Challenge GS234-1

February 2024

Gram - CSF: no neutrophils; 4+ (>50/oif) gram positive diplococci (*Streptococcus pneumoniae*)

HISTORY

A simulated CSF collected from a 74 year old alcoholic was sent to category A and C1 laboratories. Participants were expected to report no cells and gram positive cocci/diplococci.

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 - no neutrophils; 4+ (>50/oif) gram positive diplococci (Figure 1). A culture of *Streptococcus pneumoniae* was used to prepare the slides.

There were no epithelial cells added to the sample. The challenge sample lot was confirmed to be homogeneous and stable for 56 days.

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."

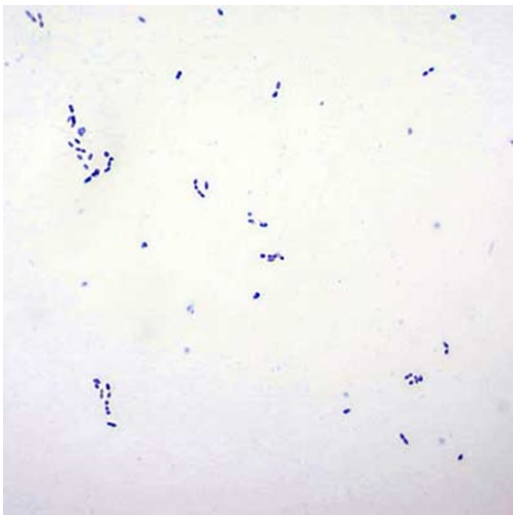

Figure 1. Gram stain of GS234-1; simulated joint fluid smear at 1000X magnification under oil immersion demonstrating gram positive diplococci.

MAIN EDUCATIONAL POINTS from GS234-1

1. Gram stains of CSF demonstrating organisms give rapid information of the course of meningeal infection.
2. The Gram reaction and morphology of the organisms observed in a CSF Gram stain are important for the presumptive identification of the causative organism.
3. The higher the organism load in CSF, the greater the likelihood of detecting the organism in the Gram stain, however this is also pathogen dependent.

Grading

Maximum grade: 8

Reporting the absence of cells was graded 4.

Reporting gram positive cocci/diplococci was graded 4.

SURVEY RESULTS

Reference laboratories

Cells: 8/10 (80%) labs reported "none seen" for neutrophils, 2 labs reported 1+ red blood cells

Bacteria: 10/10 (100%) labs reported 3+, 4+ gram positive cocci, ± pairs, ± chains (2 labs also reported suggestive of *Streptococcus* ± *Enterococcus*)

Participants

Cells: 27/35 (77%) reporting labs, indicated the absence of cells (Table 1).

Bacteria: 35/35 (100%) labs reported gram positive cocci (Table 2).

Table 1. Reported results—Cells

Reported	Cat A	Cat C1	Total	Grade
none seen/no leukocytes seen	25	2	27	4
no report*	2		2	0
1+ (rare) red blood cell	2		2	0
1+ (<1/ oif) neutrophils	1		1	0
1+ (<1/ oif) epithelial cells	1		1	0
2+ (1-5/oif) neutrophils, 2+ red blood cells	1		1	0
4+ (>10/oif) neutrophils	1		1	0
Total	**33**	**2**	**35**	

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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

Cellular component: It was expected that participants would report the absence of neutrophils, along with the presence of gram positive cocci in pairs/chains, suggestive of *Streptococcus pneumoniae*.

Overall, 77% (27/35) reported the absence of neutrophils and were graded 4; an additional 6% (3/35) reported very low numbers of either neutrophils or RBC's and were graded 0 as there were no cells of any type added to the sample. Two labs did not report on the cellular component while another three labs reported the presence of either epithelial cells or higher numbers of neutrophils ± RBC's – these labs were also graded 0.

Bacterial component: All participants (35/35) reported the bacterial component correctly and were graded 4,

CLINICAL SIGNIFICANCE

Bacterial meningitis is a medical emergency. If suspected, empiric therapy should be started immediately pending laboratory results. The 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 sensitivity of the Gram stain for the detection of organisms in patients with bacterial meningitis is dependent on the organism load in the CSF. When there are 10^3 or fewer colony-forming units per milliliter (cfu/ml) only about 25% will have a positive Gram stain, whereas when there are 10^5 cfu/ml or greater as many as 97% of cases will have a positive smear.[2,3]

The clinical utility of the Gram stain also depends on the bacterial pathogen. Adults with pneumococcal meningitis have been found to have positive Gram stains in 81–93% of cases.[4] Bacteria have been observed in 86% of meningitis cases caused 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 around 30% of patients with meningitis due to *Listeria monocytogenes* or anaerobes.[2]

The usual causes of community acquired bacterial meningitis are commonly age-group related. In people older than 16 years with bacterial meningitis, *Streptococcus pneumoniae* causes 72% of all cases and *Neisseria meningitidis* causes 11% of all cases. [5]

Invasive pneumococcal disease is typically seen in patients less than 2 or more than 50 years of age. Risk factors include splenectomy, sickle cell disease, multiple myeloma, hypogammaglobulinemia, alcoholism, chronic liver or kidney disease, malignancy, and malnutrition.[6]

Two important medical advances—antibiotics and vaccination—have dramatically decreased the incidence and the case fatality rate of bacterial meningitis, particularly within pediatric populations.[6] Currently, *S pneumoniae* is the most common cause of acute bacterial meningitis for children older than 1 month.[7]

Meningitis due to *S. pneumoniae* has been, and continues to be, associated with poor outcomes – with optimal clinical management, it has a mortality rate of approximately 20%. Additionally, approximately half of the survivors will have a long-term impact such as hearing loss. Damage to the brain is due to a combination of bacterial virulence factors and host response.[8]

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Table2. Reported results - Bacteria

Reported	Cat A	Cat C1	Total	Grade
3+, 4+ gram positive cocci ± pairs, ± chains, ± suggestive of S. pneumoniae	30	2	32	4
4+ (>50/oif) gram positive cocci suggestive of the genus Streptococcus/Enterococcus	2		2	4
4+ gram positive diplococci	1		1	4
Total	**33**	**2**	**35**	

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