



**G053 Wound smear (post-operative infection): no cells seen, 3+ (11-50/oif) gram-negative bacilli, 3+ (11-50/oif) gram-positive bacilli, suggestive of *Clostridia***

**HISTORY** This Gram smear challenge was sent to category A, B, C, and C1 laboratories with a history of being collected from a 65-year old male diabetic patient who returns to emergency with a post-operative wound infection. This sample was created to simulate an abdominal wall sample that would be compatible with gangrene containing *Escherichia coli* and *Clostridium perfringens*. This combination was selected as a sample that might be seen in the presence of infection with bacteria that produce cytotoxic enzymes that destroy inflammatory cells. It was anticipated that all laboratories would report the absence of cells and 3+ gram-negative bacilli and 3+ gram-positive bacilli. A companion sample for culture was not included in this survey.

**CMPT QA** The sample was constructed using cultured isolates of *Escherichia coli* [3+ (10-50/oif) gram-negative bacilli] and *Clostridium perfringens* [3+ (11-50/oif) gram-positive bacilli] in the presence of a heavy concentration of sterile horse serum. No cells were incorporated into the slide. The challenge was verified by internal quality control, which indicated 99% accuracy based on MIL-STD-105E<sup>1</sup>.

**RESULTS and GRADES (maximum grade = 4)** Eleven (73%) out of 15 reference laboratories correctly reported the absence of any cells; 4 reported the presence of neutrophils, either with (n=1) or without epithelial cells. One laboratory that reported neutrophils noted the slide was of poor quality. This resulted in less than the required 80 percent requirement for assessment of cellular content of the slide, therefore this component was not graded.

**GRADING –Maximum grade = 4**

**Cellular component (ungraded)**

**Bacterial component:** The percentages of category A, B, C, and C1 laboratories receiving a grade of 4/4 or 3/4 are:

Category A: 85% (61/72)

Category B: 75% (34/45)

Category C/C1: 64% (18/28)

**NOTES**

1. Regardless of which objective power is used for determination of cellular content of slides, cellular morphology should be confirmed using sufficient power that the cells can be accurately examined and interpreted.
2. The reporting of large gram-positive bacilli “suggestive of *Clostridia*” was seen as the optimal report because it provides information that would be immediately useful to the clinical team caring for the patient.

With respect to reporting of bacteria, 13 (86%) reference laboratories reported the presence of both gram-negative rods and gram-positive rods. One laboratory reported only the presence of gram-negative bacilli, with the comment the organisms were either overdecolourized or in mixed culture, while another reported only the presence of gram-positive bacilli with the comment that the slide was of poor quality. None the less the slide was deemed as satisfactory for assessment and the bacterial component received a maximum grade of 4.

Of the 148 laboratories that received the slide, three were ungraded as they do not perform Gram staining. One category B laboratory received a grade of zero for not submitting a report and another category B laboratory inadvertently heat fixed the slide so was unable to perform the Gram stain satisfactorily.

**CELLULAR COMPONENT (ungraded)** This sample did not contain cells. Those laboratories that reported numerous cells of any type were asked to return the slides to CMPT for review.

When serum dries on glass slides, it tends to dry unevenly with occasional areas of aggregated density. This is commonly seen in clinical samples as well as constructed EQA samples. Sometimes these aggregated densities may be of a size similar to a white blood cell, or produce smaller vacancies. If examined quickly and

(Continued on page 2)

**Table 1. Ungraded G053 Cellular components: Results received from category A, B, C, and C1 laboratories.**

Cells reported	A	B	C/C1	Total % out of 143	Grade if assigned.
No cells seen	42	29	11	82 (57%)	4
Rare/occ (0-1/oif)/few (0-2/oif), 1+ neutrophil	14	7	6	27 (19%)	3
1+ neutrophils, 1+-2+ epithelial cells	6	1	3	10 (7%)	3
1+ epithelial cells (2+ RBCs [2A])	2	1	4	7 (5%)	3
1+/occ (0-1/oif) neutrophils & (RBCs present [1A]); 4+RBCs [2B]	1	2	0	3	3
2+/4+ RBCs	2	0	0	2	1
2+/3+ neutrophils	7	2	2	11 (8%)	1
4+ neutrophils, 2+ epithelial cells	0	0	1	1	1
No report-slide heat fixed	0	1	0	1	0
Report not received	0	1	0	1	0
Specimen not normally processed	0	0	3	3	ungraded
<b>Total</b>	<b>74</b>	<b>44</b>	<b>30</b>	<b>148</b>	

