

Challenge G244-1

February 2025

Gram: Joint fluid: 3+ (6-10/oif) neutrophils, 3+ (11-50/oif) gram positive bacilli (*Clostridium perfringens*) + and 4+ (>50/oif) gram negative bacilli (*E. coli*)

HISTORY

A simulated joint fluid sample collected from a 32 year old athlete with knee trauma 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, and post sample delivery. The number of random samples selected is based on selection tables within Military standard 105E.¹

The sample contained: 3+ (6-10/oif) neutrophils, 3+ (11-50/oif) gram positive bacilli and 4+ (>50/oif) gram negative bacilli (*E. coli*) (Figure 1). A culture of *Clostridium perfringens* and *E. coli* was used to prepare the slides.

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

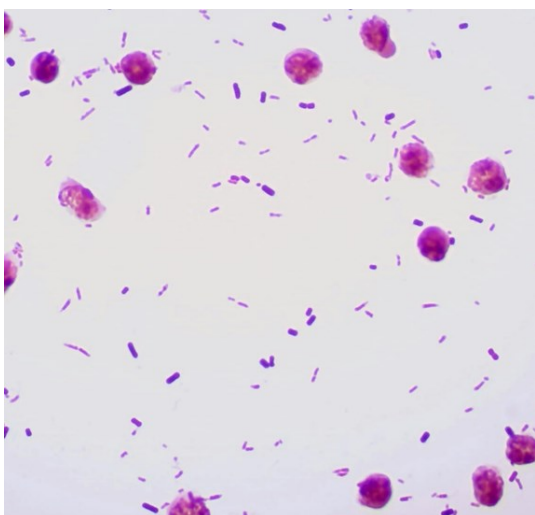


Figure 1. Gram stain of G2S44-1; simulated joint fluid smear at 1000X magnification under oil immersion demonstrating gram positive and gram negative bacilli, and neutrophils.

MAIN EDUCATIONAL POINTS from GS244-1

1. Detecting *Clostridium perfringens* and *Escherichia coli* in joint fluid following a traumatic injury suggests a polymicrobial wound infection. This could potentially lead to severe complications such as necrotizing soft tissue infections or gas gangrene.
2. While many organisms such as *E. coli* can be found as part of the normal skin flora, their presence in synovial fluid should raise suspicions and be reported accordingly.
3. Rapid and precise Gram-staining is essential, as treatment options in this case often dictate the use of broad-spectrum antibiotics that target both gram-negative and gram-positive bacteria.

The challenge sample lot was confirmed to be homogeneous and stable for at least 15 days after shipping day.

All challenge components have in-house assigned values based on the most clinically appropriate result; the most clinically appropriate result is determined by expert committee evaluation. No further statistical analysis is performed on the results beyond that described under "Suitability for grading."

Grading

Maximum grade: 4

Reporting the presence of neutrophils was graded 4.

The bacteria component of this challenge was ungraded.

SURVEY RESULTS

Reference laboratories

Cells: 10/10 (100%) labs reported >25/lpf, 3+, 4+ neutrophils/white blood cells

Bacteria: 6/10 labs reported 3+, 4+ gram positive bacilli and 3+, 4+ gram negative bacilli, 4/10 labs reported 3+, 4+ gram positive bacilli/rods - UNGRADED due to lack of consensus

Participants

Cells: 34/34 (100%) labs reported 1+ to 4+ or >25/oif neutrophils or white blood cells (Table 1).

Bacteria: 23/34 (68%) participants reported gram positive and gram negative bacilli. The rest of the laboratories reported gram positive or gram negative bacilli only, gram variable bacilli, or gram negative bacilli and gram positive cocci (Table 2).

Table 1. Reported results—Cells

Cells Reported	cat A	cat C1	Total	Grade
>25/lpf, 1+, 2+, 3+, 4+ neutrophils, white blood cells	32	2	34	4
Total	32	2	34	

COMMENTS ON RESULTS

All participant and reference labs (44/44 – 100%) were able to identify the presence of neutrophils/white blood cells on the slide smear. Since >80% of Category A labs and >50% of participant labs reached agreement.

The majority of the participant labs (23/34 - 68%) correctly reported the presence of 3+ gram-positive bacilli and 4+ gram-negative bacilli bacteria on the smear. The remaining participants reported gram-positive or gram-negative bacilli only, gram-variable bacilli, or gram-negative bacilli and gram-positive cocci. Only 6/10 (60%) of reference labs correctly reported the results. The remaining reference labs reported only gram-negative bacilli, gram-positive bacilli or gram-positive rods. Because only 60% of the Category A labs reported the correct bacterial Gram-stain morphology, consensus was not reached for this particular portion of the assessment.

CLINICAL SIGNIFICANCE

Traumatic injury can result in the introduction of soil and fecal organisms including *Clostridium* species into wounds.

Highly soiled open fractures or wounds contaminated with environmental organisms are at high risk for *Clostridium* species infection. Infections and related tissue damage result from the combination of injury and microbial virulence features, including exotoxins. *Clostridium* species account for 30% of all anaerobic organisms recovered from post-traumatic wounds.²⁻⁵

Commonly the Gram stain of tissue or fluid infected with *C. perfringens* demonstrates many characteristic gram positive bacilli, often with reduced numbers of polymorphonuclear cells

(PMNs) many of which are damaged.⁶ *Clostridium* species produce a range of toxins.⁷ The α toxin of *C. perfringens* is a phospholipase C that is both hemolytic and cytotoxic to platelets and leukocytes due to its ability to cleave sphingomyelin and the phosphoglycerides present in eukaryotic cell membranes.⁸ The *C. perfringens* θ toxin (perfringolysin O) is similarly cytolytic for PMNs, especially in high concentration.⁸

C. perfringens, the species most frequently encountered in infections, does not normally sporulate in tissue, and thus the gram positive rods seen on Gram stain of tissue or other patient specimens do not usually exhibit sporulation. Instead, the classic appearance is that of large, boxy gram positive rods with blunt ends, and may be encapsulated in smears from wounds.⁷

While *Staphylococcus aureus* and *Streptococcus pyogenes* are the common causes of soft tissue infection, *Escherichia coli*, a gram-negative facultative anaerobe, can occasionally contribute to wound infections, cellulitis, and abscess formation.⁹ If left untreated, may lead to sepsis. Certain strains of *E. coli* produce virulence factors such as endotoxins, capsules, and biofilms, which help the bacteria evade the immune system and cause tissue damage.¹⁰ In some instances, *E. coli* isolated from wounds may exhibit greater virulence than strains obtained from urinary tract infections or bacteremia.¹⁰ In polymicrobial infections, *E. coli* may interact synergistically with other organisms, increasing the risk of more severe infections. Consequently, prompt wound debridement, the administration of broad-spectrum antibiotics, and careful monitoring for systemic complications are crucial for effective management.

Table2. Reported results - Bacteria

Reported	cat A	cat C1	Total	Grade
2+, 3+, 4+ gram positive bacilli, 2+, 3+, 4+ gram negative bacilli	22	1	23	ungraded
3+ gram positive bacilli, rods \pm large	4	1	5	ungraded
4+ gram negative bacilli	3		3	ungraded
4+ gram negative bacilli - multiple morphotypes	1		1	ungraded
4+ gram negative bacilli, 4+ gram positive cocci	1		1	ungraded
4+ gram variable bacilli	1		1	ungraded
Grand Total	32	2	34	

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