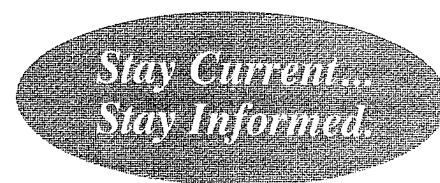


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## Best Laboratory Practices for Respiratory Cultures

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### Abstract

Respiratory specimens for culture are one of the most common test requests received in the clinical microbiology laboratory and often the most difficult to evaluate. Samples are frequently contaminated with resident upper airway flora, and it is often difficult to determine which organisms represent potential pathogens. These challenges often result in time and resources being spent to assess and report cultures that may provide little clinical value. In addition, working up and reporting results from mixed cultures may generate clinically misleading information that leads to inappropriate and unnecessary antimicrobial therapy.

This article discusses the evaluation and reporting of results on respiratory samples beginning with the direct specimen Gram stain through culture. It describes a clinically relevant interpretation and reporting scheme for direct specimen Gram stains, reviews the use of the direct Gram stain to guide respiratory culture workup, and provides several logical and easy-to-follow strategies that can be employed in the laboratory to ensure that results are clinically relevant and culture examination and work up are consistent among technologists.

### Introduction

#### Pathogenesis of Pneumonia

The respiratory tract is constantly exposed to microorganisms. However, host defenses, including hair in the anterior nares, mucociliary action, and secretory IgA, among many other attributes, protect the lower respiratory tract. The development of lower respiratory tract infection indicates either a breakdown in these defense mechanisms, exposure to a virulent organism(s), or a large microbial inoculum. Organisms can reach the lungs in one of three ways: direct inhalation of infectious respiratory droplets; aspiration of oropharyngeal contents; or, less commonly, hematogenous spread. *Mycobacterium tuberculosis* and *Legionella pneumophila*

are examples of organisms that can enter the lower respiratory tract through inhalation. Indigenous bacteria present in the oropharynx, such as *Streptococcus pneumoniae*, can be aspirated into the lungs either by oral secretions seeping into the lower respiratory tract (microaspiration) or by overt aspiration, as is seen in patients with an impaired gag and/or swallowing reflex due to increased age or alcohol intake. Pneumonia as a result of hematogenous spread is far less common but may be seen in patients with right-side bacterial endocarditis or in patients with bacteremia, most often due to *Staphylococcus aureus*.

#### Oral flora versus potential pathogen

The microbial species that make up the oral microbiome number in the hundreds (1). Some of these organisms are cultivatable, and some currently are not. Thus, it is not at all surprising to find cultivatable organisms normally found in the oral cavity growing in cultures of respiratory specimens. In addition,

there is indigenous flora that can also be pathogenic, depending on the circumstances. Many organisms that are normally thought to be pathogenic can also inhabit the upper respiratory tract without causing disease (*S. aureus*, *Streptococcus pyogenes*, and other  $\beta$ -hemolytic streptococci; *S. pneumoniae*, *Haemophilus influenzae*, *Moraxella catarrhalis*, and *Candida albicans*). In addition, the following organisms are usually considered indigenous flora of the respiratory tract: viridans streptococci, coagulase-negative staphylococci, pigmented *Neisseria* species, *Corynebacterium* species, and anaerobic organisms (2). In addition, Gram-negative bacilli (enteric and nonenteric) can colonize the upper respiratory tract without causing infec-

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tion during hospitalization and, by extension, during stays in long-term care facilities (3). Through the appropriate utilization of Gram-stained smears from respiratory specimens and a clinically relevant culture work up approach, one can effectively determine which organisms are most likely associated with an infectious process versus those that are most likely not associated with disease.

## Direct Gram Stain

### Utility of the direct Gram stain of respiratory specimens

The direct specimen Gram stain, when performed well, is an inexpensive test that rapidly provides a wealth of information to clinicians about the quality of the specimen received, as well as the organisms present. Microscopic examination and culture of respiratory secretions remain the mainstays of laboratory evaluation of pneumonia. It is important to remember that neither the Gram-stained smear nor culture of respiratory secretions is sufficient to diagnose the presence of pneumonia. However, once pneumonia has been established clinically by physical assessment and chest X ray, the direct Gram stain is useful in determining the probable etiologic agent of infection. The majority of the literature supports the clinical usefulness of Gram-stained sputum smears, despite the fact that a wide range of sensitivities (35 to 96%) and specificities (12 to 85%) are reported (4-9) (Table 1). This disparity in the literature is due in part because there is no

