CMPT Clinical Bacteriology Program

Innovation, Education, Quality Assessment, Continual Improvement

Challenge G244

Canadian

testing

microbiology proficiency

February 2025

Gram: eck abscess: 4+ (>10/oif) neutrophils and 4+ (>50/oif) gram negative bacilli (*Fusobacterium necrophorum*)

HISTORY

cmpt

A simulated neck abscess sample collected from a 20 year old in-patient with swollen neck was sent to category A and C1 laboratories.

Participants were expected to report the presence of neutrophils and gram negative bacilli.

CMPT QA/QC/STATISTICS

The samples are assessed for homogeneity and stability using in-house quality control methods and random selection of samples before and during production, a nd post sample delivery. The number of random samples selected is based on selection tables within Military standard $105E^{-1}$

The sample contained 4+ (>10/oif) neutrophils and 4+ (>50/oif) gram negative bacilli (Figure 1). A culture of *Fusobacterium necrophorum* was used to prepare the slides.

Cells were prepared from whole peripheral blood. There were no epithelial cells added to the sample.



Figure 1. Gram stain of G244; simulated neck abscess smear at 1000X magnification under oil immersion demonstrating gram negative bacilli and neutrophils.

MAIN EDUCATIONAL POINTS from G244

- Reporting both neutrophils and a description of the observed organism(s) is important. The significant number of neutrophils suggests that a good quality specimen was obtained and the description of the organism(s) aids in the empiric antimicrobial management of the patient.
- 2. Experienced technologists, observing long filamentous gram negative bacilli may be comfortable suggesting that these are potentially consistent with anaerobes ensuring clinicians include anaerobic coverage in their empiric therapy.

SURVEY RESULTS

Reference laboratories

<u>Cells:</u> 12/12 labs reported >25/lpf, 4+ neutrophils/white blood cells, 1 lab reported it does not normally process this type of sample

<u>Bacteria:</u> 12/12 labs reported 4+ gram negative bacilli ± resembling anaerobes, 1 lab reported it does not normally process this type of sample

Participants

<u>Cells:</u> 51/51 (100%) reporting laboratories reported 3+ to 4+ or >25/lpf neutrophils (Table 1)

<u>Bacteria:</u> 50/51 (98%) participants reported gram negative bacilli ± resembling anaerobes (Table 2)

Suitability for Grading

A challenge is considered suitable for grading if agreement is reached by 80 percent of selected reference group and at least 50 percent of the participants.

Table 1. Reported results—Cells

Reported	cat A	cat C1	Total	Grade
>25/lpf, 3+, 4+ neutrophils	47	4	51	4
no report	3		3	0
sample not normally processed	1		1	ungraded
Total	51	4	55	

Grading

Maximum grade: 8

Reporting the presence of neutrophils was graded 4.

Reporting gram negative bacilli was graded 4. Identification of cell and bacteria components was correctly performed by at least 80 percent of reference laboratories and greater than 50 percent of all laboratories thus, both components were determined to be suitable for grading.

COMMENTS ON RESULTS

The vast majority of participating labs did well on this challenge with most correctly reporting at least 3+ neutrophils and 4+ gram negative bacilli. Some labs included a report suggesting that the observable organisms were suggestive of anaerobic bacilli.

CLINICAL SIGNIFICANCE

Fusobacterium species belong to the family *Bacteroidaceae* and are anaerobic gram-negative 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 and while *Fusobacterium necrophorum* has a similar morphology it usually has fewer round bodies.^{2,4,5}

Both *F. nucleatum* and *F. necrophorum* are usually found in the gastrointestinal, oropharyngeal and respiratory tracts and are associated with several diseases including periodontal disease, recurrent tonsilitis, Lemierre's syndrome, brain abscesses, and bacteremia's'.^{2,4,5,6}

REFERENCES

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Reported	cat A	cat C1	Total	Grade
4+ (>50/oif) gram negative bacilli/rods/Bâtonnets gram négatif ± resembling/ suggestive of anaerobes	46	4	50	4
4+ (>50/oif) GNB	1		1	0
no report	3		3	0
sample not normally processed	1		1	ungraded
Total	51	4	55	

Table2. Reported results - Bacteria