Challenge G244-2

February 2025

Gram: CSF: 3+ (6-10/oif) neutrophils, 4+ (>50/oif) gram negative coccobacilli (*Haemophilus influenzae*)

HISTORY

A simulated joint fluid sample collected from an 8 year old with no history of vaccination was sent to category A and C1 laboratories.

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

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, 4+ (>50/oif) gram negative coccobacilli (Figure 1). A culture of *Haemophilus influenzae* was used to prepare the slides.

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

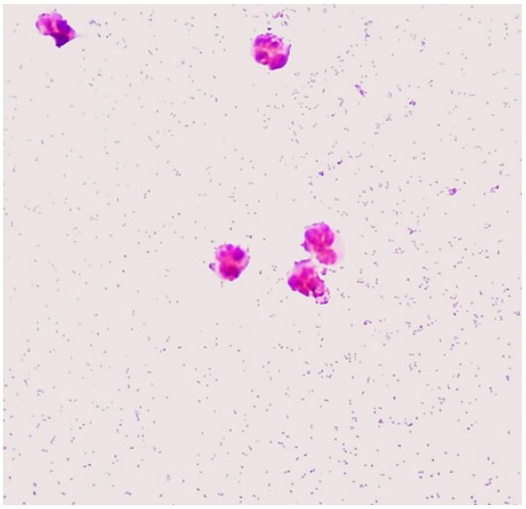

Figure 1. Gram stain of G2S44-2; simulated CSF smear at 1000X magnification under oil immersion demonstrating gram negative coccobacilli and neutrophils.

MAIN EDUCATIONAL POINTS from GS244-2

1. Gram stain examination of CSF specimens is a rapid method to visualize the presence of white blood cells (indicative of an infectious process), with higher likelihood of positive microscopy for preliminary identification of bacterial agents when there are 105 cfu/ml or greater number of organisms.
2. The presence of small gram negative organisms with pleomorphic (variable) coccobacillus appearance provides presumptive identification of *Haemophilus influenzae*.
3. Vaccination against Haemophilus influenzae type b (Hib, capsular serotype b) has led to a significant reduction of meningitis cases globally. However Hib is still an important cause of meningitis in regions with low vaccine coverage rates. Invasive diseases by other serotypes of *Haemophilus influenzae* have increased in prevalence over the last 2 decades.

The challenge sample lot was confirmed to be homogeneous and stable for at least 15 days from shipment.

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: 8

Reporting neutrophils was graded 4

Reporting gram negative bacilli/coccobacilli was graded 4.

SURVEY RESULTS

Reference laboratories

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

Bacteria: 10/10 labs reported 4+ gram negative coccobacilli ± suggestive of *Haemophilus* species

Participants

Cells: 34/34 (100%) laboratories reported 2+ to 4+ or >25/lpf neutrophils or white blood cells (Table 1)

Table 1. Reported results—Cells

Reported	cat A	cat C1	Total	Grade
>25/lpf, 10-25/lpf, 2+, 3+, 4+ neutrophils, white blood cells	32	2	34	4
Total	**32**	**2**	**34**	

Bacteria: 27/34 (79%) participants reported gram negative coccobacilli/bacilli; 4 labs reported gram negative diplococci, 1 gram negative cocci, and 2 gram positive cocci (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.

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

All labs correctly reported the presence of 2+ to 4+ (>25/low power field) white blood cells/ neutrophils.

Most labs (27/34 or 79% participants) properly characterized the gram stain with identification of coccobacillus.

The variable appearance of this bacterium often leads to classification as cocci; 5 labs reported gram negative cocci (4 labs: gram negative diplococci, 1 lab: gram negative cocci).

Two labs performed the Gram stain procedure incorrectly and reported gram positive cocci.

CLINICAL SIGNIFICANCE

Microscopic examination of CSF by Gram stain is a rapid, inexpensive test that can provide valuable information to the clinician when performed and interpreted by skilled technologists.

The presence of white blood cells (WBCs), along with the quantity and type of cell can be useful in identifying samples that require closer scrutiny by laboratory staff.

Gram stain examination of CSF permits rapid, preliminary identification of the causative microorganism in 60% to 90% of patients with bacterial meningitis (with a specificity of nearly 100%). [6]

The likelihood of detecting the organism by Gram stain correlates with the concentration of bacteria in CSF. Concentrations of 10^3 or fewer colony-forming units per milliliter (cfu/ml) are associated with positive Gram stains about 25% of the time, whereas CSF concentrations of bacteria of 10^5 cfu/ml or greater lead to positive microscopy results in up to 97% of cases.[2,3]

Use of cytospin centrifugation will not only maintain better WBC morphology, but can increase chances of observing organisms by up to 100 times when compared to unconcentrated samples, or those concentrated by conventional means. [3]

The clinical utility of the Gram stain also depends on the bacterial pathogen. Bacteria have been observed in 90% of meningitis cases caused by Streptococcus pneumoniae, 86% of cases by *Haemophilus influenzae*, 75% of cases caused by *Neisseria meningitidis*, and 50% of cases caused by gram negative bacilli.

The Gram stain of CSF is positive in less than 50% of patients with meningitis due to *Listeria monocytogenes* or anaerobes. [3]

H. influenzae was previously isolated in 45% to 48% of all cases of bacterial meningitis in the United States. Currently, this organism is now isolated in only 7% of cases. Most cases of meningitis previously occurred in infants and children younger than 6 years (peak incidence of 6 to 12 months), with 90% of cases caused by capsular *H. influenzae* type b strains.

A profound reduction has been seen in the incidence of invasive infections caused by *H. influenzae* type b, attributed to the widespread use of conjugate vaccines. The number of *H. influenzae* type b cases of meningitis since the introduction of vaccination has decreased more than 90%. [2,4] In Canada, *H. influenzae* type a has emerged as an important cause of invasive disease, including meningitis. [5]

Table2. Reported results - Bacteria

Reported	cat A	cat C1	Total	Grade
3+, 4+ gram negative coccobacilli, ± suggestive of *Haemophilus* species ± or anaerobe	24	1	25	4
4+ gram negative bacilli, small	2		2	4
4+ gram negative cocci	1		1	?
4+ gram negative diplococci	3	1	4	0
4+ gram positive cocci	2		2	0
Total	**32**	**2**	**34**	

All isolates of *H. influenzae* from cases of invasive disease should be further investigated to determine the capsular serotype. In addition to providing valuable epidemiological information, this will also assist in ruling out the possibility of a vaccine failure.

REFERENCES

1. Famum NR. Acceptance sampling. In: Modern Statistical Quality Control and Improvement. Belmont, California.: Duxbury Press; 1994: 305.

2. Tunkel AR, van de Beek D, Scheld WM. Acute meningitis. In: Bennett JE, Dolin R, Blaser MJ, ed. Principles and Practice of Infectious Diseases 8th ed. Elsevier Saunders: Philadelphia; 2015: 1097-1137.

3. Gray LD, Fedorko DP. Laboratory diagnosis of bacterial meningitis. Clin Microbiol Rev. 1992; 5: 130-145.

4. Steinhoff MC. Haemophilus influenzae type b infections are preventable everywhere. Lancet. 1997; 349: 1186-1187.

5. Tsang RSW, Ulanova M. The changing epidemiology of invasive Haemophilus influenzae disease: emergence and global presence of serotype a strains that may require a new vaccine for control. Vaccine 2017; 35: 4270-4275.

6. van de Beek D, Brouwer M, Hasbun R, Koedel U, Whitney CG, Wijdicks E. Community-acquired bacterial meningitis. Nat Rev Dis Primers. 2016 Nov 3; 2: 16074.