true gold standard for the diagnosis of pneumonia, and the reference standard used in most studies is the sputum culture. Adding to the diagnostic difficulties, there is also variation in specimen collection protocols and different criteria for determining specimen quality among published studies. The Infectious Diseases Society of America (IDSA) and American Thoracic Society (ATS) consensus guidelines on the management of community-acquired pneumonia advocate the performance of a sputum Gram stain only if a high-quality specimen can be obtained (10). The IDSA/ATS guidelines report that the benefit of a sputum Gram stain is 2-fold, in that it broadens the initial empirical coverage for less common etiologies, such as *S. aureus* or Gram-negative bacilli, and it validates subsequent sputum culture results. The IDSA/ATS consensus guidelines on the management of hospital-acquired pneumonia, ventilator-associated pneumonia, and health care-associated pneumonia point out that a reliable tracheal aspirate Gram stain may be useful in directing initial empirical therapy, since culture results are not available immediately (11). Some studies have reported the decreased use of antibiotics with no adverse outcomes and a trend toward improved mortality when the direct Gram stain is utilized (12,13). In a recent meta-analysis, O'Horo and colleagues (14) attempted to determine if the Gram stain is useful in the microbiological diagnosis of ventilator-associated pneumonia. They determined

that the negative predictive value of the Gram stain was high; however, the positive predictive value was not. However, differences in specimen quality and variability in Gram stain interpretation may have seriously impacted the study's conclusions. Heineman and colleagues (15) previously reported that half of the information gleaned from sputum cultures is clinically misleading in the absence of correlation with direct Gram stain results. In a multiyear study, Gleckman and colleagues (16) reported that when guided by bacterial morphotypes from the Gram stain, appropriate monotherapy was selected 94% of the time. This study emphasizes the importance of the collection of quality sputum specimens and the use of blood culture rather than sputum culture as a reference standard.

### Evaluation of specimen quality

Optimal smear preparation and staining are key to obtaining a smear that can be used to assess the respiratory specimen's quality; microscopic assessment can identify superficially contaminated specimens. Ideally, these poor-quality specimens should not be cultured because of the potential for producing misleading information. Knowledge of specimen quality also enhances the discrimination between samples that contain potential pathogens versus specimens that likely contain colonizing flora. The specimen quality rating can be used to guide the interpretation of culture results in the laboratory and is of value in improving the diagnostic

**Table 1. Utility of the direct respiratory Gram stain**

Study (reference)	Sensitivity (%) <sup>a</sup>	Specificity (%) <sup>a</sup>	Population <sup>b</sup>	Comment
Cao et al. (4)	Spn (81) Hflu (86) Mcat (91)	Spn (98) Hflu (95) Mcat (98)	Pediatric	
Musher et al. (5)	Spn (57)	NA	Adult	Included patients on antibiotics
Rosón et al. (6)	Spn (57) Hflu (82)	Spn (97)	Adult (CAP)	Gram stain provided presumptive Hflu (99) diagnosis in 80% of patients.
Anevlaivis et al. (7)	Spn (82) Hflu (79) Saur (76) GNB (78)	Spn (93) Hflu (96) Saur (96) GNR (95)	Adult (CAP)	Utilized specific criteria for Gram stain evaluation
Blot et al. (8)	EA (91) PTC (70)	EA (64) PTC (96)	Adult (HAP)	

<sup>a</sup> Spn, *Staphylococcus pneumoniae*; Hflu, *Haemophilus influenzae*; Mcat, *Moraxella catarrhalis*; Saur, *Staphylococcus aureus*; GNB, Gram-negative bacilli; EA, endotracheal aspirate; PTC, plugged telescoping catheter; NA, not applicable.

<sup>b</sup> CAP, community-acquired pneumonia; HAP, hospital-acquired pneumonia.

utility of culture results to clinicians.

Standardization is key to ensuring consistent smear interpretation. As demonstrated in Table 2, there are numerous criteria published in the literature for determining respiratory specimen acceptability. While each may have its own advantages and disadvantages, it is important to utilize one of these criteria consistently to determine specimen quality and acceptability. It is also important to emphasize that there are instances in which these criteria should not be applied. These circumstances include specimens submitted for *Legionella* culture and specimens from cystic fibrosis (CF) patients (18). For CF screening cultures, direct specimen Gram stains are not clinically relevant and need not be performed. In instances in which poor-quality specimens are not processed, the use of interpretive comments can provide clinicians with important information regarding the quality of the specimen (Table 3).

