

## Challenge GS114-1

February 2012

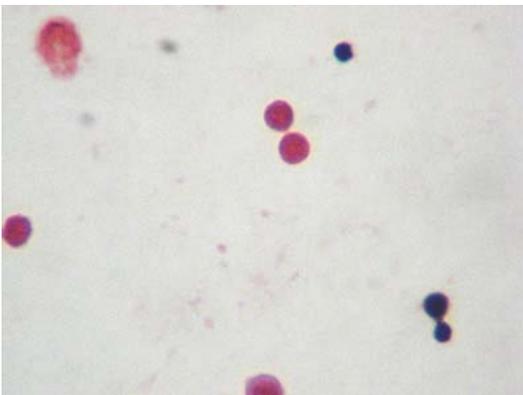
Gram: CSF – mononuclear cells – yeast elements (*Cryptococcus*)

### HISTORY

The sample was a simulated CSF obtained from a 35 year old HIV + man and was sent to participant laboratories for Gram staining. Participants were expected to report the presence of yeast elements and mononuclear cells.

### CMPT QA

The sample contained 3+ (6 – 10)/oif mononuclear cells, rare PMNs and 2+ (2-10/oif) yeast elements suggestive of *Cryptococcus* species (figure 1). The challenge was verified by internal quality control, which indicated 99% accuracy based on MID-STD-105E. <sup>1</sup>



**Figure1.** Mononuclear cells and round gram positive yeast elements.

**Table –1:** Reported results – Cell component -

Reported results	Total
1+ neutrophils, 2+ WBCs (not PMNs)/ mononuclear cells	3
1+ to 4+ neutrophils +/- 1+ to 4+ RBCs	73
1+ neutrophils, 1+ epithelial cells	1
4+ other	1
no cells seen	5
Wrong identifier	1
no report	2
<b>Total</b>	<b>86</b>

snp: sample not normally processed

### MAIN EDUCATIONAL POINTS of GS114-1

1. As previously highlighted in Challenge G113 (November 2011), it is important that laboratories correctly differentiate and report the predominant WBC type in sterile site (especially CSF) samples as this assists in identifying the most likely etiologic agent.

**If laboratories are unable to distinguish between mononuclear (eg. lymphocytes) and polymorphonuclear (eg. neutrophils) cells on the Gram stain then they should report as white blood cells (WBCs) without type differentiation.**

For quality purposes, the microbiology lab should subsequently correlate the results with the cell count performed in hematology to determine the correct white blood cell type.

2. Reporting epithelial cells or red blood cells when none are present is significant because it suggests a problem of competence in reading and interpretation of cells.

3. When round yeasts are seen in the Gram stain of a CSF sample, care should be taken to identify the presence or absence of a capsule. The presence of a thick capsule allows one to indicate that the yeasts seen are suggestive of *Cryptococcus* species.

### SURVEY RESULTS

**Reference Labs:** Only 1/11 reference labs correctly reported the predominance of mononuclear cells and therefore **the cellular component was ungraded.**

11/11 reference labs reported the presence of yeasts/yeast-like organisms but only two of those laboratories indicated that the yeasts resembled *Cryptococcus* species.

Consensus was reached for the presence of yeast elements so this alone was graded.

#### Participants

**Cells** (table 1): Only 3/86 (3.5%) laboratories correctly reported the predominance of WBCs (not PMNs)/mononuclear cells. 88% (76/86) of the laboratories reported neutrophils (PMNs) in the sample. Rare neutrophils were present in the sample but almost all labs failed to report the mononuclear cells which were the predominant WBC type on the slide.

Nine laboratories reported the presence of RBCs which were not present on the slide.

### Grading

**Maximum grade: 4**

The cellular component of the challenge was ungraded due to lack of consensus.

Reporting yeast elements +/- suggestive of *Cryptococcus* was graded 4.

Reporting other organisms in addition to the yeast elements, or alone, or the absence of organisms was graded 0.

Using the wrong identifier to report results is considered a clerical error and thus, is graded 0.

No reporting results is always graded 0.

One participant reported the presence of epithelial cells which is egregious in the setting of a CSF sample.

Five laboratories reported they saw no cells while one participant used the wrong identifier to report results.

One participant checked the “other” box in the cells section of the report form but did not specify what “other” was.

