remain open to discussion on the necessity to have further ID and/or AST performed on particular isolates.**

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Matkoski, et. al.
Conclusions (cont'd)

- **Both the Q-score and the Q234 systems showed clinical relevance, cost effectiveness and would allow for standardized approaches to the work up of wound cultures.**

- **The Q234 system proved to be a more practical procedure to implement in their laboratory.**

- **The Q234 system does not require a change in Gram stain interpretation or physician approval for specimen rejection.**

Matkoski, et. al.
Conclusions (cont'd)

- **All PP are reported from a culture with either ID or MID, allowing for further consultation with the ordering physician if clinically warranted.**

- **The Q234 system, which is more structured and cost-effective than their current method, was acceptable to the ID physicians.**

- **Will allow technologists to make more independent & consistent decisions about the significance of organisms in a wound culture.**

"Watch your P's and Q's"

1) Determine your "P's" (potential pathogens)

2) Pick one of the "Q's" (Q systems)

Report consist and clinically-relevant wound culture results.

Bronchoalveolar lavage

- **Quantitate or not**

- **What quantity to work up**

- **What to do with 'normal flora'**

- **When to do susceptibility testing**

?

Bronchoalveolar lavage

- **Quantitative cultures of BAL specimens are imperfect predictors of the presence of pneumonia in mechanically-ventilated patients.***

- **This technique had a reported sensitivity and specificity of 91% and 78%, respectively.****

- **However, despite the limitations, there are no alternatives that are clearly better.**

*Fujitani and Yu, Clin Infect Dis 43 suppl 2:S106, 2006
**Mayhall, Emerg Infect Dis 7:200, 2001

Bronchoalveolar lavage

- Quantitative BAL cultures:
  - Patients are considered to have a likelihood of pneumonia if there is "at least one microorganism obtained at a concentration of $\geq 10^4$ cfu/ml of lung fluid."*
  - Commensal flora:
    - There should be no commensal flora at this site at concentrations $\geq 10^4$ cfu/ml of lung fluid.
    - Commensal oral flora, when aspirated, can cause pneumonia.
    - Quantitation is used to determine whether this has occurred or not.
    - There should also be NO squamous epithelial cells present (this represents oral contamination).

*Mayhall, Emerg Infect Dis 7:200, 2001; Baselski & Wunderink, Clin Microbiol Rev 7:533, 1994)

Bronchoalveolar lavage

IDSA/AST guidelines*

- **For VAP state that:**

     "Significant growth of oropharyngeal commensals (viridans group streptococci, coagulase-negative staphylococci, *Neisseria* spp., and *Coryne-bacterium* spp.) from distal bronchial specimens is difficult to interpret…,

     but these organisms can produce infection in immunocompromised hosts and some immunocompetent patients."

(*Am J Respir Crit Care Med 171:388, 2005)

Bronchoalveolar lavage

POTENTIAL PATHOGENS:

Quantitate and perform ID/AST on up to 3 potential pathogens if at $\geq$10,000 for BAL and $\geq$1000 for Brush (if > 3 report MID).

Quantitate and report MID on any potential pathogens if at < 10,000 for BAL and < 1,000 for Brush.

ORAL FLORA:

1. If SEC were seen on initial GS (= possible contamination is present), quantitate and report 'oral flora' (OF) regardless of amount present.

   If no SEC were seen on initial GS, for each OF isolate at $\geq$10,000 for BAL and $\geq$1,000 for Brush, report MID.

2.

   If no SEC were seen on initial GS, for each OF isolate at <10,000 for BAL & <1000 for Brush, quantitate and report **TOTAL AMOUNT** of combined 'oral flora' (ie: 7000 diph + 8000 γ-strep = 15,000 OF) .

EXAMPLE: BAL

INITIAL GS:

Few polys, <u>no SEC</u>, NOS

CULTURE:

50,000/ml *Enterobacter* spp.

25,000/ml *Klebsiella* spp.

8,000/ml CoNS

7,000/ml α-streptococci (not S.pneumo)

REPORT:

50,000/ml *Enterobacter* (species) with AST

25,000/ml *Klebsiella* (species) with AST

15,000/ml Oral flora

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EXAMPLE: BAL

INITIAL GS:

Moderate polys, no SEC, NOS

CULTURE:

50,000/ml *Staphylococcus auerus*

15,000/ml CoNS

15,000/ml α-streptococci (not S.pneumo)

REPORT:

50,000/ml *S.aureus* with AST

15,000/ml CoNS

15,000/ml α-streptococci (not S.pneumo)

ADD CONTACT COMMENT: Contact microbiology if further work up of this culture is clinically indicated.

EXAMPLE: BAL

INITIAL GS:
Moderate polys, <u>few SEC</u>, few GPC/clusters

CULTURE:
50,000/ml *Staphylococcus auerus*
15,000/ml CoNS
15,000/ml α-streptococci (not S.pneumo)

REPORT:
50,000/ml *S.aureus* with AST
30,000/ml Oral flora