### Interpretation and reporting of organisms in direct smears

Most laboratories routinely report the Gram stain reaction and morphology/arrangement (i.e., Gram-positive cocci in clusters) when describing organisms observed in a direct smear. While this practice provides clinicians evaluating the report with information about the organisms present in a sample, it assumes that the clinician is familiar enough with bacterial morphology to understand that a report of Gram-positive cocci in clusters equates to the presence of *Staphylococcus* species. These assumptions are not always accurate.

In the 1980s and 1990s, Raymond Bartlett advocated the reporting of organism genera in direct specimen smears, as it was more useful to clinicians than a description of organism morphology (19-21). Even today, many microbiologists feel confident suggesting the presence of a specific bacterial genus but are hesitant to put it in writ-

ing, since they fear their impression may be incorrect. Nevertheless, Bartlett believed that the clinical usefulness of this specific, rapid result warranted the risk (19). Bartlett also demonstrated that Gram-negative bacilli could be reliably differentiated in Gram-stained smears as Gram-negative coccobacilli suggestive of *Bacteroides* or *Haemophilus* spp., enteric Gram-negative bacilli, and *Pseudomonas*-like Gram-negative bacilli (21). In some cases, it may not be possible to report a specific genus, in which case, the report could indicate that a distinction cannot be made.

Unlike many other specimens in which all organisms present in the direct Gram stain are reported, reporting all organisms from lower respiratory tract specimens, especially if they are in small numbers or there is a mixture of organisms, can be misleading (19). This is due to the fact that, as indicated previously, many of the organisms present in respiratory specimens, such

**Table 2. Gram stain screening criteria to determine specimen acceptability (17)**

Author (yr)	Method <sup>a</sup>	Minimum criteria <sup>a</sup>
Bartlett (1974)	Sum of PMN/LPF (10-25, 1+; >25, 2+), mucus (1+), and SEC/LPF (10-25, -1; >25, -2)	Score > 0
Murray and Washington (1975), Geckler et al. (1977)	Enumerate SEC/LPF	<10 SEC/LPF; <25 SEC/LPF
Van Scoy (1977)	Enumerate PMN/LPF	>25 PMN/LPF
Heineman and Radano (1979), Kalin et al. (1983)	Ratio of PMN to SEC	>10 PMN/SEC; >5 PMN/SEC
Morris et al. (1993)	Enumerate SEC/LPF and presence/absence of organisms/OIF	<10 SEC/LPF and organisms present
Zaidi and Reller (1996)	Presence/absence of organisms/OIF	Organisms present

<sup>a</sup>LPF, low-power field; OIF, oil immersion field; PMN, polymorphonuclear leukocytes; SEC, squamous epithelial cells.

**Table 3. Example of interpretive comment used for a poor-quality respiratory specimen**

COLLECT DATE/TIME: 11/10/12 1655 RESP CULTURE SPECIMEN: Sputum  
 RECEIVE DATE/TIME: 11/10/12 1800  
 REPORT STATUS: FINAL 11/10/12  
 DIRECT SMEAR SUGGESTS:  
 No neutrophils  
 Many squamous epithelial 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 5 days.)

as *S. pneumoniae*, *S. aureus*, *M. catarrhalis*, and Gram-negative bacilli, may represent colonizing or transient flora rather than a potential pathogen. The use of objective criteria, such as the number of organisms present per oil immersion field, was proposed by Bartlett to distinguish resident flora or colonizers from potential pathogens (19).

These reporting criteria are also supported by additional studies (22,23). A reporting scheme that includes the reporting of bacterial genera is presented in Table 4, and examples of Gram stains with genus-specific reports are seen in Fig. 1 through 3. For example, in a respiratory Gram stain, such as that seen in Fig. 3, *Pseudomonas*-like Gram-negative bacilli would only be reported if 10 or

more organisms were present in the average oil immersion field. Otherwise, "mixed flora" would be reported as a designation for organisms that were seen in the Gram stain but did not meet specific reporting criteria. Using these specific reporting criteria, certain organisms, such as Gram-positive bacilli, streptococci that do not resemble *S. pneumoniae*, and yeast would be reported as "mixed flora" routinely, regardless of the quantity present in the smear. This type of reporting greatly reduces the generation of misleading information. This is particularly true when yeasts are reported from a respiratory Gram stain. The literature indicates that pneumonia caused by *Candida* spp. is very rare, and due to the high prevalence of yeast colonizing the respiratory tract, recovery of yeast

from lower respiratory tract specimens cannot and should not be used to diagnose fungal pneumonia (24-26). Table 5 depicts a respiratory Gram stain report from the institution of one of the authors (Y.S.M.) before and after the implementation of the mixed-flora criteria. The report is clearer and highlights the potential pathogens present in significant numbers.

