

Challenge G092

August 2009

Gram: Wound - *Fusobacterium nucleatum*

HISTORY

The sample was sent to category A, B, C, and C1 laboratories as a gram smear from a neck abscess of an 18 year old patient with septic shock.

CMPT QA

The smear, reviewed at CMPT, presented 4+ (>10/oif) neutrophils and 3+ (11-50/oif) gram negative bacilli (see Figure 1). The challenge was verified by internal quality control, which indicated 99% accuracy based on MIL-STD-105E¹.

SURVEY RESULTS

Reference Labs:

cells - 14 labs reported >25/oif, 2+, 3+, 4+ neutrophils, 1 lab reported 3+ neutrophils, 1+ epithelial cells

bacteria - 15/15 labs reported >25/oif, 3+, 4+ gram negative bacilli

All 15 of the reference labs reported the presence of neutrophils and gram negative bacilli. Consensus was achieved by the reference labs thus the sample was considered for grading.

Cell component (see table 1)

The majority of the labs (95%) received a grade of 4 for the cell component, reporting neutrophils between 2 to >25/oif or 2+ to 4+.

One lab also reported the presence of mononuclear cells together with neutrophils, another reported the presence of red blood cells, three

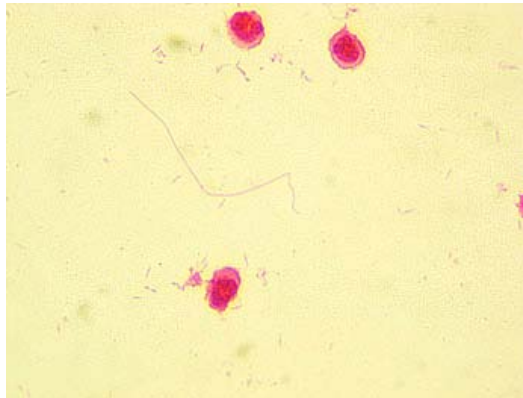


Figure1: G092 gram smear. The sample shows neutrophils and long, gram negative, fusiform bacteria

laboratories reported the presence of epithelial cells in addition to neutrophils. Mononuclear cells, red blood cells or epithelial cells were not in the preparation, but laboratories were not downgraded for these observations.

One laboratory did not report neutrophils in the sample and was given a grade of zero.

Bacterial component (see table 2)

Overall, 94% of the laboratories (97% of A, 94% of B, 80% of C and 92% of C1 laboratories) successfully identified gram negative bacilli in the smear; many of them described it as “suggestive of *Fusobacterium* species, fusiforms or anaerobes”.

One laboratory reported the presence of gram positive cocci in addition to the gram-negative bacilli and received a grade of 1.

Another lab reported gram negative coccobacilli

Table –1: Reported results for G092 smear – Cell component -

| Reported results (cell component) | Total | % | Grade |
|---|------------|------------|----------|
| 2+, 3+, 4+, 0-35, >10, 20, >25/oif neutrophils +/- 1+ RBC's | 130 | 94.9 | 4 |
| 2+ neutrophils (4+ mononuclear WBC's) | 1 | 0.7 | 4 |
| 3+, 4+ neutrophils, 1+ epithelial cells | 3 | 2.2 | 4 |
| no neutrophils seen | 1 | 0.7 | 0 |
| snp, refer | 2 | 1.4 | ungraded |
| Total | 137 | 100 | |

snp: sample not normally processed

Grading

Maximum grade: 8 (4 points each for cell and bacterial component).

- Reporting mononuclear cells, red blood cells or epithelial cells was not considered to be serious enough in this type of sample and the laboratories reporting them were not downgraded.
- Not reporting neutrophils in the sample was considered unacceptable and was graded zero.
- Reporting gram positive microorganisms with gram negative bacilli was considered serious enough to downgrade to one due to the therapeutic implications of such report.
- Reporting only gram positive microorganisms was graded as zero due to the therapeutic implications of such report.
- Reporting gram negative coccobacilli was downgraded to 1 because the sample contained typical long slender rods

instead of gram negative bacilli – although the organisms were pleomorphic, the sample contained typical long slender rods. This laboratory will receive a grade of 1. Three percent of the labs reported only gram positive bacteria and received a grade of zero. Reporting gram positive organisms may lead to inappropriate treatment. These laboratories are asked to reexamine their slides and return them to CMPT for review.

