cmpt Canadian microbiology proficiency testing

CMPT Clinical Bacteriology Program

Innovation, Education, Quality Assessment, Continual Improvement

Challenge GS242-1

August 2024

Gram - CSF: *4*+ (>10/oif) neutrophils and 4+ (>50/oif) gram negative diplococci (*Neisseria* species)

HISTORY

A simulated CSF sample collected from a 40 year old male traveler was sent to category A and C1 laboratories.

Participants were expected to report the presence of neutrophils and gram negative 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 3+ (6-10/oif) neutrophils and 3+ (11-50/oif) gram negative diplococci (Figure 1). A culture of *Neisseria gonorrhoeae* was used to prepare the slides.

Cells were prepared from whole peripheral blood. There were no epithelial cells added to the sample. The challenge sample lot was confirmed to be homogeneous and stable for 14 days.

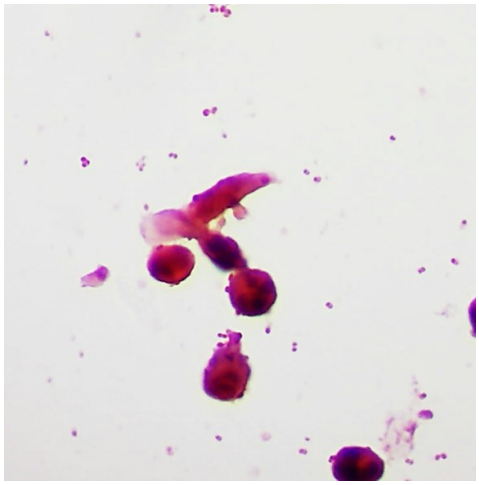

Figure 1. Gram stain of GS242-1; simulated CSF smear at 1000X magnification under oil immersion demonstrating gram negative diplococci and neutrophils.

MAIN EDUCATIONAL POINTS from GS242-1

1. An urgent cerebrospinal fluid collected in the emergency room should be examined rapidly in the microbiology lab to confirm what most likely will be either consistent with meningococcal meningitis (with many neutrophils and gram negative diplococci/ cocci), or viral (with a lymphocytosis and no bacteria observed).
2. This finding would be important to report as a critical value to the health care provider due to the need for rapidly initiating treat ment1 (if not already started) and for Public Health notification for initiation of investigation and measures to prevent spread of the pathogen within close contacts of the individual.
3. Microbiology laboratories should confer with their chemistry (low glucose) and haematology (a significant neutrophil response) colleagues to confirm the bacterial nature of the infection.

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 cocci/diplococci was graded 4.

SURVEY RESULTS

Reference laboratories

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

Bacteria: 8/10 (80%) labs reported 3+, 4+ gram negative diplococci, 2 labs reported 4+ gram negative cocci

Table 1. Reported results—Cells

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

Participants

Cells: 34/34 (100%) participants reported the presence of neutrophils (Table 1).

Bacteria: 28/34 (82%) labs reported gram negative diplococci, 3 participants reported gram negative cocci, 2 labs reported gram positive cocci, and one participant reported using acronyms (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

Overall, the participants did well in reporting this Gram Smear challenge. All labs reported a large number of neutrophils, leukocytes, and white blood cells, which is what was expected from the sample type. The majority of the 31/34 laboratories got the organism morphology part right. 82 percent of the participating laboratories got acceptable answers that are in line with the reference laboratory number (80%). The grading for the 3 laboratories that got the morphology or gram stain wrong are graded as zero, because these are critical errors and can have major treatment/intervention implication.

CLINICAL SIGNIFICANCE

Meningococcal meningitis

N. meningitidis causes sporadic cases of meningitis and outbreaks. *N. meningitidis* is the most common pathogen causing bacterial meningitis in young children beyond the neonatal period, teenagers, and in young adults. An increased incidence of invasive meningococcal disease has been observed for patients with deficiencies in the terminal complement components.

The clinical manifestations of meningococcal disease include meningoencephalitis, meningitis with or without meningococcemia, meningococcemia without meningitis, and bacteremia without sepsis. [2] Variations of these scenarios have also been reported, and the patient may progress from one manifestation to another during the course of their disease. Even for patients with culture proven meningococcal meningitis, the classical triad of neck stiffness, fever, and altered consciousness may be uncommon and found in as few as 27% of patients. [3]

In patients with meningococcemia, CSF and blood are the most commonly collected specimens. Samples from synovial, pleural, or pericardial fluid may also be submitted but have lower sensitivity. [4]

Direct examination of the CSF or other sterile sites (other than blood) using Gram stain reveals gram negative diplococci both intra and extracellular.. Use of a cytospin centrifuge has been reported to increase the sensitivity of the Gram stain. [5]

CSF Gram stains can frequently identify a bacterial pathogen when present and frequently can indicate the probable bacterial genus/species causing meningitis. In patients with appropriate clinical presentation, a negative CSF Gram stain may suggest the infection may have a viral etiology. A CSF Gram stain also demonstrates the host immune response and its composition, and provides clues useful to care providers in formulating a treatment plan.