### Respiratory Culture Work Up

There are no clear guidelines for evaluating bacterial cultures, and the literature is lacking in standard techniques. If one were to ask several colleagues how they work up respiratory cultures, you would inevitably get several different answers. Thus, there seems to be a need for standardization

**Table 4. Direct Gram stain reporting scheme**

Gram stain morphology	Criterion for reporting in respiratory direct smear <sup>a</sup>	Gram stain report	Comment
Plump Gram-negative bacilli; may be uniform in size and can sometimes show bipolar staining	≥10 organisms/OIF	<ul style="list-style-type: none"> <li>Gram-negative bacilli suggestive of enterics</li> <li>Enteric-like Gram-negative bacilli</li> </ul>	
Thin, somewhat faint staining; uniform in shape; elongated Gram-negative bacilli, may occur in pairs end to end	≥10 organisms/OIF	<ul style="list-style-type: none"> <li>Nonenteric Gram-negative bacilli</li> <li><i>Pseudomonas</i>-like Gram-negative bacilli</li> </ul>	
Gram-negative coccobacilli/pleomorphic rods; may be faint staining	≥10 organisms/OIF	<ul style="list-style-type: none"> <li>Gram-negative coccobacilli suggestive of <i>Haemophilus</i></li> </ul>	
Gram-negative diplococci with flattened sides	≥25 organisms/OIF	<ul style="list-style-type: none"> <li>Gram-negative diplococci suggestive of <i>Moraxella</i></li> </ul>	
Gram-positive cocci in pairs or short chains; usually lancet shaped and may be encapsulated	≥25 pairs of organisms/OIF	<ul style="list-style-type: none"> <li>Gram-positive cocci suggestive of <i>Pneumococcus</i></li> </ul>	
Gram-positive cocci in clusters	≥50 organisms/OIF	<ul style="list-style-type: none"> <li>Gram-positive cocci in clusters suggestive of <i>Staphylococcus</i></li> <li>Gram-positive cocci suggestive of <i>Staphylococcus</i></li> </ul>	
Gram-positive cocci in pairs or chains (not <i>S. pneumoniae</i> )		Mixed flora	Not routinely reported in respiratory Gram stains regardless of number
Large, boxcar-shaped Gram-positive bacilli		Mixed flora	Not routinely reported in respiratory Gram stains regardless of number
Small, irregular-shaped Gram-positive bacilli; may have a Chinese character appearance		Mixed flora	Not routinely reported in respiratory Gram stains regardless of number
Yeast, with or without budding, with or without pseudohyphae		Mixed flora	Not routinely reported in respiratory Gram stains regardless of number

<sup>a</sup>OIF, oil immersion field.

and consistency when working up respiratory cultures, including uniformity in work up and reporting of bacterial isolates as potential pathogens.

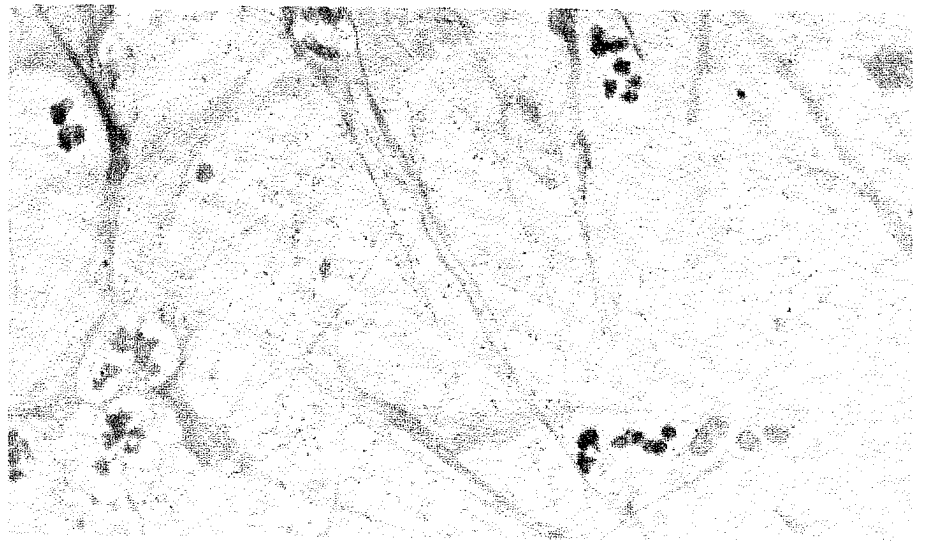
To start down this path, one must make two reasonable assumptions: the first is that polymorphonuclear leukocytes (PMNs) indicate infection or inflammation in an immunocompetent patient, and the second is that squamous epithelial cells (SECs) indicate superficial contamination. Thus, it would also hold true that if a noninvasively collected respiratory specimen contains a large number of SECs, superficial contamination is likely, and the specimen should be re-collected. Likewise, extensive testing on heavily mixed respiratory cultures should not routinely be performed.

