Challenge G234

February 2024

Gram - Joint fluid: 4+ (>10/oif) neutrophils; 4+ (>50/oif) gram positive cocci (group C Streptococcus)

HISTORY

A simulated wound sample collected from 20 year old male with sore elbow was sent to category A and C1 laboratories. Participants were expected to report the presence of neutrophils and gram positive cocci.

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 4+ (>10/oif) neutrophils; 4+ (>50/oif) gram positive cocci (Figure 1). A culture of group C Streptococcus 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 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."

SURVEY RESULTS

Reference laboratories

Cells: 13/13 (100%) labs reported >25/oif, 4+ neutrophils/white blood cells ± 1+ red blood cells

Bacteria: 13/13 (100%) labs reported 3+, 4+ gram positive cocci, ± pairs, ± chains

Participants

Cells: 52/55 (95%) reporting laboratories reported the presence of leucocytes/neutrophils; two laboratories reported low amounts of epithelial cells in addition to the neutrophils (Table 1).

Bacteria: 55/55 (100%) reporting laboratories reported the presence of gram positive cocci (Table 2)

MAIN EDUCATIONAL POINTS from G234

1. Joint fluids are collected by aspiration and therefore are not expected to show epithelial contamination.
2. Gram stains of joint fluids generally show a purulent picture but may not always show the causative organism due to lack of sensitivity. They are positive in about 71% of cases of gram-positive septic arthritis, and are more likely to be negative in those cases caused by gram-negative organisms, with less than 25% sensitivity in gonococcal septic arthritis.
3. Culture is much more sensitive and crucial for pathogen identification. Blood cultures should always be obtained when septic arthritis is suspected as it generally results from the organism spreading to these sites via the bloodstream.

Grading

Maximum grade: 8

Reporting the presence of neutrophils was graded 4.

Reporting gram positive cocci was graded 4.

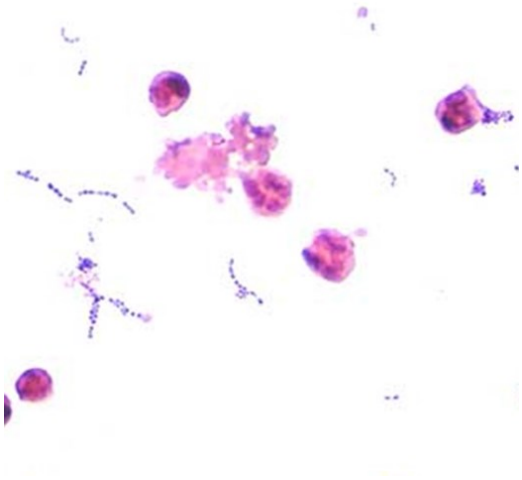

Figure 1. Gram stain of G234; simulated joint fluid smear at 1000X magnification under oil immersion demonstrating gram positive cocci and neutrophils.

Table 1. Reported results—Cells

Reported	Cat A	Cat C1	Total	Grade
>25/lpf, >25/oif, 2+, 3+, 4+ neutrophils/leukocytes/white blood cells ± 1+ red blood cells	47	4	51	4
5-30/champs (fields) leucocytes	1		1	4
>25/lpf, 4+ (>10/oif) neutrophils, <10/lpf, 1+ (<1/oif) epithelial cells	2		2	1
none seen	1		1	0
no report	2		2	0
sample not normally processed	1		1	ungraded
Total	**54**	**4**	**58**	

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 participants did very well in reporting this Gram smear on the joint fluid that was a companion to the M234-5 culture. Most labs reported a large number of neutrophils and a large number of gram-positive cocci in chains ± suggestive of Streptococcus/ Enterococcus species and were given full marks. A few labs did not report these expected findings and were given a grade of 0.

Two labs reported the presence of scant epithelial cells (which would be unexpected in a joint fluid aspiration and were not present in the smear) and was given a grade of 1 due to this reporting error. Those that do not process this type of specimen were not graded.

CLINICAL SIGNIFICANCE

Joint infection, also known as septic, suppurative, or pyogenic arthritis, is caused by a wide range of microorganisms with bacterial pathogens being the most common etiology. Several factors may affect the type of bacteria causing arthritis, e.g., age, immunization status, presence of prosthesis, and laboratory detection method. *Staphylococcus aureus* remains the most common cause of bacterial arthritis in children followed by *Streptococcus pyogenes,* [2,3] or *Kingella kingae* in some areas of the world.[4]

Positive Gram stains of joint fluids are usually highly specific, but the sensitivity is as low as 17%.[5] Gram stains are positive in 71% of gram-positive septic arthritis, 40% to 50% of cases of gram-negative septic arthritis, and fewer than 25% of cases of gonococcal septic arthritis. [7]

Culture is more sensitive than direct Gram stain and is crucial for pathogen identification and antimicrobial susceptibility testing, although it might be falsely negative with fastidious organisms. Blood cultures should be obtained in all cases of septic arthritis since it might be a consequence of occult bacteremia. Blood cultures are positive in at least 14% of patients with septic arthritis and in some instances might be the only positive bacteriologic confirmation.[6]

Table2. Reported results - Bacteria

Reported	Cat A	Cat C1	Total	Grade
2+, 3+, 4+ gram positive cocci/cocci gram positif, ± pairs ± in chains/en chaine ± suggestive of Streptococcus/Enterococcus species	**50**	4	54	4
cocci gram positif en chaîne: présence	1		1	4
no report	2		2	0
sample not normally processed	1		1	ungraded
Total	**54**	**4**	**58**	

REFERENCES

1. Famum NR. Acceptance sampling. In: *Modern Statistical Quality Control and Improvement.* Duxbury Press; 1994:305.
2. Klosterman MM, Villani MC, Hamilton EC, Jo C, Copley LA. Primary Septic Arthritis in Children Demonstrates Presumed and Confirmed Varieties Which Require Age-specific Evaluation and Treatment Strategies. *Journal of Pediatric Orthopaedics*. 2022;42(1):e27. doi:10.1097/BPO.0000000000001976
3. Ceroni D, Cherkaoui A, Combescure C, Francois P, Kaelin A, Schrenzel J. Differentiating osteoarticular infections caused by Kingella kingae from those due to typical pathogens in young children. *PediatrInfectDisJ*. 2011;30(10):906-909. doi:10.1097/INF.0b013e31821c3aee;
4. Gouveia C, Duarte M, Norte S, et al. Kingella kingae Displaced S. aureus as the Most Common Cause of Acute Septic Arthritis in Children of All Ages. *The Pediatric Infectious Disease Journal*. 2021;40(7):623. doi:10.1097/INF.0000000000003105
5. Gbejuade H, Elsakka M, Cutler L. How well does synovial fluid gram staining correlate with cultures in native joint infections? *Orthop Rev (Pavia)*. 2019;11(4):8156. doi:10.4081/or.2019.8156
6. Manz N, Krieg AH, Heininger U, Ritz N. Evaluation of the current use of imaging modalities and pathogen detection in children with acute osteomyelitis and septic arthritis. *Eur J Pediatr*. 2018;177(7):1071-1080. doi:10.1007/s00431-018-3157-3
7. Ross JJ. Septic Arthritis of Native Joints. *Infectious Disease Clinics*. 31(2):203-218. doi:10.1016/j.idc.2017.01.001