cmpt Canadian microbiology proficiency testing

CMPT Clinical Bacteriology Program

Innovation, Education, Quality Assessment, Continual Improvement

Challenge G241

May 2024

Gram - Sputum: 4+ (>10 /oif) neutrophils – 3+ (11-50/oif) gram positive cocci (*Streptococcus viridans*) and 4+ (>50/oif) gram negative coccobacilli (*Haemophilus influenzae*). Sample suitable for culture.

HISTORY

A simulated sputum sample collected from a 75 year old male with exacerbation of COPD was sent to category A and C1 laboratories. Participants were expected to report the presence of neutrophils, gram positive cocci and gram negative coccobacilli.

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 4+ (>10 /oif) neutrophils, 3+ (11-50/oif) gram positive cocci and 4+ (>50/oif) gram negative coccobacilli (*Haemophilus influenzae*) (Figure 1). A mixed culture of *S. viridans* and *H. influenzae* was used to prepare the slides.

MAIN EDUCATIONAL POINTS from G241

1. When the Gram stain morphology of organisms is fairly predictive of their identity, that information, or at least an accurate description, is useful to provide in the report.
2. Examination of a well prepared and stained sputum Gram smear can provide valuable information to clinicians as to the inflammatory response, the presence or absence of contamination, and the type of bacterial flora present which, in turn, could be useful in guiding antimicrobial therapy until culture results are available.
3. The two main sputa screening processes are based on the presence of squamous epithelial cells either with or without an assessment on the presence of neutrophils.

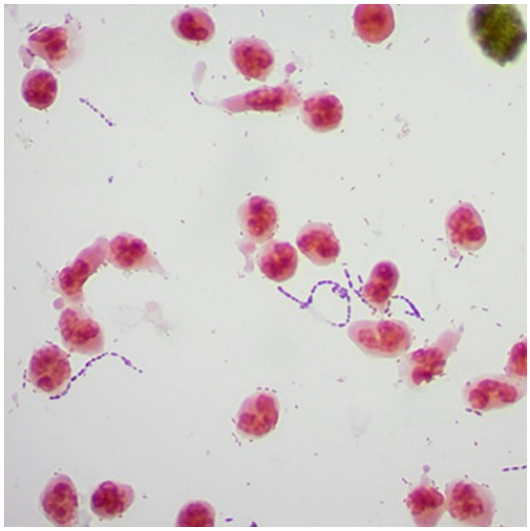

Figure 1. Gram stain of G241; simulated sputum smear at 1000X magnification under oil immersion demonstrating gram positive cocci, gram negative coccobacilli, and neutrophils.

Grading

Maximum grade: 12

Reporting neutrophils was graded 4.

Reporting the sample suitable for culture, was graded 4.

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

Table 1. Reported results—Cells

Reported	Cat A	Cat C1	Total	Grade
>25/lpf, 4+ neutrophils/white blood cells	34	3	37	4
>25/lpf neutrophils, <1/lpf epithelial cells	2		2	4
>25/lpf, 4+ neutrophils, <10, <25/lpf epithelial cells	11	1	12	4
>25/lpf, 4+ neutrophils, 1+ (<1/lpf) epithelial cells, ± 1+ red blood cells	3		3	4
sample not normally processed	1		1	ungraded
Total	**51**	**4**	**55**	

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 56 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."

SURVEY RESULTS

Reference laboratories

Cells: 13/13 (100%) labs reported >25/lpf, 4+ neutrophils (6 of which also reported <1, <10, <25/lpf epithelial cells)

Suitability for culture: 13/13 (100%) labs reported the sample was suitable for culture

Bacteria: 11/13 (85%) labs reported 3+, 4+ gram negative coccobacilli and 3+, 4+ gram positive cocci, 2 labs reported 3+, 4+ gram negative coccobacilli and 3+, 4+ mixed organisms and/or usual flora or organisms suggestive of oropharyngeal flora.

Participants

Cells: 54/54 (100%) reporting labs indicated the presence of neutrophils and no or low count of epithelial cells (Table 1).

Suitability for culture: 54/54 (100%) participants reported the sputum suitable for culture (Table 2).

Bacteria: 46/54 (85%) laboratories reported both gram positive cocci and gram negative bacilli/coccobacilli (Table 3).

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, as was the suitability for culture component thus, all three components were determined to be suitable for grading.

Table2. Reported results - Suitability for culture

Reported	Cat A	Cat C1	Total	Grade
Yes	50	4	54	4
snnp	1		1	ungraded
Total	**51**	**4**	**55**	

Snnp: sample not normally processed

COMMENTS ON RESULTS

Overall, participating labs performed very well on this challenge. All participants received a grade of 4 for the cellular component as well as a grade of 4 for the suitability component.

For the bacterial component, 45 of participating labs received a grade of 4 for reporting both gram negative coccobacilli/bacilli/rods ± *Haemophilus* and gram positive cocci/mixed organisms/usual flora.

Labs that reported only one of the bacterial components were graded 1. Labs that implied that the Gram stain showed only normal flora or used abbreviations for the bacterial morphotypes were graded 0.

CLINICAL SIGNIFICANCE

The sputum Gram stain is done to simultaneously provide initial rapid biopsy-like examination to observe for the type of host inflammatory response and possible bacterial etiology for a lower respiratory tract infection, and also provide an approach to determine suitability of the specimen for culture. This screening process is not designed as a technique to save time, but rather to avoid providing confusing information for samples that are mainly saliva.