Two systems utilize the direct respiratory Gram-stained smear, along with culture results, to effectively distinguish potential pathogens from indigenous flora in noninvasively collected respiratory specimens (sputum, tracheal aspirates, nasotracheal aspirates, etc.). These two systems are the Quality (Q) score system and the Q234 system (17, 27). Both systems are described here, and examples are presented.

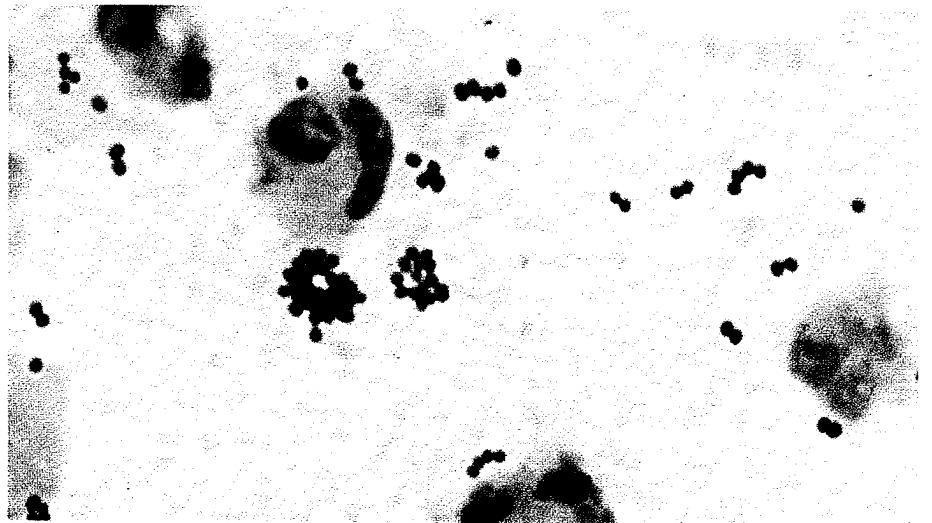
#### Q Score System

The Q score system was first introduced by Bartlett in 1974 (27). Since its introduction, it has been modified slightly so that lower respiratory tract Gram stains are examined at  $\times 10$  magnification for the presence of PMNs and SECs, which are quantitated as depicted in Fig. 4. The presence of PMNs is recorded as a positive value, and that of SECs is recorded as a negative value. The addition of these two numbers generates a Q score for that specimen. The Q score equates to the number of potential pathogens that would be worked up (i.e., complete identification and antimicrobial susceptibility testing, if appropriate) from that specimen. Specimens with a Q score of 0 would not be processed.

