

G074 Tissue smear for Gram staining (companion to M074-3): no cells seen, 2+ (2-10/oif) gram-positive bacilli, 2+ (2-10/oif) gram-negative bacilli

HISTORY This Gram smear challenge was sent to category A, B, C, and C1 laboratories with a history of being collected from a 64-year old diabetic with a gangrenous foot. Category A and B laboratories also received the companion specimen M074-3 for culturing. The smear was developed to simulate a sample collected from gangrenous tissue that did not contain cells, but did include two bacterial components. It was anticipated that all laboratories would stain and report ‘no cells seen’ and gram-positive bacilli and gram-negative bacilli. A representative view of the Gram smear may be seen on the CMPT web site.

CMPT QA The Gram stain challenge was created without cells and with 2+ (2-10/oif) gram-positive bacilli (*Clostridium perfringens*) and 2+ (2-10/oif) gram-negative bacilli (*Pseudomonas aeruginosa*). The challenge was verified by internal quality control, which indicated a 99% accuracy based on MIL-STD-105E¹.

GRADING (maximum grade = 8) The cellular and bacterial component each received a maximum grade of 4. The cellular component was validated by 87% (13/15) of the reference laboratories that reported ‘no cells or neutrophils seen’. Two reference laboratories reported low numbers (either < 1/oif or 1+) neutrophils seen. The bacterial component was validated by the reference laboratories, with all 15 reporting both gram-positive and gram-negative bacilli.

Of the 137 laboratories that received the slide, six were ungraded; three do not (routinely) perform Gram staining, two laboratories called CMPT to say due to staff shortage samples would not be processed, and one laboratory reported ‘no smear received*’. **In future challenges, in the event a compromised sample is received, laboratories that do not contact CMPT as soon as possible for replacement samples will receive a grade of zero. As PT samples are to be processed**

GRADING –Maximum grade = 8

Cellular Component: 97% (74/76) of category A, 95% (36/36) of category B, and 100% (18/19) of category C laboratories received a grade of 4/4 or 3/4.

Bacterial Component: 95% (72/76) of category A, 78% (28/36) of category B, and 37.5% (9/24) of category C laboratories received a grade of 4/4.

NOTE *C. perfringens* appear in Gram smears as large, relatively short, fat gram-positive bacillus with blunted ends, often referred to as "boxcar" shaped. Other than *Bacillus* species, there are no other common clinical isolates that resemble *Clostridia* species, particularly *C. perfringens*.

as routine samples, failure to report results even when there is a staff shortage will result in a grade of zero*.

CELLULAR COMPONENT 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. Reports received and grades assigned are summarized in Table 1.

As reported in a previous critique, G053, 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 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.

BACTERIAL COMPONENT Although reports varied, 131 laboratories submitted a report noting bacteria present; 119/131 (91%) reported both gram-negative bacilli and gram-positive bacilli. Reports received and grades assigned are shown in Table 2.

Overall, 22 (17%) laboratories reported a single bacterial component, of these 17 reported only gram-positive bacilli, 3 laboratories only reported gram-negative bacilli, 1 only reported gram-variable bacilli, and one category C laboratory reported only gram-positive cocci. These laboratories missed an essential element in the slide and received a grade of zero. The slides should be returned to CMPT for review.

Table 1. G074 Cellular components: Results received from all laboratories and grades assigned.

Cells reported	# labs	Grade
no cells seen (94); no neutrophils seen (23)	117 (89%)	4
rare/<1/oif neutrophils	2	3
1+ epithelial cells	1	3
1+ neutrophils	8	3
2+ neutrophils (A); 2+ neutrophils, 1+ epithelial cells (A)	2	1
No report (1B)	1	0
Specimen not normally processed (3C); *No report—staff shortage, called CMPT (2C); *No report-no smear received (1A)	6	Ungraded
Total	137	

(continued on page 2)

CLINICAL SIGNIFICANCE ^{2,3} It was anticipated that all laboratories would report the absence of cells and 2+ gram-negative bacilli and 2+ gram-positive bacilli. *Pseudomonas* are gram-negative, non-spore-forming bacilli that are usually thinner than *Enterobacteriaceae* measuring 0.5-0.8 µm by 1.5-3.0 µm. *C. perfringens* appear in Gram smears as large, relatively short, fat gram-positive bacilli with blunted ends, often referred to as "boxcar" shaped. Spores are rarely seen in direct swabs from tissue, but readily after several days anaerobic incubation. Other than *Bacillus* species, there are no other common clinical isolates that resemble *Clostridia* species, particularly *C. perfringens* ⁴. Therefore it is appropriate to report suggestive of *Clostridium* or *Bacillus* and ensure that both aerobic and anaerobic media are inoculated.

