



## G061 Sputum smear for Gram staining: 3+ neutrophils, 2+ yeast cells, 3+ gram-negative diplococci – Suitable for culturing.

**HISTORY** This simulated sputum smear, sent with the history that it was collected from a 40-year old lymphoma patient, returning from a cross country camping trip, was sent to category A, B, C, and C1 laboratories for Gram staining. A companion sample for culture was not included in this survey. Participants were expected to stain, read, and interpret the Gram stain, including whether or not the sample was considered suitable for culture.

**CMPT QA** The smear was created using cultured isolates of *Cryptococcus neoformans* (2+ [2-10/oif]) and *Moraxella catarhalis* (3+ [11-50-]). The sample contained 3+ (6-10/oif) neutrophils, but did not contain epithelial cells to ensure that it would be considered “suitable for culture.” The smear was verified by internal quality control, which indicated 99% accuracy based on MIL-STD-105E<sup>1</sup>.

**GRADING (maximum grade = 4)** It was anticipated that a grade of 4 be assigned to interpretation of sample suitability, cellular component, and bacterial component for a maximum grade of 12, however due to lack of consensus between the reference laboratories only the cellular component was suitable for grading.

The slide was received and reported upon by 145 laboratories, with 7 reporting that the challenge was not completed because the sample was not one normally processed in their laboratory. In addition, 2 laboratories reported the slide as of poor quality and commented that they would normally create a new slide, or ask for a new sample. Participants are reminded that they may contact CMPT for a new slide up to 3 days prior to the due date.

### GRADING –Maximum grade = 4

#### Cellular Component:

93% (70/75) of category A, 93% (41/44) of category B, and 85% (17/20) of category C laboratories received a grade of 4/4 or 3/4.

#### NOTES

1. The ability of laboratory personnel to recognize and be able to identify *Cryptococcus* on the sputum smear is related to the likelihood of infection with the organism in the population in the area the laboratory is situated.
2. Differentiation of *Cryptococcus* from *Blastomyces* is important both clinically and because culture of *Blastomyces* on an open bench is hazardous.
3. In future sputum Gram smear challenges, sample assessment ‘suitability to culture’ component will be graded.

### SAMPLE ASSESSMENT ‘suitability to culture’

**(ungraded)** As only 8 out of 15 (53%) reference laboratories indicated that the Gram smear would be suitable for culture this component of the challenge was not graded. In total, 55 laboratories failed to report on the suitability for culture. In addition, 2 laboratories reported the sample would not be considered for culture based on Q-score. When CMPT submits a Gram stain along with a companion sample for culture processing, an argument can be made that the presence of a culture result implies the decision to consider the sample suitable for culture. Such cannot be the case when a companion culture sample is not provided. Laboratories are reminded to indicate on the sputum Gram smear report the suitability to culture. In

future sputum Gram smear challenges, this component will be graded.

### CELLULAR COMPONENT

**(grade = 4)** The cellular component was considered suitable for grading as all of the reference laboratories reported neutrophils with or without 0-1, <10, < 25/lpf, or 1+ epithelial cells. Of 136 laboratories, 128 (94%) reported the presence of neutrophils in a variety of concentrations. Of these 47 (37%) reported the presence of epithelial cells. As the sample was constructed without epithelial cells, these laboratories were down graded to reflect this false positive reporting of epithelial cells. One laboratory reported only the presence of epithelial cells and received a grade of 1

**Table 1. G061 Cellular components: Results received from category A, B, C and C1 laboratories and grades assigned.**

Cells reported	A	B	C/C1	Total (% out of 136)	Grade
neutrophils (+/- rbcs)	55	25	11	91 (67%)	4
neutrophils (+/- rbcs), ≤ 0-1/lpf, < 10-25/lpf, 1+ epithelial cells	13	14	6	33 (24%)	3
neutrophils (+/- rbcs), 2+ epithelial cells	2	2		4	3
Red blood cells only	2	0		2	1
No neutrophils seen; 1+, < 25/lpf epithelial cells only	2		1	3	1
No cells seen	1	2		3	1
No report received			1	1	0
unable to process, heat fixed slide (1B)		1		1	0
Poor quality slide, unable to differentiate components (1C)			1	1	0
Gram smears not normally performed	0	0	7	8	ungraded
<b>Total</b>	<b>75</b>	<b>44</b>	<b>27</b>	<b>146</b>	

(Continued on page 2)

because this would represent a falsely negative report on the degree of inflammation present. No penalty was incurred for the small number of laboratories reporting the presence of few blood cells. Table 1 summarizes the reports received and grades assigned.

