

## Challenge G101

May 2010

Gram: Sputum smear: >25/lpf PMN, no epithelial cells; 11-50/oif gram negative diplococci (companion to M101-4). Sample suitable for culture.

### HISTORY

A simulated sputum smear from a 64 year old male patient with community acquired pneumonia (CAP).

### CMPT QA

The sample contained 4+ (>25/lpf) PMN's, no epithelial cells, and 3+ (11-50/oif) gram negative diplococci/cocci (Figure 1).

The challenge was verified by internal quality control, which indicated 99% accuracy based on MIL-STD-105E.<sup>1</sup>

### SURVEY RESULTS

#### Reference Laboratories

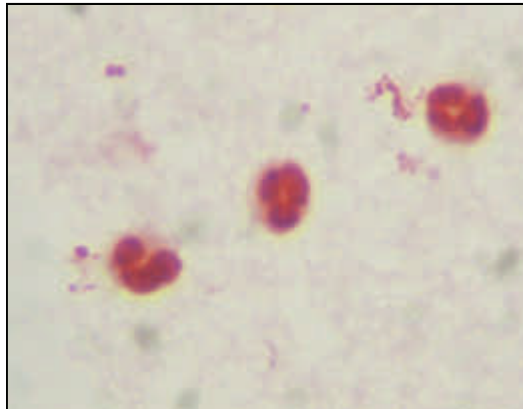
**Cells:** 13/15 laboratories reported the presence of neutrophils (1 to >25/lpf); six of those laboratories also reported the presence of epithelial cells (<10/lpf) and one reported red blood cells (RBCs). One laboratory reported the presence of 2+ mononuclear white blood cells (WBCs) with neutrophils and RBCs and another laboratory reported 4+ neutrophils and <25/lpf epithelial cells.

**Suitability for culture:** 13 labs indicated sample was suitable to culture, 1 lab reported Qscore 3, 1 lab did not report.

**Table -1:** Reported results for G101 smear – Cell component -

Reported results	Total	%	Grade
1+ to 4+, 1-9/lpf to >100/lpf, 10-25/oif, heavy neutrophils, +/- 1+ to 4+ RBCs	86	65	4
3+ neutrophils, 2+ mononuclear WBCs, 2+ RBCs	1	1	4
10 to >40/lpf, 2+ to 4+ neutrophils, occasional to <10/lpf epithelial cells	27	20	4
4+, <25 to >40/lpf neutrophils, <15/lpf, <25/lpf	4	4	4
4+ neutrophils, 4+ epithelial cells*;	1	1	0
no report**	8	6	0
no cells	1	1	0
snp +/- refer	3	2	ungraded
did not receive sample	1	1	ungraded
<b>Total</b>	<b>132</b>	<b>100</b>	

\* failed to report culture suitability. \*\* 6 labs did not report culture suitability, one lab reported unsuitable for culture, one lab reported suitable for culture. RBCs: red blood cells; WBCs: white blood cells; snp: sample not normally processed.



**Figure 1:** G101 gram smear. The smear shows neutrophils and gram negative diplococci.

**Bacteria:** 15/15 laboratories reported 1+ to 4+, 10-25/oif, or heavy gram negative cocci or diplococci +/- suggestive of *Neisseria/Moraxella* species.

Consensus was reached by the reference laboratories thus all components of this challenge (cells, culture suitability, and bacteria) were graded.

#### Participants

**Cells component** (Table 1): 65% of the participants reported the presence of neutrophils in the sample and were given a grade of four. One

### Grading

#### Maximum grade: 12

Reporting neutrophils with or without RBCs was graded 4.

Reporting the presence of 4+ epithelial cells was graded, especially because this laboratory did not report if the sample was suitable for culture or not.

Not reporting the cell component of the Gram stain or reporting "no cells" was graded 0.

Reporting the sample as suitable for culture was graded 4.

Reporting a Qscore without clarifying if the sample was suitable was downgraded to 3.

Not reporting suitability or reporting the sample as not suitable for culture was graded 0.

Reporting gram negative diplococci was graded 4.

Reporting gram negative coccobacilli was downgraded to 3.

