

Challenge G093

November 2009

Gram: knee aspirate - *Corynebacterium jeikeium* (companion to M093-4)

HISTORY

The sample was sent to category A, B, C, and C1 laboratories as a gram smear from a knee aspirate of a 27 year old elite athlete with a prosthetic knee implant.

CMPT QA

The smear, reviewed at CMPT, presented 4+ (>10/oif) neutrophils and 3+ (11-50/oif) gram positive pleomorphic bacilli (see Figure 1). The challenge was verified by internal quality control, which indicated 99% accuracy based on MIL-STD-105E¹⁰.

SURVEY RESULTS

Reference Laboratories:

Cells - 15/15 labs reported 1+ to 4+ neutrophils (1 reported 10-25/oif, 1 reported moderate).

Bacteria - 14/15 labs reported gram positive bacilli, 1 lab reported gram positive cocci.

Consensus was achieved by the reference labs, thus the sample was considered for grading.

Cell component (see table 1)

The majority of the participants (91%) received a grade of 4 for the cell component, reporting neutrophils between 1 to 4+, 10-25/oif, or moderate.

Three participants reported the presence of epithelial cells in the smear although they hadn't been added to the preparation. These laboratories were given a grade of 3. One labora-

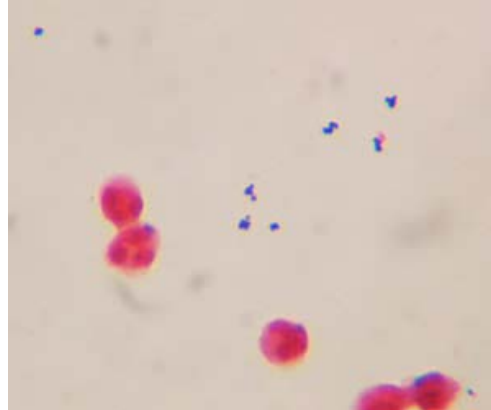


Figure1: G093 gram smear. The smear shows neutrophils and short, pleomorphic gram positive rods.

tory reported <1/oif neutrophils, <1/oif epithelial cells and was given a grade of 1.

Two participants reported "no cells seen" and were given a grade of zero. The laboratory that reported "sample not normally processed" was not graded. Those participants not reporting a cell component (4) were given a grade of zero.

Bacterial component (see table 2)

Overall, 60% of the participants successfully identified gram positive bacilli/coccobacilli in the smear. Figure 2 shows the percentage of laboratories correctly reporting gram positive bacilli/coccobacilli. Category A laboratories did quite well while participants in the other categories have more difficulty accurately reporting coryneform bacteria in the Gram stain.

Approximately 6% of the participants reported the presence of gram positive cocci in addition

Grading

Maximum grade: 8 (4 points each for cell and bacterial component).

Those laboratories reporting 1+ to 4+ neutrophils were given a grade of 4. One participant reported "moderate" neutrophils. This laboratory received a grade of 4 but it is recommended laboratories give a more specific report regarding the amount of neutrophils observed

Reporting epithelial cells was downgraded to 3. The Committee thinks it is important for the laboratories to be able to properly identify the type of cells present in the sample.

Those laboratories reporting very few or no neutrophils in the sample were given an unacceptable grade.

Reporting gram positive bacilli, coccobacilli, suggestive of *Corynebacterium* was graded 4.

Table -1: Reported results for G093 smear - Cell component -

Reported results	No of labs	%	Grade
1+, 2+, 3+, 4+, 6-10, 10-25/oif neutrophils	122	91	4
moderate neutrophils	1	1	4
2+, 3+ neutrophils, 1+, 2+ epithelial cells	3	2	3
<1/oif neutrophils, <1/oif epithelial cells	1	1	1
no cells seen	2	1	0
no report	4	3	0
snp	1	1	ungraded
Total	134	100	

