

**Challenge G171**

May 2017

Gram: Peritoneal fluid: neutrophils, gram negative bacilli (*Klebsiella pneumoniae*), gram positive cocci (*Streptococcus constellatus*) (c. M171-5)

**HISTORY**

A simulated peritoneal fluid smear collected from a 50 year old patient with intra-abdominal sepsis was sent to category A, B, C, and C1 laboratories for Gram staining.

Participants were expected to report neutrophils, gram negative bacilli, and gram positive cocci.

**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. <sup>1</sup>

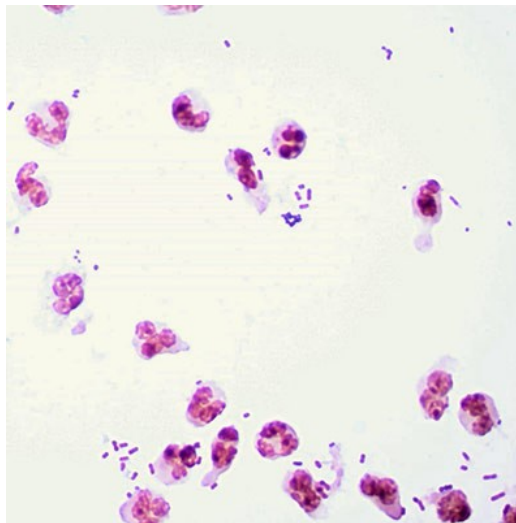
The sample contained 4+ (>10/oif) neutrophils, 3+ (11-50/oif) gram negative bacilli, and 3+ (11-50/oif) gram positive cocci (Figure 1). A culture containing *Klebsiella pneumoniae* and *Streptococcus anginosus* group (*S. constellatus*) was used to prepare the slides.

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 at least 90 days.

**MAIN EDUCATIONAL POINTS from G171**

1. Most infections in the peritoneal cavity have a mixture of micro-organism, often with anaerobic morphotypes involved.
2. Participants should carefully review Gram smears from these types of specimens to ensure that the various morphological types are not missed.
3. In this example there were no anaerobic species involved, but a mixed culture of gram negative bacilli and gram positive cocci. This information is clinically valuable for early primary treatment regimens.



**Figure 1.** Gram stain of G171; simulated peritoneal fluid smear at 1000x magnification under oil immersion demonstrating gram negative bacilli, gram positive cocci and neutrophils.

**Grading**

**Maximum grade: 8**

Reporting neutrophils, white blood cells, leukocytes was graded 4.

Reporting epithelial cells was graded 0.

Not reporting neutrophils was graded 0.

Reporting gram negative bacilli and gram positive cocci was graded 4.

**Table 1.** Reported results—Cells

Reported	A labs	B labs	C labs	C1 labs	Total	Grade
>10/oif /3+, 4+ / many neutrophils / polymorphonuclear cells	51	1	4	13	69	4
4+ white blood cells/leukocytes	9				9	4
4+ pus cells	1				1	4
4+ neutrophils (1)			1		1	ungraded
10-25/oif epithelial cells	1				1	0
no polymorphonuclear cells seen	1				1	0
snp	1				1	ungraded
<b>Total</b>	<b>64</b>	<b>1</b>	<b>5</b>	<b>13</b>	<b>83</b>	

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."

## SURVEY RESULTS

### Reference laboratories

**Cells:** 13/13 (100%) laboratories reported 3+, 4+, many, abundant neutrophils/polymorphonuclear cells/white blood cells.

**Bacteria:** 13/13 (100%) laboratories reported 10 - >25/oif, 3+, 4+ gram negative bacilli, 10 - >25/oif, 2+ to 4+ gram positive cocci.

### Participants

**Cells** (Table 1): 79/82 (96%) processing laboratories reported 3+, 4+, many, abundant neutrophils/polymorphonuclear cells/white blood/pus cells; these laboratories were graded 4. One participant reported only epithelial cells and another reported "no neutrophils" these laboratories were graded 0.

**Bacteria** (Table 2): 76/82 (93%) laboratories reported 10 - >50/oif, 2+, 4+ gram negative bacilli, 10 - >50/oif, 2+ to 4+ gram positive cocci and were graded 4. Other participants reported gram positive bacilli, gram negative coccobacilli, mixed flora; for grading please see 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 bacteria 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.

**Table2.** Reported results—Bacteria

Reported	A labs	B labs	C labs	C1 labs	Total	Grade
10- >50/oif, 2+ to 4+ gram negative bacilli ± resembling coliforms/enteric bacilli; 10- >50/oif, 2+ to 4+ gram positive cocci ± suggestive of <i>Streptococcus</i> or <i>Enterococcus</i> species	62		3	11	76	4
3+ gram negative bacilli, 3+ gram positive cocci (1)			1		1	ungraded
4+ gram negative coccobacilli, 3+ gram positive cocci		1			1	1
4+ gram negative bacilli, 2+ gram positive bacilli				1	1	1
Mixed flora	1				1	1
≥50/oif gram negative bacilli				1	1	1
4+ gram positive bacilli, 4+ gram positive cocci, snnp			1		1	0
snp	1				1	ungraded
<b>Total</b>	<b>64</b>	<b>1</b>	<b>5</b>	<b>13</b>	<b>83</b>	

(1) (late report 1st time participant)

## COMMENTS ON RESULTS

Laboratories performed very well on the cellular portion of the challenge. The laboratories that reported only epithelial cells or no polymorphs should review their smears. Patients with infections in the peritoneal cavity invariably have associated pus and no epithelial cells since the fluid is aspirated.

The morphotypes in the Gram smear were also identified very well (94%) with grades of 4. The one laboratory that reported gram-negative coccobacilli was given a grade of 1. This initial report to the physician from an intra-abdominal sample could be misleading as to the type of organism involved with the potential for inappropriate primary antimicrobial therapy.

## CLINICAL SIGNIFICANCE

Peritoneal fluid should be sent to the laboratory in an anaerobic transport system for Gram stain and aerobic and anaerobic bacterial cultures. <sup>2</sup>

Secondary peritonitis is by far the most common form of peritonitis encountered in clinical practice; it is related to a pathologic process in a visceral organ, such as perforation or trauma, including iatrogenic trauma.

This intra-abdominal infection case is an example of secondary peritonitis which is normally caused by organisms from the gastrointestinal flora and is typically polymicrobial in nature. <sup>3</sup>

## REFERENCES

1. Famum NR. Acceptance sampling. In: *Modern Statistical Quality Control and Improvement*. Belmont, California.: Duxbury Press; 1994:305.
2. Baron EJ, Miller JM, Weinstein MP, et al. A Guide to Utilization of the Microbiology Laboratory for Diagnosis of Infectious Diseases: 2013 Recommendations by the Infectious Diseases Society of America (IDSA) and the American Society for Microbiology (ASM). *Clinical Infectious Diseases*. 2013.
3. McClean KL, Sheehan GJ, Harding GKM. Intraabdominal Infection: A Review. *Clinical Infectious Diseases*. 1994;19:100-116.