Challenge G242

August 2024

Gram — Joint fluid**:** 3+ (6-10/oif) neutrophils and 3+ (11-50/oif) gram negative diplococci (*Neisseria gonorrhoeae*)

HISTORY

A simulated wound 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 42 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.

MAIN EDUCATIONAL POINTS from G242

1. Gonococcal arthritis results from the bacteremic spread of *Neisseria gonorrhoeae* accompanied by infiltration of white blood cells in joints such as knees, ankles, wrist, and elbow. Typically, a synovial fluid analysis will show a white blood cell (WBC) count of 50,000 cells/mm3 or more; although, a cell count below 10,000 cells/mm3 is not uncommon.
2. Presumptive diagnosis of disseminated gonococcal infection or gonococcal arthritis can be made through gram stain of joint fluid, to visualize the etiologic agent *Neisseria gonorrhoeae* appearing as gram negative diplococci (pair of red staining spherical cells or cocci) that are typically coffee-bean or kidney-bean shaped with a small central clearing.
3. The finding of gram negative diplococci/diplococcus in sterile site gram smears is an important presumptive identification that guides diagnosis, interpretation and clinical management in the context of patient history, risk factors and symptoms.

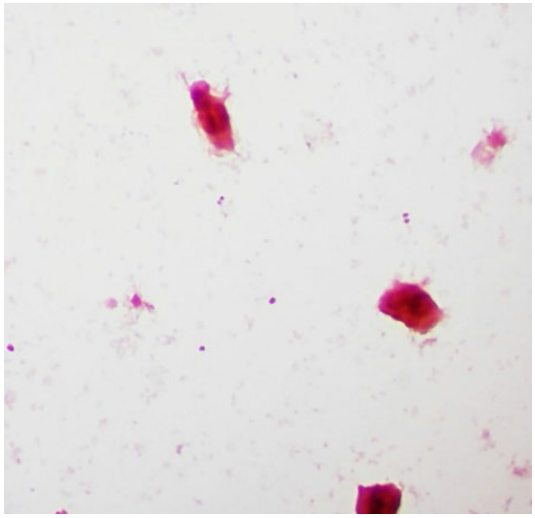

Figure 1. Gram stain of G242; simulated joint fluid smear at 1000X magnification under oil immersion demonstrating gram negative diplococci and neutrophils.

Grading

Maximum grade: 8

Reporting the presence of neutrophils was graded 4.

Reporting gram negative cocci/diplococci was graded 4.

Table 1. Reported results—Cells

Reported	A Labs	C1 Labs	Total	Grade
10-25, >25/lpf, 3+, 4+ neutrophils/white blood cells	49	4	53	4
3+ (6-10/oif) neutrophils, 2+ (1-5/oif)|Cellules mesothéliales	1		1	1
sample not normally processed	1		1	ungraded
Total	**51**	**4**	**55**	

SURVEY RESULTS

Reference laboratories

Cells: 13/13 (100%) labs reported >25/lpf, 3+, 4+ neutrophils/ white blood cells

Bacteria: 10/13 labs reported 3+, 4+ gram negative diplococci, 3 labs reported 2+, 3+ gram negative cocci (100% reported acceptable morphology).

Participants

Cells: 54/54 (100%) reporting participants reported the presence of neutrophils or white blood cells, one participant did not report results (see Table 1 for grading).

Bacteria: 51/54 (94%) reporting participants reported gram negative diplococci or gram negative cocci. One lab each reported using acronyms, gram positive cocci, and gram negative bacilli (see Table 2 for grading); one participant did not report results

Suitability for Grading

A challenge is considered suitable for grading if agreement is reached by 80 percent of the 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

The majority of participant labs (54/55) were able to identify the presence of neutrophils/white blood cells on the slide smear. One Category A lab reported that this type of sample is “not normally processed”. Category A labs are large laboratories with a broad complex testing menu capable of testing critical specimens (i.e. blood cultures and CSF) and specimens requiring specialized workup (i.e. joint fluids, deep wound, tissue and anaerobic cultures). It is unclear why this Category A lab was unable to process this specimen as it should be part of their standard testing menu.