snp: sample not normally processed

Table –2: Reported results for G093 smear – Bacteria component -

Reported results (bacteria component)	A	B	C	C1	Total	%	Grade
2+, 3+, 4+, >50/oif, moderate gram positive bacilli, +/- suggestive of <i>Corynebacterium</i> spp. +/- suggestive of diphtheroids	49	8	3	2	62	46	4
2+, 3+, 4+ gram positive coccobacilli, +/- snnp, +/- diphtheroid-like / 3+ gram variable coccobacilli (pleomorphic)	12	4	2	1	19	14	4
2+, 3+, 4+, 1-10/oif gram positive bacilli, 1+,2+, 4+, 11-50/oif gram positive cocci	2	1	2	3	8	6	1
2+ diphtheroids, 1+ gram positive cocci, 1+ gram negative bacilli	0	1	0	0	1	1	0
1+, 2+, 3+, 4+, 10-25/oif gram positive cocci +/- snnp,+/- refer	13	10	7	6	36	27	0
4+ gram positive diplococci	0	1	0	0	1	1	0
2-10/oif gram negative coccobacilli / 3+ gram negative bacilli / 2+ gram negative cocci	0	2	0	1	3	1	0
no organisms seen, snnp, refer	0	0	0	1	1	1	0
no report	0	1	0	1	2	1	0
snnp	0	0	1	0	1	1	ungraded
Total	76	28	15	15	134	100	

snnp: sample not normally processed

to gram positive bacilli. These laboratories were given a grade of 1.

Quite a few laboratories reported gram positive cocci only (26%) and were given a grade of 0.

Other reports included gram positive diplococci, gram variable coccobacilli, gram negative bacilli, and gram negative cocci. Please refer to table 2 for the grades assigned in each case.

One participant laboratory reported “no organisms seen” and was given a grade of zero. The laboratory that reported “sample not normally processed” was not graded. Two participants did not report results and were given a grade of zero.

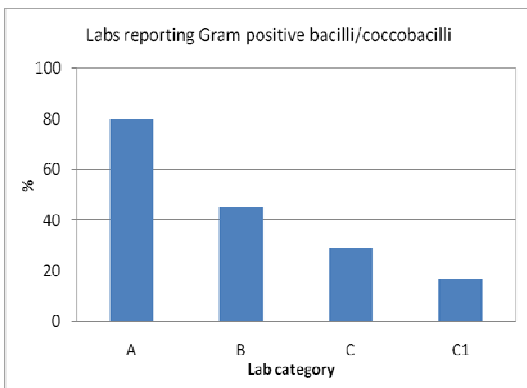


Figure 2: Percentage of laboratories reporting gram positive bacilli / coccobacilli.

COMMENTS ON RESULTS

Corynebacteria: Gram stain characteristics

When seen in Gram stain, corynebacteria generally appear as slightly curved, gram-positive rods with sides not parallel and sometimes slightly wider ends. Cells infrequently stain unevenly. If *Corynebacterium* cells are taken from fluid media and visualized using the Gram stain, they are often arranged as single cells, in pairs, in V forms, in palisades, or in clusters with a so-called Chinese letter appearance ¹. The gram stain, G093, presented a challenge because *C.jejkeium* is very pleomorphic and it requires significant expertise to differentiate the coccobacilli forms from cocci. For category B and C labs who did not accurately differentiate this as a coccobacilli, it emphasizes the need to have difficult-to-interpret gram stains reviewed by senior staff to ensure proper interpretation.

The Committee recommends that all Proficiency Testing samples should be processed as routine samples even when there is a staff shortage or high workload.

CLINICAL SIGNIFICANCE

As outlined by Funke and Bernard ¹, there are currently 67 species in the *Corynebacterium* genus and, of these, 40 are clinically relevant for humans.

Corynebacterium group JK was first recognized as a distinct species in 1976 ² and was later designated as *C. jeikeium* in 1987.

C. jeikeium is a pleomorphic gram-positive rod, which varies from coccobacillary to bacillary and club shaped. It is nonhemolytic on standard media, and forms small gray-white colonies on blood agar plates incubated under CO₂ 1, 2, 3, 4.

Corynebacteria are always catalase positive, and the medically relevant species are all non-motile. The genus *Corynebacterium* includes both fermenting and nonfermenting species 1.