Reporting the presence of gram positive cocci, gram negative bacilli, gram positive bacilli, or oropharyngeal flora was considered unacceptable and was graded 0.

category A laboratory reported the presence of mononuclear WBCs in addition to neutrophils and RBCs.

Although the sample did not contain epithelial cells, 24% of the participants reported their presence in the smear. 20% of the participants reported <10/lpf while 4% reported <15 to <25/lpf or 4+ epithelial cells. These laboratories were not downgraded for reporting epithelial cells because 7 of the 15 reference laboratories also reported epithelial cells.

Surprisingly, 6% (8/132) of the laboratories did not report the cell component and were graded zero.

Culture suitability (Table 2).

13/15 reference laboratories reported the sample as suitable for culture, one reported a Q score of 3 and one did not report culture suitability. Overall 64% of the participants clearly indicated that the sample was suitable for culture and thus received a grade of 4. Three laboratories reported Q scores of 2 or 3 but did not indicate final interpretation (see Qscore text box). These labs received a grade of 3.

Although in a previous critique ([G063](#)) it was clearly stated that **“In all future sputum Gram smear challenges, a companion culture result WILL NOT be taken as inference of acceptability. Failure to report the Interpretation Component will be negatively graded,”** 26/132 (20%) laboratories failed to report interpretation. These participants were graded zero.

Five (4B and 1C) participants (4%) reported the sample was not suitable for culture. Four of those laboratories reported <10/lpf neutrophils and one laboratory did not report cells. These laboratories were graded zero.

Bacteria component (Table 3).

Overall 70% of the participants reported the presence of gram negative cocci or diplococci with some of the laboratories suggesting *Neisseria/Moraxella* sp. These laboratories were graded 4.

Three laboratories reported gram negative coccobacilli and were given a grade of 3.

Two laboratories reported the presence of 4+ gram negative bacilli with 1+ gram negative cocci and were graded 1 because the clinician would likely treat for the presence of gram negative bacilli and not *Neisseria/Moraxella*.

Nine percent of the participants reported the presence of gram positive cocci in addition to gram negative cocci or diplococci and were graded 0 and 11% of laboratories reported gram positive cocci alone and were graded 0. Although *M. catarrhalis* may sometimes be somewhat resistant to decolorization, laboratories should also look at the morphology to determine if there are two populations or just gram variable staining. Correct decolorization is essential to correct gram stain interpretation.

One participant reported “oropharyngeal flora” and was graded 0 as this was a single bacterial morphotype with polymorphs present. The four participants that did not report the bacterial component were graded zero.

**COMMENTS ON RESULTS**

Eight laboratories reported gram positive cocci. It is recommended that laboratories review their decolorizing process. Five laboratories reported coccobacilli or gram negative bacilli. These laboratories should review their slide.

**Qscore**

In 1974, Bartlett was the first to publish criteria for evaluating the quality of lower respiratory tract secretions. He reported a quality score system that produced scores ranging from 0 to 3 based on the observed numbers of squamous epithelial cells (10 to 25 per 10x field, -1; >25, -2), mucus (+1), and neutrophils (10 to 25 per 10x field, +1; >25, +2).

Specimen recollection was requested when a score of ≤0 was obtained. The quality score was subsequently used to determine the extent of work to be performed on the culture; for scores of 1, 2, and 3, a maximum of one, two, and three potential pathogens were identified to the species level and tested for susceptibility, respectively.<sup>7</sup>

**Important**

The Q-score should not be used in the report to the clinician unless a comment explaining its meaning is added.

**Table-2:** Reported results for G101 smear – Culture suitability -

Reported results	Total	%	Grade
yes	86	65	4
Qscore 3	1	1	3
Qscore 2	1	1	3
lab did not receive samples	1	1	ungraded
refer, snnp +/- refer (reported cells and bacteria)	9	7	ungraded
snnp	3	2	ungraded
we do not determine or report suitability	1	1	0
no report	25	19	0
culture not suitable*	5	4	0
<b>Total</b>	<b>132</b>	<b>100</b>	

\*4 out of 5 of these labs reported <10 neutrophils/lpf; one lab did not report cells; snnp: sample not normally processed.