Organisms may show considerable size variation and tend to resist decolourization. The morphology of the diplococci may be useful to suggest the possibility of under decolourization. Heavily encapsulated strains may have distinct pink halos around cells. Quantitation of white blood cells and organisms should be reported as this has value in prognostic scoring. [6]

The detection of gram-negative diplococci in CSF is presumptive evidence of meningococcal meningitis. Although the initial treatment of bacterial meningitis is based on an algorithm, the Gram stain findings are essential to establish a bacterial etiology. It also suggests the type of bacteria causing infection which may allow treatment to be narrowed. Culture can be less sensitive than the Gram stain if treatment was initiated before lumbar puncture was performed. [7] If the CSF culture is negative, nucleic acid amplification (NAA) assays are useful, particularly for partially treated meningitis, and can also provide information regarding the serogroup of the isolate of *N. meningitidis* detected.

The clinical utility of the Gram stain may depend on the bacterial pathogen. Bacteria have been observed in 90% of Gram stains

Table2. Reported results - Bacteria

Reported	c at A	cat C1	Totals	Grade
2+, 3+, 4+ gram negative diplococci ± suggestive of Neisseria ± or Moraxella	26	2	28	4
4+ gram negative cocci (pairs)	1		1	4
4+ gram negative cocci	2		2	4
3+ GNDC	1		1	0
4+ gram positive cocci	2		2	0
Total	**32**	**2**	**34**	

of meningitis cases caused by *Streptococcus pneumoniae*, 86% of cases by *Haemophilus influenzae*, 75% of cases caused by *N. meningitidis*, and 50% of cases caused by gram negative bacilli.

CSF Gram stain is positive in less than 50% of patients with meningitis due to Listeria monocytogenes or anaerobes. [8]

The mortality of meningococcal meningitis has been reported to be 4 to 8% in children and up to 7% in adults. [9] Most patients that die because of an infection with *N. meningitidis* die because of systemic complications, primarily sepsis. [10]

Antimicrobial chemoprophylaxis of close contacts (household members, day care centre contacts) or anyone exposed to oral secretions of sporadic cases of meningococcal disease is important for the prevention of spread of disease. [11] The attack rate for close contacts exposed to patients who have sporadic meningococcal disease is 500-800 times greater than for the general population. [12]

REFERENCES

1. Famum NR. Acceptance sampling. In: Modern statistical quality control and improvement. Belmont, California.: Duxbury Press; 1994:305.

2. Elias J, Frosch M, Voguel U. Neisseria. In: Jorgensen et. al., ed. Manual of clinical microbiology. Vol 1. 11th ed. Washington, DC: ASM; 2015:635.

3. Stephens DS, Greenwood B, Brandtzaeg P. Epidemic meningitis, meningococcaemia, and Neisseria meningitidis. The Lancet. 2007;369 (9580):2196-2210.

4. Sneed J. Processing and interpretation of upper respiratory tract specimens. In: Pezzlo Marie, ed. Clinical microbiology procedures handbook. Vol 1. 2nd ed. American Society for Microbiology (ASM); 2004.

5. Shanholtzer C, P Schaper, L Peterson. Concentrated Gram stain smears prepared with a cytospin centrifuge. J Clin Microbiol 1982;16:1052-6.

6. Manchanda V, Gupta S, Bhalla P. Meningococcal disease: History, epidemiology, pathogenesis, clinical manifestations, diagnosis, antimicrobial susceptibility and prevention. Indian J Med Microbiol. 2006;24(1):7-19.

7. Ragunathan L, M Ramsay, R Borrow, et al. Clinical features, laboratory findings, and management of meningococcal meningitis in England and Wales: report of a 1997 survey. J Infect 2000;40:74-9.

8. Tunkel AR, Scheld WM. Acute meningitis. In: Mandel G, Douglas R, Bennett J., ed. Principles and practice of infectious diseases. Vol 1. 6th ed. Churchill Livingstone: Elsevier; 2005:1083.

9. Pelkonen T, Roine I, Monteiro L, et al. Risk factors for death and severe neurological sequelae in childhood bacterial meningitis in Sub-Saharan Africa. Clin Infect Dis. 2009;48(8):1107-1110.

10. Brouwer MC, Tunkel AR, van de Beek D. Epidemiology, diagnosis, and antimicrobial treatment of acute bacterial meningitis. Clin Microbiol Rev. 2010;23(3):467-492.

11. CDC. Control and Prevention of Meningococcal Disease: Recommendations of the Advisory Committee on Immunization Practices (ACIP), MMWR 2005; 54(RR-7) 1-28

12. The Meningococcal Disease Surveillance Group. Analysis of endemic meningococcal disease by serogroup and evaluation of chemoprophylaxis. J Infect Dis 1976;134:201-4.