To determine suitability for culture, it is important to examine the specimen for the presence of squamous epithelial cells (SEC) which indicates salivary contamination.

The Clinical Microbiology Procedures Handbook [2] advocates that this screening should be done using a low power objective (x10). However, this is based on the assumption that the slide reader is competent and capable of distinguishing epithelial cells from white blood cells at this lower power. Many laboratories base suitability on sample on the observation of fewer than 10 SECs per low power field,[3] with others report using <25 SEC/lpf which was the original studied cut-off criteria. [4]

There are few clinical samples that result in discordant interpretations. CMPT supports laboratories that use <10 or <25 SEC/lpf as an appropriate cut-off. The vast majority of samples defined as "suitable for culture" have far fewer than 10 SEC/lpf and similarly, the vast majority of rejected samples have greater than 25 SEC/lpf. There are, however, a small minority of samples in which the cell count falls in between.

In a study by Anevlavis et. al.,[5] the sputum Gram stain was demonstrated to be a dependable diagnostic test for the early etiological diagnosis of bacterial community acquired pneumonia (CAP). The study only included CAP cases with positive blood cultures as the gold standard. The sensitivity and specificity, respectively, of sputum Gram stain was 82% and 93% for pneumococcal pneumonia, 76% and 96% for staphylococcal pneumonia, 79% and 96% for *Haemophilus influenzae* pneumonia, and 78% and 95% for pneumonia due to gram negative bacilli.

Table3. Reported results - Bacteria

Reported	Cat A	Cat C1	Total	Grade
2+ to 4+ gram negative coccobacilli ± *Haemophilus*, 2+ to 4+ gram positive cocci ± pairs ± chains ± streptococcus	36	1	37	4
3+, 4+ gram negative bacilli/rod/bâtonnet gram négatif, ± suggestive of *Haemophilus*, 2+, 3+, 4+ gram positive cocci/cocci gram positif ± pairs ± chains ± suggestive of *Streptococcus/Enterococcus*	9		9	4
3+, 4+ gram negative coccobacilli, resembling *Haemophilus*, 3+, 4+ mixed organisms and/or usual flora, ± organisms suggestive of oropharyngeal flora	2		2	4
4+ gram negative coccobacilli		1	1	1
4+ gram negative coccobacillus, 3+ gram variable coccobacillus	1		1	1
3+ GNCB, 4+ GPC pr ch, snnp		1	1	0
3+ gram positive cocci in chains	1	1	2	0
3+ normal flora	1		1	0
Sample not normally processed	1		1	ungraded
Total	**51**	**4**	**55**	

Criteria for rejection of sputa

Exclusion tools have evolved over time. Presently there are two main systems used in clinical laboratories to evaluate sputa for rejection.

The original system was based on microscopic examination of the Gram stain of the sputum samples and quantification of only squamous epithelial cells (SEC). In this system, sputa with squamous epithelial cells of 10 or more per average 10X field are rejected. The evolved system, Q score, incorporated analysis of quantification of neutrophils and epithelial cells and the presence of mucus. [6,7]

One of the challenges of working with Q scores is the variability and non-specificity of neutrophil response that is seen in the wide variety of in respiratory disorders.[6] When working up specimens from immunosuppressed patients or critically ill patients with other causes of leucopenia, laboratories employing Q score, as a rule, should modify the criteria and base rejection on the amount of squamous epithelial cells only. This however can be a problematic when that information is not provided on the sample requisition.

Murray and Washington (1975) [7] and Geckler (1977) [4] found the number of isolates correlate well with number of epithelial cells when compared to isolates from concurrent transtracheal aspirates. In this study the number of white blood cells (WBC) bore no relationship to the number of isolates. Although it has been elsewhere reported that samples with > 10 SECs and a combination of large number of pus cells (i.e. ratio of 10 X pus to epithelial cells), and a single morphotype consistent with a pathogen can grow a pure growth of a potential pathogen.[8,9]

The second system uses the number of SEC only, to exclude sputa with > 10 SEC per 10 X (low power) field except for those with many pus cells and single morphotype consistent with a pathogen.

As the number of immunosuppressed patients increases in the health care system, the challenge of having risk factor information available to the laboratory increases correspondingly.

A system that uses only epithelial cells and not WBC will have less interference from causes of immunosuppression. In addition, a system measuring fewer variables is more intuitive and easier to standardize. This in turn, leads to less inter-operator variability, which is an important aspect in ensuring quality assurance.

By applying exclusion criteria, patients with a clinical diagnosis of pneumonia should produce cultural results consistent with the etiology of infection. In addition, bacterial morphotypes may be seen in the Gram stain that are suggestive of aspiration pneumonia. For example, stained smears showing many polymorphonuclear leukocytes and many mixed respiratory flora morphotypes, especially those suggesting streptococci or anaerobes, would be consistent with aspiration pneumonia – which can be seen in hospitalized patients as well as those admitted directly from the community.

Following culture incubation, it is also useful to review plates for relative quantities of each isolate, correlating the culture results with the Gram-smear results. Failure of a morphotype to grow may be a result of treatment, culture conditions, or the media used for culture (e.g. *Legionella* species).

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