**Table 3.** Bacteria component G101 challenge

Reported results	Total	%	Grade
1+ to 4+, 10 to >50/oif, heavy gram negative diplococci, cocci +/- resembling <i>Neisseria/Moraxella</i> sp.	93	70	4
4+ gram negative coccobacilli, resembling <i>Neisseria/Moraxella</i> sp.	1	1	3
1+ gram negative coccobacilli	2	2	3
1+, 2+ gram negative cocci, 1+, 4+ gram negative bacilli	2	2	0
3+ gram negative cocci, diplococci, coccobacilli; 1+ to 3+ gram positive cocci, diplococci +/- snnp, refer	9	7	0
rare, occasional, 2+, 3+ gram positive cocci	14	11	0
1+ , 4+ gram negative cocci, 1+ gram positive bacilli +/- 2+ gram positive cocci, +/- refer	2	2	0
oropharyngeal flora	1	1	0
no report	4	3	0
lab did not receive sample	1	1	ungraded
snp	3	2	ungraded
<b>Total</b>	<b>132</b>	<b>100</b>	

snp: sample not normally processed

The quality score is really a measure of the extent of squamous epithelial cells. These increase in number as oropharyngeal and upper respiratory tract cells get collected with the sample. The literature is fairly clear that when such contamination occurs, the likelihood of finding a true pathogen diminishes significantly. In this challenge, over a quarter of laboratories reported varying numbers of epithelial cells that would skew the estimation of the quality score towards not processing the sample. There were no epithelial cells in the sample. It is important to review what is thought to be an epithelial cell. Disintegrating neutrophils may appear as a conglomeration thus a larger cell, but would not have the distinctive nucleus of an epithelial cell. Further, neutrophils are multinucleated whereas epithelial cells are mononuclear. Laboratories that appeared to observe squamous epithelial cells should review their smears to clearly differentiate the cell types they saw. If questions still arise the areas in question of the slides should be marked and sent back to CMPT for further review. It is also important to re-iterate that reporting of a Q-score is of limited meaning without a comment about the level of quality (i.e., that the sputum is suitable – or not for culture).

### CLINICAL SIGNIFICANCE

Gram-stained smears of the lower respiratory tract secretions provide rapid presumptive information to guide the initial selection of antimicrobial therapy. Interpretation of gram-stained smears allows the discrimination between superficially contaminated specimens and sam-

ples likely to harbor organisms associated with infection; and can validate the subsequent sputum culture results. <sup>2, 3</sup>

In typical Gram stains, *M. catarrhalis* presents itself as a gram-negative diplococcus with flattened abutting sides.<sup>4</sup> *M. catarrhalis* has a tendency to resist decolorization and thus show in smear stains as gram positive diplococci. <sup>5, 6</sup>

### REFERENCES

1. Farnum NR. 1994. Ch 11. Acceptance sampling, p. 305-361. Modern Statistical Quality Control and Improvement. Duxbury Press, Belmont, CA.
2. Sharp SE, Robinson A, Saubolle M, Santa Cruz M, Carrol K, Baselski V. Lower respiratory tract infections; coordinating ed. SE sharp; Cumitech 7B. Washington DC: ASM press; 2004.
3. Mandell L, Wunderink R, Anzueto A, et al. Infectious diseases society of America/American thoracic society consensus guidelines on the management of Community Acquired pneumonia in adults. Clinical Infectious Diseases. 2007;44:S27-S72.
4. Murphy T, Parameswaran G. Clinical practice: *Moraxella catarrhalis*, a human respiratory tract pathogen. Clinical Infectious Diseases. 2009;49:124-131.
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6. Ainsworth SM, Nagy SB, Morgan LA, Miller GR, Perry JL. Interpretation of gram-stained sputa containing *Moraxella (Branhamella) catarrhalis*. J Clin Microbiol. 1990;28:2559-2560.
7. Bartlett RC. A plea for clinical relevance in medical microbiology. Am J Clin Pathol. 1974;61:867-872.

The Committee recommends that all Proficiency Testing samples should be processed as routine samples even when there is a staff shortage or high workload.