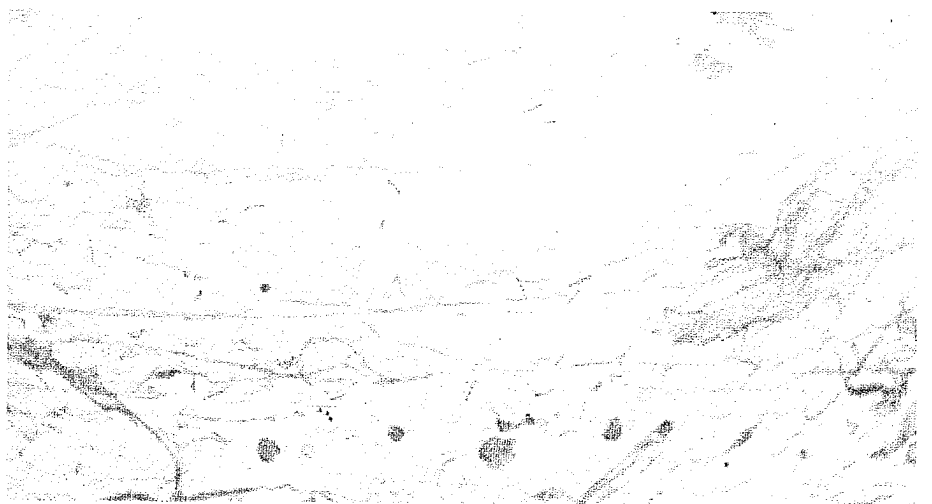
It is important to note that any specimen without SECs, regardless of the number of PMNs, would always be given the highest Q score, since some patients may not be able to mount an adequate PMN response. With the Q score system, a maximum of 3 potential pathogens can be worked up from a high-quality (i.e., Q3) specimen. The lower the quality of the specimen (i.e.,



**Figure 1.** Gram-stained smear of sputum demonstrating Gram-negative coccobacilli suggestive of *Haemophilus* spp. Magnification,  $\times 1,000$ .



**Figure 2.** Gram-stained smear of sputum demonstrating Gram-positive cocci in clusters suggestive of *Staphylococcus*. Magnification,  $\times 1,000$ .



**Figure 3.** Gram-stained smear of sputum demonstrating non-enteric or *Pseudomonas*-like Gram-negative bacilli. Magnification,  $\times 1,000$ .

**Table 5. Reporting respiratory Gram stain using mixed-flora criteria**

Conventional Gram stain report	Gram stain report using criteria for mixed flora
Direct smear suggests:	Direct smear suggests:
Cells:	Cells:
Moderate neutrophils	Moderate neutrophils
No squamous cells	No squamous cells
Bacteria:	Bacteria:
Few Gram-negative rods	Gram-positive diplococci suggestive of <i>S. pneumoniae</i>
Many Gram-positive diplococci	Mixed flora
Moderate Gram-negative diplococci	
Few Gram-positive rods	
Few Gram-negative coccobacilli	
Rare Gram-positive cocci in clusters	
Few yeast cells	

the more SECs present), the fewer organisms are worked up. For example, if 2 potential pathogens are present in a Q3 specimen, then both would be worked up. However, if the number of potential pathogens were to exceed the Q score, the culture work up is based on the correlation of the organisms grown in culture with the organisms seen in the direct Gram-stained smear. Organisms that were seen in the direct smear (up to the Q score) would be worked up, while organisms that were not seen in the direct smear would be identified based only on spot tests or other rapid tests (morphological identification [MID]), with no susceptibility testing performed. If all potential pathogens in the culture are seen in Gram stain, none of the isolates are worked up (i.e., all receive MID). A flow diagram of this system is presented in Fig. 5.

**Q234 System**

When utilizing the Q234 system, one can use whichever method is currently employed in the laboratory for acceptance of respiratory specimens for bacterial culture. In the Q234 protocol, if 2 potential pathogens are grown in culture, complete identification and susceptibility testing is performed, if appropriate; if ≥4 potential pathogens are grown in culture, all isolates receive a MID; if the culture grows 3 potential pathogens, the culture work up is based on the correlation of the organisms grown in culture with the organisms seen in the direct Gram-stained smear. If 1 or 2 of the 3 potential pathogens grown in

culture are seen in the direct specimen Gram stain, they are worked up. If all 3 of the potential pathogens grown in the culture are observed in the direct Gram stain, their work up is limited to MID. Additionally, if the growth of potential pathogens in culture is in a smaller quantity than the indigenous colonizing flora, potential pathogens work up is limited to MID. A flow diagram of this system is presented in Fig. 5.

