cmpt Canadian microbiology proficiency testing

CMPT Clinical Bacteriology Program

Innovation, Education, Quality Assessment, Continual Improvement

Challenge G233

November 2023

Gram: CSF: 2+ (1-5 /oif) white blood cells – 2+ (1-10/oif) yeast elements (*Cryptococcus neoformans*)

HISTORY

A simulated wound sample collected from 40 year old male newly diagnosed HIV positive patient was sent to category A and C1 laboratories.

Participants were expected to report the presence of white blood cells and yeast elements resembling *Cryptococcus* species.

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 2+ (1-5 /oif) white blood cells– 2+ (1-10/oif) yeast elements (Figure 1). A culture of *Cryptococcus neoformans* was used to prepare the slides.

Figure 1. Gram stain of G233; simulated joint CSF smear at 1000X magnification under oil immersion demonstrating yeast elements resembling Cryptococcus and lymphocytes.

MAIN EDUCATIONAL POINTS from G233

1. The finding of yeast in CSF can be crucial for expediting appropriate treatment for patients with cryptococcal meningitis.
2. If the laboratory is able to indicate that the yeast resembles *Cryptococcus* spp, that gives added usefulness to the report.
3. It is important to be able to distinguish cryptococci from cells that originate in the patient.

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 41 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: 8

Reporting white blood cells, neutrophils, or lymphocytes was graded 4.

Reporting yeast elements was graded 4.

SURVEY RESULTS

Reference laboratories

Cells: 10/13 labs reported 10-25, 1+, 2+, 3+ neutrophils; 1 lab reported >25/lpf neutrophils, 10-25/lpf mononuclear cells, 2 labs did not report cells.

Organisms: 13/13 (100%) labs reported yeast, ± suggestive of *Cryptococcus* species ± encapsulated ± budding

Participants

Cells: 49/52 (94%) reporting laboratories reported neutrophils and or lymphocytes or white blood cells. Three participants reported the absence of cells/leukocytes (Table 1); 4 participants did not report results.

Bacteria: 53/53 (100%) reporting laboratories reported the presence of yeast, some suggesting *Cryptococcus* species. 3 participants did not report (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 cell and fungal 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

Table 1. Reported results—Cells

Reported	Cat A	Cat C1	Total	Grade
1-5, 5-10, 10-25, >25/lpf, 1+, 2+, 3+ neutrophils ± RBCs	40	3	43	4
1+, 2+, 3+ white blood cells	3		3	4
>25/lpf, 1+, 2+ neutrophils, 10-25/lpf, 1+, 2+ white blood cells/lymphocytes/ mononuclear cells	2	1	3	4
leukocytes none seen	1		1	0
no cells seen	2		2	0
no report	4		4	0
shipping delay, no report	1		1	ungraded
sample not normally processed	1		1	ungraded
Total	**54**	**4**	**58**	

COMMENTS ON RESULTS

Laboratories reporting white blood cells, neutrophils or lymphocytes were graded 4. Laboratories which did not report cells were graded 0. Laboratories that reported yeast were graded 4, laboratories that gave no report were graded 0, and those that did not process the sample, or had a shipping delay were ungraded.

CLINICAL SIGNIFICANCE

The results of Gram stain of cryptococci can be variable. While in many cases the yeast cells will stain gram positive, penetration of the stain may be variable leading to stippling of the cells, or even a gram negative appearance. The capsule may stain gram negative and form a halo around the yeast cells[4]. Occasionally elongation of the cells may be seen[5]. In a comparison of the Gram stain with India ink staining, the gram stain was slightly more sensitive at detection. Both methods were highly specific in experienced hands[6].

Presumptive microscopic diagnosis of *Cryptococcus* in CSF may be based on the morphology of the yeast with budding, and by observation of the characteristic extracellular polysaccharide capsule. Capsules are best demonstrated with an India ink preparation. Sediment examination of CSF is usually positive in approximately 40% in patients without AIDS and in 80% of patients with severe immunosuppression. False positives are usually due to confusion with lymphocytes.[1]

The capsules vary from 2-10 um but they tend to be smaller in specimens from immunocompromised patients. The production of capsular material may be increased by growth in 1% peptone solution.[2,3] Unfortunately high-quality India ink is difficult to find, expensive and must be filtered often. Most laboratories rely on testing for capsular antigen for rapid detection of *Cryptococcus* infection. Nucleic acid amplification is beginning to be used.

The advantage of the Gram stain is it is used for all sterile fluid specimens and is not dependent on clinical suspicion, whereas the India ink stain needs to be requested. Infection can therefore be detected using the Gram stain when it is not clinically suspected. Rarely cryptococcal capsular antigen detection may be falsely negative as a result of a "prozone" phenomenon, but the Gram stain enables detection of the organism[7].

REFERENCES

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Table2. Reported results - Bacteria

Reported	Cat A	Cat C1	Total	Grade
1+, 2+, 3+, 4+ (<1/oif) yeast, ± suggestive of *Cryptococcus* species ± encapsulated ± budding ± gram positive	49	4	53	4
no report	3		3	0
shipping delay, no report	1		1	ungraded
sample not normally processed	1		1	ungraded
Total	**54**	**4**	**58**	