



G044 Endotracheal aspirate for Gram staining: no neutrophils seen, 0-1+ epithelial cells, 4+ (>50/oif) gram-negative bacilli (Companion to M044-2)

HISTORY This challenge was sent to category A, B, and C laboratories as a simulated sample of endotracheal lavage prepared for examination by Gram stain. It was submitted as a sample from a patient known to be intubated and to have severe burn injuries. This challenge was designed with no neutrophils, trace numbers of epithelial cells, and many (4+) gram-negative bacilli. In the presence of a burn injury this patient would be considered as immunosuppressed, and as such the absence of neutrophils does not necessarily represent absence of infection. This sample could either reflect colonization, or infection. G044 was the companion sample to M044-2, which yielded 4+ *Serratia marcescens*. A representative view of the Gram smear may be seen on the CMPT web site.

CMPT QA The sample was created using > 50/oil immersion field (oif) of gram-negative bacilli (*Serratia marcescens*) and scant-1+ epithelial cells. Attached to the epithelial cells were rare-to-1+ gram-positive cocci, which were only part of the production process and were not included as an organism to be identified in the companion culture. Slides were internally tested for homogeneity (15% random sampling). A very small number of neutrophils may have entered with epithelial cells and not be detected with random sampling.

RESULTS and GRADES (maximum grade = 8) The sample was considered suitable for grading as all 13 reference laboratories reported the presence of 4+ gram-negative bacilli (3 reported trace gram-positive cocci). Four reference laboratories reported no neutrophils and 1+ epithelial cells, 4 reported 'no cells seen', 4 reported 0-1 epithelial cells, and 1 reported < 25 neutrophils/low power field (lpf) and < 10 epithelial cells/lpf as their laboratory protocol. The sample was sent to 151 laboratories; 144 laboratories processed the sample. Six laboratories stated they do not process smears for Gram staining and one participant did not submit a result by the due date.

-GRADING-Maximum grade = 8

The percentages of category A, B, C, and C1 laboratories receiving an acceptable grade are as follows:

Category	Cellular Component	Bacterial Component	Total (8/8 or 7/8)
A	96%	100%	98%
B	93%	96%	95%
C & C1	100%	67%	83%

NOTES

1. A complete Gram smear report should make comment of neutrophils and epithelial cells.
2. It is appropriate to correlate Gram smear and culture results.

CELLULAR COMPONENT (grade=4) Reports received and grades assigned are shown in Table 1. Of 144 laboratories reporting, only 5 received an unacceptable grade for reporting 2+ or 3+ neutrophils. It was allowed that a very small number of neutrophils may have entered with epithelial cells and not be detected with random sampling, however the reporting of 2+ or 3+ neutrophils was considered a definite reporting error. Absence of a report on neutrophils was considered as inappropriate, as was reporting > 25 epithelial cells/lpf. Those laboratories (n=15) that either did not comment on the absence of neutrophils (or report 0-1+ neutrophils) were downgraded to a grade of 3.

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Report received	A	B	C/C1	Total (% out of 144)	Grade
No cells seen	25	27	15	67 (46.5%)	4
No neutrophils seen (specific comment), 0-2+ epithelial cells	37	13	8	57 (39.5%)	4
No comment on neutrophils, 0-2+ epithelial cells	8	3	4	15 (10%)	3
2+ or 3+ neutrophils (1B reported 3+ neutrophils and 3+ epithelial cells and another included 4+ rbc)	3	2	0	5 (3.5%)	0
No report by due date	0	1	0	1	0
Gram smears not processed, refer	0	0	6	6	ungraded
Total	72	46	33	151	

BACTERIAL COMPONENT (grade=4) Table 2 lists the results received and the grades assigned. With respect to microbial content, absence of a report of many gram-negative bacilli was considered an error. Specifically the reporting of predominantly gram-positive or gram-variable bacteria was considered as a very major staining error with potential negative patient consequences. Of 144 laboratories reporting, 138 (96%) laboratories reported the presence of gram-negative bacilli. A variety of terminologies was used including 3+, 4+, and greater than 50/oif. All were considered acceptable responses. Thirty-three also reported the presence of 1+ - 2+ gram-positive cocci &/or gram-positive bacilli. As stated previously, attached to the epithelial cells were rare-to-1+ gram-positive cocci. Laboratories receiving major errors should return their slides to CMPT. If their reading is confirmed, these scores will be amended.

NOTES

Table 2. G044 Bacterial component-results received and grades assigned to category A, B, C and C1 laboratories.					
Report received	A	B	C/C1	Total (% out of 144)	Grade
4+ gram-negative bacilli	53	37	11	101 (70%)	4
4+ gram-negative bacilli with 1+ gram-positive cocci &/or gram-positive bacilli &/or gram negative cocci/diplococci	18	6	6	30 (20%)	4
4+ gram-negative bacilli with 1+ gram-positive bacilli and 2+ gram-variable bacilli	0	1	0	1	4
4+ gram-negative bacilli with 3+ gram-positive bacilli, refer	0	0	1	1	1
4+ gram-negative bacilli with 2+, 3+, 4+ gram-positive cocci &/or gram-positive bacilli &/or gram-negative cocci	1	0	4	5	1
3+, 4+ gram-positive cocci (in pairs)	0	1	1	2	0
4+ gram-positive bacilli, diphtheroids	0	0	1	1	0
4+ gram-variable bacilli with possible spores	0	0	2	2	0
4+ gram-positive coccobacilli	0	0	1	1	0
No report by due date	0	1	0	1	0
Gram smears not processed, refer	0	0	6	6	ungraded
Total	72	46	33	151	