**Examples of use of Q Score and Q234 systems in respiratory culture work up**

Examples of similarities and differences in reporting based on the Q score and Q234 systems are presented in Fig. 6 to 8. Figure 6 demonstrates a scenario in which there are 3 potential pathogens in the culture. Using the Q score system, the number of potential pathogens exceeds the Q score, so the results of

		Squamous epithelial cells (-)			
Report value		0	-1	-2	-3
Neutrophils (+)	0	3	0	0	0
	+1	3	0	0	0
	+2	3	1	0	0
	+3	3	2	1	0

**Key:**  
 0 no cells  
 1 1-9 cells/low power field  
 2 10-24 cells/low power field  
 3 ≥ 25 cells/low power field

**Q-SCORE = Number of potential pathogens (PP) worked up in culture**

Q0 = Culture not performed  
 Q1 = Up to 1PP worked up in culture  
 Q2 = Up to 2PP worked up in culture  
 Q3 = Up to 3PP worked up in culture

**Figure 4.** Method used for determining the Q score. (Modified from reference 27.)

the direct Gram stain are reviewed to determine which potential pathogens are worked up. In this example, the same is true for the Q234 system. Thus, the cultures would be worked up in the same fashion with both Q systems. In Fig. 7, given the low quality score in the Q score system (Q1), only one potential pathogen may be worked up; thus, the *Pseudomonas aeruginosa* isolate seen in the direct Gram stain has identification and susceptibility testing preformed, and the *S. aureus* isolate would receive MID, as it was not seen in the direct Gram stain. Using the Q234 system, both potential pathogens are worked up, since  $\leq 2$  potential pathogens are present in culture. In Fig. 8, both potential pathogens are worked up using the Q score system, since the number of potential pathogens is less than or equal to the Q score. However, with the Q234 system, potential pathogens are in smaller quantity than the mixed flora, and thus, the *Escherichia coli* and *S. aureus* work up would be limited to MID.

#### Advantages of the Q Systems

The Q systems are based on three premises: (i) the prevalence of potential pathogen colonization of the oropharynx, with the reasoning that the more superficially contaminated the specimen, the higher the number of colonizing organisms present; (ii) the quality of the specimen is important in determining the acceptability of the specimen for culture, as well as the extent of culture work up; and (iii) if organisms are seen in the direct Gram stain, there is a greater chance they are associated with an infective process (organisms seen in the direct Gram stain should be present in significant number in the specimen to be seen microscopically, i.e.,  $\geq 10^5$ /ml). It is important to note that the College of American Pathologists (CAP) has recently added a question to their microbiology checklist pertaining to the use of the direct Gram stain for direct culture work up. CAP MIC.22100 (phase I) states that "a gram-stained smear is performed routinely on expectorated sputa to determine acceptability of a specimen for bacterial culture and as a guide for culture workup" ([http://www.cap.org/apps/cap.portal?\\_nfpb=true&\\_pageLabel=eLABLAP\\_page&eLabSol=eLABLAP\\_page](http://www.cap.org/apps/cap.portal?_nfpb=true&_pageLabel=eLABLAP_page&eLabSol=eLABLAP_page)). Either of the previously discussed Q systems would meet this CAP directive, and they both offer

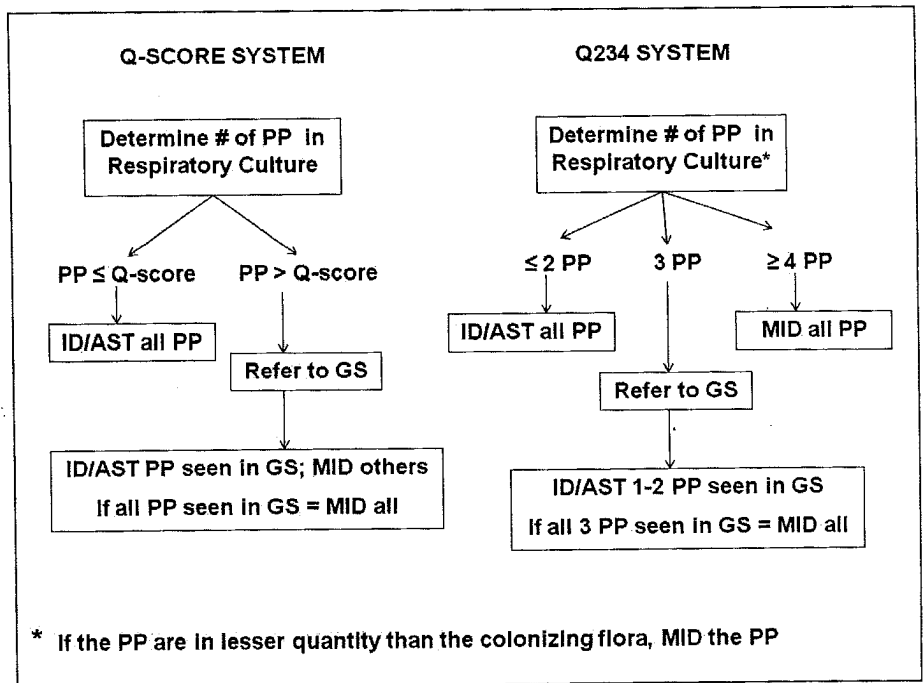


Figure 5. Flow diagram demonstrating the work up of potential pathogens with the Q score and Q234 systems.