Corynebacterium jeikeium is a normal commensal organism in healthy adults. It is a lipophilic organism, which explains its predilection for the axilla and groin (both sites of sebaceous glands). *C. jeikeium* is one of the most frequently detected corynebacteria in clinical specimens. *C. jeikeium* most commonly causes sepsis, followed by skin lesions and nodular pulmonary infiltrates, but cases of endocarditis, osteomyelitis, arthritis, visceral abscesses, meningitis, otitis, and other infections have been described. Mortality has been reported to be as high as 34% 6. It is becoming well characterized as a pathogen in neutropenic hosts with indwelling catheters, by far the two strongest risk factors for infection 5, 6.

Prosthetic joint infections (PJI) occur in approximately 1.5% to 2.5% of all primary hip or knee arthroplasties 7. Treatment of PJI usually requires either removal of the prosthesis followed by a disabling arthrodesis, or resection of the bioprosthetic materials, stabilization of the joint and administration of 6 weeks of intravenous antibiotics followed by implantation of a new joint prosthesis. Few cases of PJI by *C. jeikeium* have been reported to date 8,9.

Estimating the clinical significance of coryneform bacteria isolated from clinical specimens is often difficult for clinical microbiologists. Current recommendations suggest that coryneform bacteria should be identified to the species level when; (i) detected from normally sterile body sites, (ii) from adequately collected clinical material where they are the predominant organism and iii) from urine specimens where they are the only organism at > 10⁷ cfu/L or are the predominant organism at > 10⁸ cfu/L.1

The clinical significance of coryneform bacteria is strengthened when multiple specimens are positive for the same coryneform bacteria and/

or the coryneform bacteria are seen in the direct Gram stain with a strong presence of leukocytes 1.

Identifiers

CMPT has observed that a common error by participant laboratories is to mix samples and results.

Participants are encouraged to incorporate two CMPT identifiers on the results report form to prevent these types of errors.

REFERENCES

1. Funke G, Bernard K. A. *Coryneform gram-positive rods*. In: Murray ea, ed. *Manual of Clinical Microbiology*. Vol 1. 9th ed. ed. Washington, DC.: ASM; 2007:485.
2. Hande KR, Witebsky FG, Brown MS, et al. Sepsis with a new species of corynebacterium. *Ann Intern Med*. 1976;85:423-426.
3. Riley PS, Hollis DG, Utter GB, Weaver RE, Baker CN. Characterization and identification of 95 diphtheroid (group JK) cultures isolated from clinical specimens. *J Clin Microbiol*. 1979;9:418-424.
4. Meyer D.K, Reboli A. C. *Other coryneform bacteria and rhodococcus*. In: Mandell D, Bennett, ed. Vol 2. 6th ed. Philadelphia; Churchill Livingstone: Elsevier; 2005:2465.
5. Mookadam F, Cikes M, Baddour LM, Tleyjeh IM, Mookadam M. *Corynebacterium jeikeium* endocarditis: A systematic overview spanning four decades. *Eur J Clin Microbiol Infect Dis*. 2006;25:349-353.
6. Olson JM, Nguyen VQ, Yoo J, Kuechle MK. Cutaneous manifestations of corynebacterium jeikeium sepsis. *Int J Dermatol*. 2009;48:886-888.
7. Lentino J. Prosthetic joint infections: Bane of orthopedists, challenge for infectious disease specialists. *Clinical Infectious Diseases*. 2003;36:1157-1161.
8. Yildiz S, Yildiz HY, Cetin I, Ucar DH. Total knee arthroplasty complicated by corynebacterium jeikeium infection. *Scand J Infect Dis*. 1995;27:635-636.
9. Tleyjeh IM, Qutub MO, Bakleh M, Sohail MR, Virk A. *Corynebacterium jeikeium* prosthetic joint infection: Case report and literature review. *Scand J Infect Dis*. 2005;37:151-153.
10. Farnum NR. 1994. Ch 11. Acceptance sampling, p. 305-361. *Modern Statistical Quality Control and Improvement*. Duxbury Press, Belmont, CA.