**MICROBIAL COMPONENT (ungraded)** All of the reference group reported the bacterial component as intended, although reporting styles varied: 15/15 reported yeast cells (rare,

1+/2+/3+/4+) and 13 of these 15 reported 3+ ,4+ or many gram-negative diplococci and 1/15 reported 1+ gram positive cocci. Two/15 reported gram-negative cocci as either 4+ or 3+ (resembling *Neisseria* or *Branhamella*). One laboratory reported > 25 mixed organisms and/or usual flora (rare yeast cells and many gram-negative diplococci seen, not reported due to protocol, diplococci not intracellular.) Four reference laboratories added 'resembling *Cryptococcus* spp.' to describe the yeast cells seen. These results were considered

acceptable making this component of the challenge suitable for grading, *however* since there was such variation in microbial reporting between the participants this component was not graded. Reports are shown in Table 2.

This specimen had two microbial components. The first was yeast cells (*Cryptococcus neoformans*) that were present in numbers to allow them to be seen in most fields. Of 135 laboratories reporting the presence of microbes, 105 (78%) laboratories reported yeast, in varying concentrations. The most common concentration reported was 2+. Many were comfortable providing a supplementary comment on the yeast -"resembling *Cryptococcus* species". The second was gram-negative diplococci that were present in all fields. In total, 79% (106/135) of participants reported this component although a variety of terms was used including gram-negative diplococci, gram-negative cocci, and gram-negative coccobacilli. Of concern were the number of laboratories reporting 2+-4+ gram-positive cocci, indicating inadequate decolorization of the smear.

**CLINICAL SIGNIFICANCE** *Cryptococcus neoformans* causes pneumonia, meningitis and may disseminate to involve the skin, bones and more widely in some immunocompromised patients. In the immunocompetent patient it may cause pneumonia, which may be subclinical. In this group meningitis is not usually accompanied by pulmonary disease. In immunocompromised patients, especially those patients with AIDS, *Cryptococcus* can cause aggressive pulmonary disease and may cause hemoptysis. It may disseminate to the meninges or widely throughout the body. In an immunocompromised patient this infection is life threatening<sup>1</sup>. In recent years *C. gattii* (which was formerly *C. neoformans* var. *gattii*, most often serotype B) has been recognized as endemic on Vancouver Island<sup>2</sup>. It has been associated with pulmonary disease in immunocompetent

(Continued on page 3)

Report received	A	B	C/C1	Total (% out of 136)
few, 1+/2+/3+/4+ yeast cells, 1+/2+/3+/4+ gram-negative diplococci (+/- 1+ gram-positive cocci [3-1 A, B, and C])	34	13	3	50 (38%)
1+/2+/3+ yeast cells, 2+/3+/4+ gram-negative diplococci with 2+ (4A, 1C) or 3+/4+ gram-negative cocci (15A, 4B, 3C) or 2+/3+ gram-negative coccobacilli (2A, 2B), With 2+ or more gram-positive cocci; mixed respiratory organisms (1B)	21	11	4	36 (26%)
3+ yeast cells, 3+ gram-negative diplococci with 3+ gram-positive diplococci			1	1
4+ fungal-like forms, 4+ gram-negative diplococci		1		1
3+ yeast cells only	1	2		3
3+,4+ gram-negative diplococci only	5	3	2	10
4+ gram-negative coccobacilli only	1			1
3+ (A), 4+ (B,C) gram-negative cocci , with 1 comment oropharyngeal flora, not normally quantitated (B)	1	2	1	3
2+ yeast cells, 3+ mixed bacterial flora	1			1
2+/4+ yeast cells, 2+/3+/4+ gram-positive cocci	4	6	4	14
2+>50/oif gram-negative diplococci & 1+ gram-negative cocci (1A) or <1/oif gram-positive cocci (1A, 1B)	2	1		3
4+ gram-negative cocci, 2+/3+ gram-positive cocci (1A); 4+ gram-negative diplococci, 2+ gram-positive cocci	1	1	1	2
3+, 4+ gram-positive cocci only	3	2	2	7
> 25 mixed organisms and/or usual flora (rare yeast cells and many gram-negative diplococci seen, not reported due to protocol, diplococci not intracellular.)	1			1
No organisms seen		1		1
No report received			1	1
unable to process, heat fixed slide		1		1
Poor quality slide, unable to differentiate components			1	1
Gram smears not normally performed			7	7
<b>Total</b>	<b>75</b>	<b>44</b>	<b>27</b>	<b>146</b>

patients, and may represent a hypervirulent clone<sup>3</sup>.

In microbiology specimens *Cryptococcus neoformans* may be detected microscopically in sterile fluids, especially CSF, or less commonly in sputum. The most commonly used stain is the Gram stain, and this organism can be less predictable in its staining characteristics than other yeast. Although *Cryptococcus neoformans* is usually described as a gram-positive yeast, the thick polysaccharide capsule can interfere with the penetration of the stain. Cryptococci may appear pale lavender to red and may contain gram-positive granular inclusions<sup>4</sup>. Organisms may appear to be gram-negative. Capsular material may be unstained or may appear gram-negative. The presence of round yeast with thin-based buds is suggestive of cryptococci. Pseudohyphae are rarely seen, in contrast to many *Candida* species. *Blastomyces dermatitidis* also appear as round yeast but the cells are larger and the buds have a broad base<sup>5</sup>. Differentiation of *Cryptococcus* from *Blastomyces* is important both clinically and because culture of *Blastomyces* on an open bench is hazardous.