To assist in the identification of isolates it is recommended to record, at least on the microbiology worksheet, descriptive details beyond the basic classification of bacilli and cocci. For example, include the relative size, and depict bacilli as slender, thick, filamentous, branching, coccobacillary, club-shaped, tapered, blunt ends, boxcar-like or other adjectives as befit the observations ⁵.

Quality control of the Gram stain procedure includes a daily visual check of all reagents to ensure no precipitate has formed and staining a slide of known positive and negative organisms. The most persistent problem encountered in Gram staining is over-decolourization. It can result from cell wall damage of an organism due to prolonged exposure to solvents, the age of the organism, and use of antibiotics, which

may cause gram-positives to stain gram-negative. Over-decolorization is still possible once the counterstaining step is reached. Basic dyes applied in sequence will replace each other in bacterial cells if left on too long; the second dye simply replaces the first. Researchers found that basic dyes do this consistently regardless of the order in which they are applied, though the time required for complete replacement varies ⁶. The use of a mordant, such as iodine, significantly slows this process; however, over time, replacement occurs therefore the counterstain should not be left on the slide for prolonged periods, but used as indicated in the laboratory's SOP.

REFERENCES

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2. Blondel-Hill E, Henry DA, Speert DP. 2007. *Pseudomonas* p.734-748.. In PR Murray, et al. (eds.) *Manual of Clinical Microbiology*. 9th ed. ASM Press, Washington, DC.
3. Johnson EA, Summanen P, Finegold SM. 2007. *Clostridium*. p. 889-910. *ibid*.
4. 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*.
5. Kwong A. 1998. The Gram smear-the century old test enters the new millennium. CMPT Connections. 2:1. URL http://www.cmpt.ca/pdf_archived_articles/connections_gram_spring_98.pdf
6. McClelland R. 2001. Gram's stain: The key to microbiology. (isolate identification method) (Tutorial). 4:1. Med Lab Observer.

Table 2. G074 Bacterial components: Results received from all laboratories and grades assigned.

Bacteria	A	B	C/C1	Total	Grade
gram-positive bacilli, sugg. of <i>Clostridium/Bacillus</i> sp. (2+/3+/4+) and gram negative bacilli +/- sugg. of coliforms / <i>Pseudomonas</i> sp. (2+/3+/4+)	24	5	1	30 (23%)	4
gram-positive bacilli (1+/2+/3+/4+, 8-10, 10-20, 11-50, >25/oif) and gram-negative bacilli (1+/2+/3+/4+, 2-10, 10-25/oif)	47	22	6	75 (57%)	4
3+ gram positive bacilli, sugg of <i>Clostridium</i> or <i>Lactobacillus</i> sp. and 2+ gram negative bacilli	1	0	0	1	4
3+ gram-positive bacilli, and 1+ gram-negative bacilli, and 1+ gram-positive cocci, snnp, refer	0	1	2	3	3
2+ gram positive bacilli, sugg of <i>Clostridium</i> sp.	1	1	1	3	0
2+/3+ gram-positive bacilli (6 C refer)	3	4	7	14	0
2+/3+ gram-negative bacilli	1	2	0	3	0
3+ gram-variable bacilli, snnp	0	0	1	1	0
2+ gram-positive cocci, snnp	0	0	1	1	0
No report received (1B)	0	1	0	1	0
Specimen not normally processed, refer (3); *No report—staff shortage, called CMPT (2C); *No report-no smear received (1A)	1	0	5	6	ungraded
Total	77	36	24	137	