



G052 Bronchial wash smear for Gram staining: + (0-1/oif) neutrophils, 1+ (0-1/oif) epithelial cells, 3+ (11-50/oif) gram-negative coccobacilli, 1+ (1-5/oif) gram-positive cocci

HISTORY This Gram smear challenge was sent to category A, B, C, and C1 laboratories with a history of being a bronchial wash collected from a 70-year old male with ventilator-associated pneumonia. It was anticipated that all laboratories would stain and report the gram-negative coccobacilli. The companion sample for culture, M052-4, was sent to category A laboratories and contained 4+ *Acinetobacter baumannii* and 1+ viridans group streptococci.

CMPT QA The smear was developed to simulate a sample collected from an elderly patient with ventilator-associated pneumonia and contained a small number of cells, including 1+ (0-1/oif) epithelial cells and 1+ (0-1/oif) neutrophils and 3+ (11-50/oif) gram-negative coccobacilli and 1+ (1-5/oif) gram-positive cocci. The challenge was verified by internal quality control, which indicated 99% accuracy based on MIL-STD-105E¹.

The reference group of laboratories all reported either few or no cells present therefore this component of the challenge was considered suitable for grading. The reference group did not meet consensus in reporting the bacterial component.

CELLULAR COMPONENT (maximum grade = 4) The cellular component received a maximum grade of 4. Of the 149 laboratories that received the slide, five were ungraded as they do not perform Gram staining. One category B laboratory received a grade of zero for not submitting a report.

Laboratories that submitted a report indicating cells reported with quantitation although reporting styles varied e.g., 1+, 1+/lpf, 0-1/oif, < 1/lpf. Reports received and grades assigned are summarized in Table 1.

BACTERIAL COMPONENT (ungraded) The reference group did not meet consensus in reporting the bacterial component. Only 4 reference laboratories reported gram-negative coccobacilli (all reported 4+); 2 out of these 4 did not report gram-positive cocci, while 1 reported gram-positive cocci and 1 reported gram-positive cocci and gram-positive bacilli. Six reference laboratories reported gram-negative bacilli (noting 2+-4+)

GRADING –Maximum grade = 4

Cellular component: All of the laboratories that processed the sample received a grade of 4/4. One laboratory (category B) received a grade of zero for not submitting a result.

Bacterial component (ungraded): 27/143 (19%) of laboratories reported only gram-positive bacteria.

NOTES

- All laboratories are encouraged to correlate Gram smear and culture results.
- It is important, for the purpose of smear and culture correlation, to recognize *Acinetobacter* as coccobacilli and different from the typical morphology of *Neisseria* species or *Moraxella* species.
- The CMPT Microbiology Advisory Committee strongly reinforces that the term 'gram-negative diplococci' be reserved for organisms suggestive of *Neisseria* or *Moraxella*.

with 1+ (n=4), 2+ (n=1) or 4+ (n=1) gram-positive cocci. Two reference laboratories reported gram-negative cocci (1 of these reported 4+ gram-positive cocci). One reported 3+ gram-positive cocci only.

The organism used in this sample was *Acinetobacter baumannii*, a gram-negative coccobacillary rod, frequently arranged in pairs. It is important, for the purpose of smear and culture correlation, to recognize these organisms as coccobacilli and different from the typical morphology of *Neisseria* species or *Moraxella* species. As stated previously in critique G033, the terms gram-negative coccobacilli and gram-negative cocci may be considered as subtle variations, but could result in different approaches to investigation, and could mislead initial therapy. Specific notes regarding the cellular morphology and Gram staining characteristics of *Acinetobacter* species are available in the companion bronchial wash specimen M052-4 critique. Results received and grades assigned are shown in Table 2.

In this challenge, 27/143 (19%) of laboratories reported only gram-positive bacteria.

Only category A laboratories received the companion culture sample and it is disappointing that the 3 category A laboratories that reported only gram-positive organisms and the 1 that reported 3+ mixed bacterial flora did not amend their reports or recheck after they all reported *A. baumannii* correctly on

(Continued on page 2)

Table 1. G052 Cellular components: Results received from category A, B, C and C1 laboratories and grades assigned.

Cells reported	A	B	C/C1	Total	Grade
0-1+ neutrophils, and/or 0-1+ epithelial cells	56	28	15	99	4
No cells seen	15	16	11	42	4
2+ neutrophils only	1	0	0	1	4
2+ neutrophils and no epithelial cells	1	0	0	1	4
Report not received-lost slide	0	1	0	1	0
Specimen not normally processed	0	0	5	5	ungraded
Total	73	45	31	149	

the culture sample. Additionally, 12 laboratories reported the presence of gram-negative diplococci. The CMPT Microbiology Advisory Committee strongly reinforces that this term be reserved for organisms suggestive of *Neisseria* or *Moraxella*. Four laboratories (1 A, 2 B, 1 C) included a report of 1+ yeast. Yeast was not included in this sample. All laboratories are encouraged to correlate Gram smear and culture results. It is appropriate to correlate the results of the two, and consider sending an amended Gram smear report. While this does not provide early useful information, it ensures consistency between the two permanent reports.

CLINICAL SIGNIFICANCE Ventilator associated pneumonia is a common infection in the intensive care unit, occurring as often as 40 episodes per 1000 patient days. In an intensive care unit with 10 ventilated patients, that would represent as many as 10 episodes every month. Often the source of the infection is the patients own aspirated flora, however commonly colonization from flora from the ICU environment, water, or personnel is the source.

The cellular composition of this slide was intentionally set as very low. As microscopists, we tend to correlate the presence of neutrophils with the presence of infection, and their absence more likely associated with colonization or contamination. While this is often a reasonable assumption, it is important to appreciate that a lung host response can often be suppressed in lung infection resulting in sparse or absent neutrophils. Neutrophil suppression can result from bone marrow suppression, medications such as steroids, antibiotics, or drugs such as alcohol, or as a consequence of massive endotoxin release from gram-negative bacteria^{2,3,4}.

REFERENCES

1. Farnum NR. 1994. Ch. 11. Acceptance sampling. p. 305-361. *Modern Statistical Quality Control and Improvement*. Duxbury Press, Belmont, California.
2. Nelson S, Chidiac C, Bagby G, Summer WR. 1990. Endotoxin-induced suppression of lung host defenses. *J Med*. 90;21(1-2):85-103.
3. Nelson S, Summer WR, Terry PB, Warr GA, Jakab GJ. 1987. Erythromycin-induced suppression of pulmonary antibacterial defenses. A potential mechanism of superinfection in the lung. *Am Rev Respir Dis*. Nov;136(5):1207-12.
4. Zhang P, Nelson S, Summer WR, Spitzer JA. 1997. Acute ethanol intoxication suppresses the pulmonary inflammatory response in rats challenged with intrapulmonary endotoxin. *Alcohol Clin Exp Res*. Aug;21(5):773-8.

Bacteria reported	A	B	C/C1	Total
Gram-negative coccobacilli	32	11	3	46
Gram-negative bacilli	32	3	1	36
Gram-negative diplococci	3	8	3	14
Gram-negative cocci	2	3	6	11
Combination of gram-negative organisms (if equal amt)	0	1 (gram-negative diplococci & gram-negative bacilli)	3 (gram-negative cocci & gram-negative diplococci [2], gram-negative coccobacilli & gram-negative cocci)	4
Mixed flora, nos; oropharyngeal flora	1	1	0	2
Gram-positive organisms only	3	15	9 (3C1)	27
No bacteria seen	0	2	0	2
No report, not processed	0	1	6	7
Total	73	46	31	149