The majority of participant labs (45/55) correctly reported the presence of 1+ to 4+ gram negative diplococci on the smear. 1 lab reported 3+ bacteria with the acronym GNDC; laboratories should spell out the results of the Gram reaction and avoid using acronyms which may be unfamiliar to physicians/healthcare practitioners who must act on lab results. 6 labs reported 1+ to 4+ gram negative cocci, without denoting the characteristic 2 cell formation of *Neisseria* species; while this is semantically correct, it is not the optimal way to report the gram findings for *Neisseria* species. The finding of gram negative diplococci/ diplococcus in sterile site smears is an important presumptive identification that guides diagnosis, interpretation and clinical management in the context of patient history, risk factors and symptoms. Hence labs should take care to verify and report the presence of characteristic 2 cell diplococci formation.

1 lab reported 2+ gram negative bacilli, indicating an inability to recognize the distinctive diplococcus morphology. 1 lab reported 4+ gram positive cocci which implies a critical error with the Gram staining procedure, most likely due to inadequate decolorization with ethanol and/or counterstaining with safranin dye. The inability to correctly identify gram negative cocci represents a major error for these 2 Category A labs as the incorrect gram stain result would drastically change specimen workup for culture and subsequent reporting.

1 Category A lab did not report any result citing “sample not normally processed”. See note above about the expected testing menu for Category A labs.

CLINICAL SIGNIFICANCE

Clinically, gonorrhea has several manifestations. Urogenital disease is the most common, but primary infections can also present as pharyngitis, conjunctivitis, proctitis, and disseminated infections as asymmetric arthritis, tenosynovitis, and dermatitis. Disseminated gonococcal infection typically results from bacteremic spread of *N. gonorrhoeae* from a genitourinary source and usually presents in one of two ways: 1) as an arthritis-dermatitis syndrome that occurs within 2-3 weeks of a genitourinary infection and is characterized by fever, chills, generalized malaise, asymmetric polyarthralgia (symmetric joint involvement

Table2. Reported results - Bacteria

Reported	A Labs	C1 Labs	Total	Grade
1+, to 4+ gram negative diplococci/ diplocoques gram négatif ± suggestive of *Neisseria* spp	41	4	45	4
1+ to 4+ gram negative cocci/cocci Gram négatif	6		6	4
3+ (11-50/oif) GNDC	1		1	0
2+ (2-10/oif) gram negative bacilli	1		1	0
4+ (>50/oif) gram positive cocci	1		1	0
sample not normally processed	1		1	ungraded
Total	**51**	**4**	**55**	

is uncommon and there is a migratory nature to the arthralgias), tenosynovitis, and dermatitis or 2) as a purulent mono- or oligoarthritis with an abrupt onset, pain, and swelling in one or more joints (polyarthritis, when present, is typically asymmetric; most patients are afebrile). Amongst infected cases, disseminated gonococcal infection and subsequent inflammation and/or arthritis of joints is estimated to occur in 0.5 to 3% of otherwise healthy young adults (<40 years of age) who are sexually active. Gonococcal arthritis has a marked predilection for women (4:1 ratio compared with men) which likely reflects their higher propensity for asymptomatic genital infections (and subsequent lack of medical attention and appropriate antibiotic treatment). [2] Men who have sex with men (MSM) represent another at-risk population for disseminated gonococcal infection.

Over the past decade, the incidence of sexually-transmitted infections (including gonorrhea, chlamydia, and syphilis) has increased in Canada and elsewhere. While Gram stain may often provide a rapid presumptive identification, labs should also be aware that gram stain of synovial fluid lacks sensitivity for the diagnosis of septic arthritis with <25% positivity in cases of gonococcal septic arthritis. Hence for suspected cases of monoarticular arthritis, aspirated joint fluids should be sent for cell count, Gram stain, and culture. Culture should include media to recover fastidious pathogens including *N. gonorrhoeae*.[3]

The majority of positive gram smears in nongonococcal septic arthritis are due to gram positive cocci (70-80%) or other gram negative organisms[3–5] (40-50%). *Staphylococcus aureus* is the most common organism in native and prosthetic joint infections, followed by streptococci[4].

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