The ability of laboratory personnel to recognize and be able to identify *Cryptococcus* on the smear is related to the likelihood of infection with the organism in the population in the area the laboratory is situated. *Cryptococcus gattii* is endemic on Vancouver Island and adjacent areas of the mainland of British Columbia. As there is frequent local travel to areas of endemicity it has been increasingly recognized as a cause of infection. Laboratories in these areas or in other areas that encounter Cryptococcal infection more frequently than non-endemic areas should be able to identify the organism microscopically to provide the clinician with this important information. In laboratories that do not encounter the organism frequently in sputum specimens, for example in Central or Eastern Canada, the ability to identify the organisms as *Cryptococcus* is not as critical as the incidence of infection is very low and false positive results are more likely, giving misleading information. In these areas it may be important to be able to identify *Blastomyces* microscopically, depending on local prevalence of infection. In Atlantic Canada, where neither infection is endemic or commonly seen, an identification as yeast is appropriate, although an experienced microscopist may choose to be more definitive. Discussion with the clinician may be helpful in determining the potential for significance. Although yeast are not common pathogens causing pneumonia in Atlantic Canada, when seen in a sputum Gram stain the report should indicate their presence which may reflect oro-pharyngeal, if not deeper, infection.

Gram-negative cocci in sputum are most commonly associated with the normal flora, but when present in large numbers and in good quality specimens, they may reflect pneumonia with *Moraxella catarrhalis* or occasionally *Neisseria meningitidis*<sup>6</sup>. The presence of intracellular organisms can be helpful to determine the significance of gram-negative

diplococci seen on a smear in a good quality specimen. The gram-negative cocci in this specimen resembled *M. catarrhalis*, or *N. meningitidis*. The organisms were present in pairs with the long axis of the organisms in a parallel orientation in the pair. This morphology is distinct from gram-positive diplococci like *Streptococcus pneumoniae* where the long axis of the pair of organisms is the same to give the classical lancet shape. This difference in morphology can be useful to determine if pairs of cocci are overdecolourized or underdecolourized.

Laboratories that reported gram-positive cocci, or did not report the bacterial component of the specimen should review their procedures, as should laboratories that did not report, or provide information to the detail appropriate for their area for the yeast component.

## REFERENCES

1. Diamond RD. 2000. *Cryptococcus neoformans*. p. 2707-2718. In GL Mandell, JE Bennet, R Dolin (eds.) *Mandell, Douglas and Bennett's Principles and Practice of Infectious Diseases*. 5th ed. Churchill Livingstone, Philadelphia, PA.
2. Fraser JA, Giles SS, Waning EC, Geunes-Boyer SG, Wright JR, Diezmann S, Allen A, Stajich JE, Dietrich FS, Perfect JR, Heitman J. 2005. Same-sex mating and the origin of the Vancouver Island *Cryptococcus gattii* outbreak. *Nature*. 437(7063):1360-4.
3. Hoang LM, Maguire JA, Doyle P, Fyfe M, Roscoe DL. 2004. *Cryptococcus neoformans* infections at Vancouver Hospital and Health Sciences Centre (1997-2002): epidemiology, microbiology and histopathology. *J Med Microbiol*. 53 935-40.
4. Bottonne EJ 1980. *Cryptococcus neoformans*: Pitfalls in diagnosis through evaluation of gram stained smears of purulent exudates. *J. Clin Microbiol*. 12:6, 790-1.
5. Hazen KC, SA Howell. 2003. *Candida*, *Cryptococcus*, and other yeasts of medical importance. pp. 1693-1711. In PR Murray, Editors: EJ Baron, JH Jorgensen, MA Pfaller, H Tenover, H Tenover (eds.) *Manual of Clinical Microbiology* 8<sup>th</sup> ed. ASM Press Washington, DC.
6. Murphy TF 2000. *Moraxella (Branhamella) catarrhalis* and other gram-negative cocci. p.2259-2266. In GL Mandell, JE Bennet, R Dolin (eds.) *Mandell, Douglas and Bennett's Principles and Practice of Infectious Diseases*. 5th ed. Churchill Livingstone, Philadelphia, PA.

**ADDENDUM** As noted in CMPT critique G043 sputum from November 2004, since the information of the Gram smear is available before the culture result, and since a critical laboratory decision has been made based on the smear, it is imperative that a smear interpretation be provided. **Failure to check the suitability of the sample will be considered as a post-analytic reporting error. However, in this sample, as only 53% (8/15) of reference laboratories submitted comments about sample suitability based on Gram smear interpretation this component was ungraded.**