Two laboratories did not report a result.

**Organisms** (table 2): 73/86 (85%) participants reported yeast / yeast-like elements, 24 of those suggested *Cryptococcus* species.

Three laboratories reported the presence of gram negative bacilli in addition to the yeast elements, these labs were graded with a 1.

One participant reported “gram positive, very large, round” without specifying type of organism, this lab was graded 0.

Five laboratories did not observe organisms, one reported gram negative bacilli, and one laboratory used the wrong identifier; all these laboratories were graded 0. Not sending a report is always graded 0.

### COMMENTS ON RESULTS

Microscopic examination of CSF specimens is one of the first-line methods for the diagnosis of cryptococcosis. <sup>2</sup>

*Cryptococcus* is a round to somewhat oval yeast-like fungus ranging from 3.5 to 8µm or more in diameter, with single, narrow-neck budding daughter cells. <sup>3</sup>

Cells are characterized by the presence of a polysaccharide capsule. The capsule is often seen in primary clinical samples but may be lost with repeated subculturing.

On Gram stain, the yeast may stain gram positive or variable. The capsular component of the yeast often stains gram negative or may exhibit an absence of stain uptake.

More sensitive methods for direct microscopy include fluorescent stain, calcofluor white, mucicarmine, alcian blue, and India ink. A disadvantage with this last method is that leukocytes are sometimes difficult to differentiate from *Cryptococcus* cells. <sup>4,5</sup>

Typically, CSF examination from patients with cryptococcosis often reveals modestly elevated or normal white cell counts (usually lymphocytes), elevated protein, and low or normal glucose. <sup>6</sup>

### CLINICAL SIGNIFICANCE

Microscopic examination of CSF specimens is one of the first-line methods for the diagnosis of cryptococcosis. <sup>2</sup>

*Cryptococcus* is a round to somewhat oval yeast-like fungus ranging from 3.5 to 8µm or more in diameter, with single, narrow-neck budding daughter cells. <sup>3</sup>

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### Reporting using the wrong form

Laboratories are reminded that reporting results using the wrong reporting form is considered a transcription error and will be graded as 0.

Please contact CMPT, if in doubt, or fax the results using a hard copy of the report form.

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

**Table-2:** Reported results - Organisms component -

Reported results	A Labs	B Labs	C Labs	C1 Labs	Total	Grade
2+ to 4+ yeast (± budding), suggestive of <i>Cryptococcus neoformans</i> /species	20	2	1	1	24	4
3 to >25/oif, 1+ to 4+ yeast/yeast-like organisms +/- not <i>C. albicans</i>	40	2	1	6	49	4
2+ to 3+ yeast, 2+ to 4+ gram negative bacilli	1		1	1	3	1
3+ gram positive, very large, round	1				1	0
2+ gram negative bacilli				1	1	0
no organisms seen	3	1		1	5	0
Wrong identifier				1	1	0
no report		1	1		2	0
<b>Total</b>	<b>65</b>	<b>6</b>	<b>4</b>	<b>11</b>	<b>86</b>	

snp: sample not normally processed;

yeast often stains gram negative or may exhibit an absence of stain uptake.

More sensitive methods for direct microscopy include fluorescent stain, calcofluor white, mucicarmine, alcian blue, and India ink. A disadvantage with this last method is that leukocytes are sometimes difficult to differentiate from *Cryptococcus* cells.<sup>4,5</sup>

Typically, lumbar puncture from patients with cryptococcosis often reveals modestly elevated or normal white cell counts (usually lymphocytes), elevated protein, and low or normal glucose.<sup>6</sup>

Cryptococci are encapsulated basidiomycetous yeasts that can cause disease in both humans and animals. There are two predominant species that are pathogenic to humans: *Cryptococcus neoformans* and *C. gattii*.<sup>4</sup>

Inhalation of small, thinly encapsulated yeasts, or basidiospores, may lead to an initial pulmonary infection. *C. neoformans* infections occur mostly in immunodeficient individuals, including patients with AIDS, transplant recipients or other patients receiving immunosuppressive medications, and patients with hematological malignancies. *C. gattii*, on the other hand, infects mostly immunocompetent hosts, primarily causing pulmonary infection, but can also lead to extrapulmonary infections.<sup>4</sup>

## REFERENCES

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