only under low power (X 10 objective) these densities and vacancies might be misinterpreted as degenerated white blood cells or erythrocytes. In the presence of cytotoxicity as would be expected with *Clostridium perfringens*, this misinterpretation, while technically incorrect would not be a very major error because it would not significantly influence patient outcome. It does however indicate a problem with the accurate assessment of cells on Gram stains. **Regardless of which objective power is used for determination of cellular content of slides, cellular morphology should be confirmed using sufficient power that the cells can be accurately examined and interpreted.** While grading of the slides for cells can not be done because of failed validation, Table 1 indicates how grading would have been assessed.

**BACTERIAL COMPONENT** Although reports varied, 143 laboratories submitted a report noting bacteria present; 113/143 (79%) reported both gram-negative bacilli and gram-positive bacilli. Reports received and grades assigned are shown in Table 2.

With respect to examination of bacteria, the reporting of large gram-positive bacilli “suggestive of Clostridia” was seen as the optimal report because it provides information that would be immediately useful to the clinical team caring for the patient. Reporting gram-positive bacilli is similarly accurate but would lack the value-added nature of the interpreted comment, and was thus seen as a correct, but less optimal report.

Twenty (14%) laboratories reported the presence of gram-positive bacilli but did not mention the gram-negative bacilli.

These slides should be returned to CMPT for review.

The 7 laboratories that reported only the presence of gram-negative bacilli, or gram-negative bacilli with coccobacilli, have missed an essential element in the slide. These laboratories received a grade of zero for not reporting gram-positive bacilli.

#### REFERENCES

Farnum NR. 1994. Ch. 11. Acceptance sampling. p. 305-361. *Modern Statistical Quality Control and Improvement*. Duxbury Press, Belmont, California.

**Table 2. G053 Bacterial components: Results received from category A, B, C, and C1 laboratories and grades assigned.**

Bacteria	A	B	C/C1	Total (% out of 143)	Grade
1+-4+ gram-negative bacilli, 1+-4+ gram-positive bacilli (w/wo large, box car shape suggestive of anaerobes/Clostridia/ <i>Bacillus</i> sp. – A & B labs only)	59	32	17	108 (76%)	4
4+ gram-negative bacilli, 3+ gram-positive bacilli with spores	1	0	0	1	4
2+ gram-negative bacilli, 2+ gram-positive bacilli, few gram-negative coccobacilli, suggestive of anaerobes	1	0	0	1	3
3+ gram-negative bacilli, 3-4+ gram-positive bacilli with either 2+ gram-negative cocci or 2+ gram-negative cocci & 2+ gram-positive cocci, or 1+ yeast (1C)	0	2	1	3	3
1+-4+ gram-positive bacilli with large, box-car shape suggestive of anaerobes/Clostridia/ <i>Bacillus</i> sp. or Clostridia or lactobacillus	5	2	0	7 (5%)	1
4+ gram-positive bacilli, 3+ gram-negative cocci, refer	0	0	1	1	0
1+ gram-positive bacilli, 1+ gram-negative coccobacilli, & 1+ gram-positive cocci	0	1	0	1	0
4+ gram-positive bacilli & few gram-positive cocci, refer	0	0	1	1	0
4+ gram-positive bacilli	6	3	3	12 (8%)	0
1+-4+ gram-negative bacilli	2	2	3	7 (5%)	0
2+ gram-positive cocci, refer	0	0	1	1	0
Slide inadvertently heat fixed – no report	0	1	0	1	0
Not processed, no results, no comment received	0	1	0	1	0
Specimen not normally processed, refer	0	0	3	3	un-graded
<b>Total</b>	<b>72</b>	<b>45</b>	<b>31</b>	<b>148</b>	