**Gram Stain**  
Many PMN (+3), few SEC (-1), many enteric-like Gram negative bacilli, many Gram positive cocci suggestive of Staph, few mixed flora (yeast)

**Culture** (3 PP present)  
Moderate *P. aeruginosa*, moderate *E. coli*, moderate *Staph aureus*, few yeast

**WORK UP:**

Q-Score (Q2=2PP): Work up *E. coli* and *S. aureus*  
MID *P. aeruginosa*; report Mixed flora

---

Q234 (3PP): Work up *E. coli* and *S. aureus*  
MID *P. aeruginosa*; report Mixed flora

Figure 6. Comparison of specimen work up between the Q score and Q234 systems in which the overall work up is the same.

several advantages. First, they offer a consistent approach for interpreting cultures. Both systems are based on specimen quality and rely on the organisms seen in the direct Gram stain to direct culture work up. Thus, they limit the number of organisms worked up from mixed cultures and minimize the reporting of misleading information. This approach can play an important role in minimizing the unnecessary use of

antimicrobials, yet no potential pathogen is ever ignored. All potential pathogens are reported, although complete identification and susceptibility testing may not be performed, depending on the score. These systems can also be modified to work up pathogens that some believe should always be worked up, such as *S. aureus*,  $\beta$ -hemolytic streptococci, and *P. aeruginosa*, or to include screening for organisms such as methi-

cillin-resistant *S. aureus* and vancomycin-resistant enterococci. While the Q systems offer guidelines for a systematic approach to culture interpretation, the guidelines are just that—guidelines. Exceptions can be made, and a concerned physician can consult with the microbiology laboratory to have further work performed on any culture if clinically indicated.

### Conclusion

The Q system culture protocols are Gram stain-directed, limit the identification and susceptibility testing of organisms in mixed cultures, and can be used for the standardization of work up and reporting of noninvasively collected respiratory specimens. It should be noted that, although not all potential pathogens are fully identified or have susceptibility testing performed, they are always reported and never ignored. These protocols can also be modified so that particular organisms can always be worked up and/or others can be ruled out. The protocols can also be used for the consistent, cost-effective, clinically relevant work up of wound specimens (28).

In conclusion, the performance of direct Gram stains on respiratory specimens is very useful, so it should be done well and used to the fullest, not only to assess specimen quality, but also to determine rapid presumptive organism identification and to guide culture work up. Thus, as your mother would remind you, watch your Ps and Qs: determine your "Ps" (potential pathogens) and pick a "Q" (Q system) to report consistent, clinically relevant, and cost-effective results from respiratory cultures.

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**Gram Stain**  
 Many PMN (+3), moderate SEC (-2), many nonenteric-like  
 Gram negative bacilli, moderate mixed flora

**Culture** (2 PP present)  
 Many *P. aeruginosa*, moderate *Staph aureus*, few viridans streptococci

**WORKUP:**

Q-Score (Q1=1PP): Work up *P. aeruginosa*  
 MID *S. aureus*; report Mixed flora

---

Q234 (2PP): Work up *P. aeruginosa* and *S. aureus*;  
 report Mixed flora

**Figure 7.** Comparison of specimen work up between the Q score and Q234 systems in which the overall work up for each system differs.

**Gram Stain**  
 Many PMN (+3), few SEC (-1), many Mixed flora

**Culture** (2 PP present)  
 Moderate diphtheroids, moderate coagulase negative Staph,  
 few *E. coli*, rare *Staph aureus*

**WORKUP:**

Q-Score (Q2=2PP): Work up *E. coli* and *S. aureus*;  
 report Mixed flora

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Q234 (2PP): Report Mixed flora  
 MID *E. coli* and *S. aureus* \*\*

\*\* If mixed flora > PP = MID PP

**Figure 8.** Comparison of specimen work up between the Q score and Q234 systems demonstrating the Q234 caveat of not working up potential pathogens if they are in smaller quantity than the mixed flora.

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