cmpt Canadian microbiology proficiency testing

CMPT Clinical Bacteriology Program

Innovation, Education, Quality Assessment, Continual Improvement

Challenge GS242-2

August 2024

Gram - Joint fluid - *4*+ (>10/oif) neutrophils and 4+ (>50/oif) gram positive bacilli (*Cutibacterium acnes*)

HISTORY

A simulated wound sample collected from a 27 year old post shoulder surgery was sent to category A and C1 laboratories.

Participants were expected to report the presence of neutrophils and gram positive 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 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 21 days.

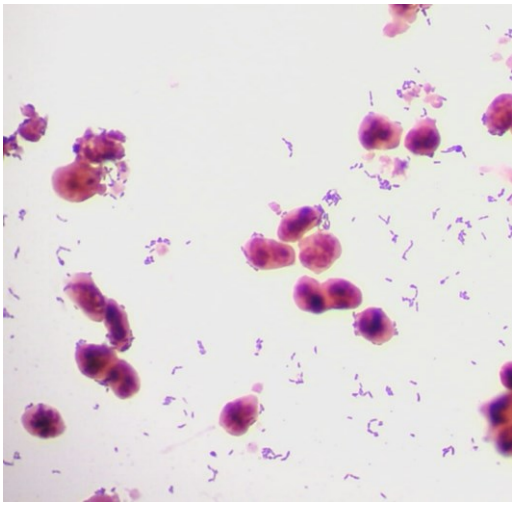

Figure 1. Gram stain of GS242-2; simulated joint fluid smear at 1000X magnification under oil immersion demonstrating gram positive ba-

MAIN EDUCATIONAL POINTS from GS242-2

1. Identifying *Cutibacterium acnes* (*C. acnes*) in shoulder hardware infections is crucial due to its indolent nature, often presenting with subtle symptoms like low-grade pain and stiffness, which can complicate diagnosis.

2. Accurate identification is essential for appropriate treatment, as these infections may require specific antibiotic regimens and sometimes surgical intervention.

3. Misdiagnosis or delayed diagnosis can lead to prolonged pain and hardware failure.

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."

SURVEY RESULTS

Reference laboratories

Cells: 10/10 (100%) labs reported >25/oif, 3+, 4+ neutrophils/white blood cells (1 lab also reported red blood cells)

Bacteria: 10/10 (100%) labs reported 4+ gram positive bacilli/rods

Participants

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

Bacteria: 30/34 (88%) labs reported gram positive bacilli; two labs reported gram positive cocci in addition to the gram positive bacilli (Table 2).

Grading

Maximum grade: 8

Reporting neutrophils was graded 4.

Reporting gram positive bacilli was graded 4.

Table 1. Reported results—Cells

Reported	Cat A	Cat C1	Total	Grade
>25/lpf, 1+, 4+ neutrophils/leukocytes/ white blood cells	31	2	33	4
3+ white blood cells, 2+ red blood cells	1		1	4
Total	**32**	**2**	**34**	

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 a well-collected wound sample.

About 88 percent of the laboratory identified the Gram stain enough to have gotten a completed score. Two laboratories that identified cocci was given a score of 0. The reason for this was that the identification of gram positive cocci in the joint fluid could have lead the physicians down the wrong path. The identification of the GN bacilli was given a score of zero.

CLINICAL SIGNIFICANCE

The pleomorphic nature of these bacteria occasionally leads the laboratories to misinterpret the smear as a mixed infection.[2]

Although not in this case, some authors[2] have reported difficulty in detecting this organism on direct microscopy by Gram smear from clinical samples especially when growth was only moderate. This has been attributed to cell wall deterioration due to the inflammatory response.[3]

C. acnes is known to be a common causative organism in infections after shoulder instability surgery and arthroplasty. The shoulder is thought to have a propensity for infection with *C. acnes* because the organism is the dominant anaerobic bacteria isolated from healthy skin in moist areas such as the axilla.[4,5] However, diagnosis may be difficult because this opportunistic pathogen has historically been considered a contaminant.

REFERENCES

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

2. Esteban J, Garcia-Calvo G, Jimenez-Castillo P, Soriano F. Failure of gram stain to detect *Propionibacterium acnes* in specimens from clinically significant infections. *J Clin Microbiol*. 1996;34(8):2051.

3. Butler-Wu SM, She RC. *Actinomyces, Lactobacillu*[1]*s, Cutibacterium*, and other non-spore-forming anaerobic gram positive rods. In: Carrol et al, ed. *Manual of clinical microbiology*. 12th ed. ed. Washington DC: ASM; 2019:938.

4. Zeller V, Ghorbani A, Strady C, Leonard P, Mamoudy P, Desplaces N. *Propionibacterium acnes*: An agent of prosthetic joint infection and colonization. *J Infect*. 2007;55(2):119-124.

5. Dodson CC, Craig EV, Cordasco FA, et al. *Propionibacterium acnes* infection after shoulder arthroplasty: A diagnostic challenge. *Journal of Shoulder and Elbow Surgery*. 2010;19(2):303-307.

Table2. Reported results - Bacteria

Reported	Cat A	Cat C1	Total	Grade
3+, 4+ (>50/oif) gram positive bacilli/rods ± suggestive of Cutibacterium spp. ± branching	29		29	4
4+ (>50/oif) gram positive bacilli resembling diphtheroids		1	1	4
4+ (>50/oif) gram positive bacilli - suggestive of Cutibacterium spp., 2+ (2-10/oif) gram positive cocci	1		1	0
4+ (>50/oif) gram positive bacilli, 1+ (<1/oif) gram positive cocci	1		1	0
4+ (>50/oif) gram negative bacilli		1	1	0
3+ (11-50/oif) GNB	1		1	0
Total	**32**	**2**	**34**	