Two participants indicated this kind of sample is not normally processed in their laboratories. One of these labs commented they had problems with the smear and suspected the sample

COMMENTS ON RESULTS

Some laboratories reported the presence of gram positive cocci in addition to the gram negative bacilli. Crystal violet precipitate may appear as small gram-positive cocci in dense clumps and these clumps could be misinterpreted as gram-positive cocci.

Four laboratories reported gram-positive bacilli which suggests the smear may not have been sufficiently decolorized.

CLINICAL SIGNIFICANCE

Fusobacterium species belong to the family *Bacteroidaceae* and are non-spore-forming gram-negative anaerobic bacilli. The term is derived from the Latin word *fusus*, referring to its spindle-shaped appearance.^{2,3}

The typical gram stain of *Fusobacterium nucleatum* shows slender gram-negative rods with pointed ends. The microscopic morphology of *Fusobacterium mortiferum* reveals filaments containing swollen areas with large, round bodies and exhibits irregular staining. *Fusobacterium necrophorum* has a similar morphology to

F. mortiferum but usually has fewer round bodies^{2,4,5}.

F. nucleatum is usually found in the gastrointestinal, oropharyngeal and respiratory tracts and is most commonly associated with periodontal disease^{2,4,6}. Oropharyngeal infection can get complicated in some cases by neck-vein thrombosis.⁵

Like *F. necrophorum*, *F. nucleatum* is capable of causing thrombophlebitis of the internal jugular vein in previously healthy young adults, usually following pharyngo-tonsillar infection (Lemierre's syndrome), sometimes with metastatic suppuration.^{5,7}

REFERENCES

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3. Citron DM, Poxton IR, Baron EJ. *Bacteroides, porphyromonas, prevotella, fusobacterium*, and other anaerobic gram-negative rods. In: Murray et. al, ed. *Manual of Clinical Microbiology*. Vol 2. 9th ed. ed. Washington, DC.: ASM; 2007:911.
4. El Braks R, Harnois F, Boutros N, et al. Mesenteric adenitis and portal vein thrombosis due to *Fusobacterium nucleatum*. *Eur J Gastroenterol Hepatol*. 2004;16:1063-1066.
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6. Bultink IE, Dorigo-Zetsma JW, Koopman MG, Kuijper EJ. *Fusobacterium nucleatum* septicemia and portal vein thrombosis. *Clin Infect Dis*. 1999;28:1325-1326.

Gram Stain

The Gram stain is still the fastest, simplest, and the best way to yield significant information among rapid methods. Gram stains should be prepared from all specimens accepted for anaerobic culture.

The morphotypes and relative quantities of both the host and the bacterial cells present in the preparation should be reported.

Standard gram stain procedures and reagents are used. Basic fuchsin (0.1 to 0.5%) enhances the staining of gram negative anaerobes and can be substituted for safranin as the counterstain.³

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

Table-2: Reported results for G092 smear – Bacterial component -

| Reported results (bacterial component) | A | B | C | C1 | Total | % | Grade |
|--|-----------|-----------|-----------|-----------|------------|--------------|----------|
| 2+, 3+, 4+, 0-2/oif gram negative bacilli, >25 to >100/oif gram negative bacilli, suggestive of <i>Fusobacterium</i> species/ fusiforms/anaerobes +/- snnp,+/- refer | 76 | 29 | 12 | 12 | 129 | 94.2 | 4 |
| 4+ gram negative bacilli, fusiform, 1+ gram positive cocci | | 1 | | | 1 | 0.7 | 1 |
| 3+, 4+ gram positive bacilli +/- 1+ gram positive cocci | 2 | | 1 | 1 | 4 | 2.9 | 0 |
| 4+ gram negative coccobacilli | | 1 | | | 1 | 0.7 | 1 |
| snp, refer | | | 2 | | 2 | 1.4 | ungraded |
| Total | 78 | 31 | 15 | 13 | 137 | 100.0 | |

snp: sample not normally processed