Challenge GS234-2

February 2024

Gram - Joint fluid: 3+ (6-10) neutrophils; 3+ (11-50/oif) gram negative bacilli (*Kingella kingae*)

HISTORY

A simulated wound sample collected from a 8 year old with painful knee was sent to category A and C1 laboratories. Participants were expected to report the presence of neutrophils and gram negative 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) neutrophils; 3+ (11-50/oif) gram negative bacilli (Figure 1). A culture of *Kingella kingae* was used to prepare the slides.

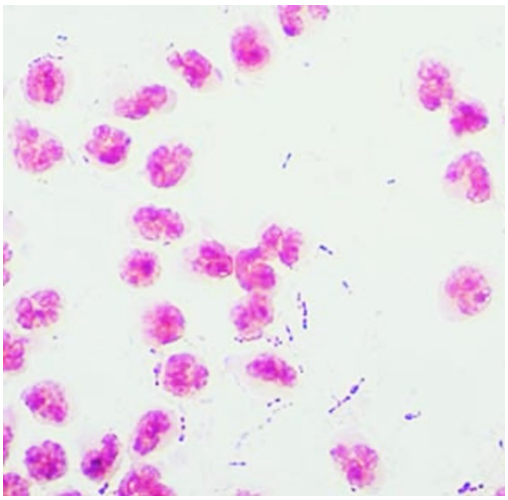

Figure 1. Gram stain of GS234-2; simulated joint fluid smear at 1000X magnification under oil immersion demonstrating gram negative bacilli and neutrophils.

NOTE: K. kingae may appear gram-positive due to its propensity to resist decolorization and retain crystal violet

MAIN EDUCATIONAL POINTS from GS234-2

1. Rapid and accurate interpretation of Gram stains from sterile fluids, including synovial fluids, is crucial for appropriate patient management.
2. Reporting epithelial cells incorrectly may suggest skin contamination of the sample, which might lead to misinterpretation of the Gram stain results.
3. *K. kingae* has propensity to resist decolorization and retain crystal violet dye, therefore, it may be, erroneously, identified as gram-positive on Gram stain.

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."

Grading

Maximum grade: 4

Reporting the presence of neutrophils was graded 4.

The bacteria component of this challenge was ungraded.

SURVEY RESULTS

Reference laboratories

Cells: 9/10 labs reported >25/lpf, 3+ to 4+ neutrophils/white blood cells, 1 lab reported 4+ neutrophils and 2+ epithelial cells

Bacteria: 7/10 (70%) labs reported 3+ to 4+ gram negative bacilli/rods, 1 lab reported 4+ gram negative bacilli and 3+ gram positive cocci pairs and chains, 2 labs reported 3+/4+ gram positive cocci - Ungraded

Table 1. Reported results—Cells

Reported	Cat A	Cat C1	Total	Grade
10 to >25/lpf, 3+, 4+ neutrophils/white blood cells	30	2	32	4
4+ neutrophils, 1+ (<1/ oif) epithelial cells	1		1	1
4+ neutrophils, 2+ (1-5/oif) epithelial cells	1		1	1
none seen	1		1	0
Total	**33**	**2**	**35**	

Participants

Cells: 32/34 (94%) reporting labs reported the presence of neutrophils; 2 labs also reported epithelial cells (Table 1).

Bacteria: 26/34 (76%) reporting labs reported gram negative bacilli; 3 participants reported gram positive cocci in addition to the gram negative bacilli and 5 reported only gram positive cocci (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 cells was correctly performed by at least 80 percent of reference laboratories and greater than 50 percent of all laboratories; bacteria component did not reach consensus as there was not enough agreement amongst reference laboratories. Only the cell component was suitable for grading.

COMMENTS ON RESULTS

The majority of laboratories did very well in reporting the cellular component of this challenge. Two laboratories reported epithelial cells when none were present in the sample, so these were downgraded to 1.

On the other hand, the bacterial component was not graded due to lack of consensus among the reference laboratories despite having 76% of the participant laboratories reporting the correct answer. The Gram stain was misread as follow: four laboratories correctly reported the gram-negative bacilli but also reported the presence of gram-positive cocci, and five laboratories only reported gram positive cocci.

It is important to note that *K. kingae* may appear as gram-positive on Gram stain due to its propensity to resist decolorization and retain crystal violet. Laboratories that incorrectly reported the bacterial component of this challenge should review their Gram stain procedures and interpretations.

CLINICAL SIGNIFICANCE

The accurate interpretation of Gram stain of synovial fluids is essential for appropriate patient management. The presence of a cellular response, e.g., neutrophils, is important to initiate empiric antimicrobial therapy, although this will not differentiate infectious from non-infectious causes.

K. kingae is fastidious, gram-negative bacilli or coccobacilli. It is an important cause of invasive infections in young children causing septic arthritis, osteomyelitis and spondylodiscitis.[2-4] Routine culture media are not optimal for *K. kingae* isolation, but this can significantly be improved by inoculating samples into blood culture bottles[5]. Furthermore, the availability of nucleic acid amplification tests provides a rapid and highly sensitive method for *K. kingae* detection in clinical samples.

On Gram stain, *K. kingae* appears as gram-negative bacilli or coccobacilli with squared ends, and they are generally seen in pairs or short chains (Fig 1). [5,6] However, *K kingae* has propensity to resist decolorization and retain crystal violet,[5,7] which might have caused some laboratories to report it as gram positive.

REFERENCES

1. Famum NR. Acceptance sampling. In: Modern statistical quality control and improvement. Belmont, California.: Duxbury Press; 1994:305.
2. Vásquez MA, Palacián MP, Cruz Villuendas M, Marne C, Paz RuizEcharri M, Revillo MJ. Kingella kingae pediatric septic arthritis. Archivos argentinos de pediatría 2012;110(6):e126.
3. Long SS. Kingella kingae, a surprising and stealthy osteoarticular pathogen. J Pediatr 2014;165(5):879-881.
4. Ceroni D, Cherkaoui A, Ferey S, Kaelin A, Schrenzel J. Kingella kingae osteoarticular infections in young children: Clinical features and contribution of a new specific real-time PCR assay to the diagnosis. J Pediatr Orthop. 2010;30(3):301-304.
5. Zbinden R, von Graevenitz A. Aggregatibacter, Capnocytophaga, Eikenella, Kingella, Pasteurella, and other fastidious or rarely encountered gram-negative rods. In: Carrol K. C., et. al., ed. Manual of clinical microbiology. Vol 1. 10th ed. ed. Washington, DC.: ASM; 2019:656.
6. Araya DI, Camponovo CR. Kingella kingae. Rev Chilena Infectol. 2006;23(1):55.
7. Yagupsky P. Outbreaks of Kingella kingae infections in daycare facilities. Emerging Infectious D

Table2. Reported results - Bacteria

Reported	Cat A	Cat C1	Total	Grade
3+, 4+ gram negative bacilli/rods	25	1	26	ungraded
3+ (11-50/oif) gram negative bacilli, 3+ (11-50/oif) gram positive cocci, pleomorphic	1		1	ungraded
4+ gram negative bacilli, 2+, 3+ gram positive cocci ± pairs, ± chains	3		3	ungraded
2+, 3+, 4+ gram positive cocci	3	1	4	ungraded
4+ (>50/oif) gram positive cocci suggestive of *Streptococcus/Enterococcus*	1		1	ungraded
Total	**33**	**2**	**35**	