

Challenges G251 and M251-4

Sample: Blood culture

Target

- Gram: 4+ (>50/oif) gram negative bacilli (*Campylobacter coli*) – cells: not applicable
- Culture: *Campylobacter coli*
- Susceptibility testing: not required

HISTORY

A simulated blood culture sample collected from a 37 year old in-patient with diarrhea and fever was sent to participant laboratories; category A labs received a Gram smear for staining and culture sample while category C1 labs only received the Gram smear.

SAMPLE STABILITY, HOMOGENEITY AND QUALITY CONTROL

Sample composition

- Gram: 4+ (>50/oif) gram negative bacilli (*Campylobacter coli*) (Fig. 1) Cells were prepared from whole peripheral blood. There were no epithelial cells added to the sample.
- Culture: *Campylobacter coli* – pure culture

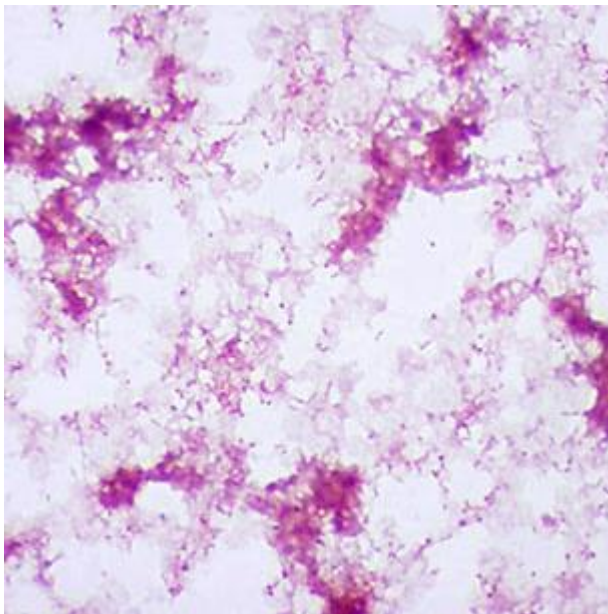


Fig.1 Gram stain of G251; simulated blood culture smear at 1000X magnification under oil immersion demonstrating gram negative bacilli.

Stability: **Gram:** 14 days from shipping day; **Culture:** 7 days from shipping day

Organism identification was confirmed by a reference laboratory before shipping to participants.

All simulated samples are produced at in house according to CMPT internal protocols. The samples are assessed for homogeneity and stability using in-house quality control methods and random selection of samples during production, before and post sample delivery. The number of random samples selected from culture samples is 15% of the total production batch; and from Gram smears is based on selection tables within Military standard 105E.¹

GRAM STAIN - RESULTS

Reference laboratories

Cells: Not applicable. The blood culture systems tend to destroy WBCs

Bacteria: 13/13 (100%) labs reported no quantity, 3+, 4+ gram negative bacilli/rods ± gull-wing, curved ± resembling/suggestive of *Campylobacter* species

Participants' results

Table 1. Gram stain Reported results - Cells

Not applicable

Table 2. Gram stain Reported results - Organisms

Reported	cat A	cat C1	Total	Grade
(2+ - 4+) gram negative bacilli/rods, ± gull-winged/curved/spiral shaped ± resembling <i>Campylobacter</i> ± or <i>Vibrio</i> spp. ± anaerobe	45	2	47	4
gram negative bacilli, gram negative diplococci/cocci	1		1	1
3+ (11-50/oif) GNR, curvy	1		1	0
GNR	1		1	0
curved rods	1		1	0
no organisms seen	2		2	0
sample not normally processed	1		1	ungraded
Total	52	2	54	

GRAM STAIN - COMMENTS ON RESULTS

A Gram smear would not be performed on a blood culture submitted for culture prior to incubation of the blood culture. Therefore, the Gram smear results should only be related to the Gram stains performed on growth from the blood culture. Almost all laboratories performed very well on this aspect of the challenge. The three laboratories that reported gram-negative bacilli

(diplococci/cocci) were given a score of 1. This result did not identify the curved nature of *Campylobacter* but does identify that the isolate was Gram negative. The laboratories that reported GNR +/- curvy, or only curved rods without an identification of gram-negative were also given a result of 0. The laboratory that reported no organisms seen was also graded as 0. Precise reporting of a Gram smear from a positive blood culture is important for early clinical management of infected patients.

CULTURE - RESULTS

Reference laboratories

12/12 (100%) labs reported *C. coli* or *Campylobacter* species, refer, 1 lab indicated it does not normally process blood cultures

Participants' results

Table 3. Reported results – Identification

Reported	Total	Grade
<i>Campylobacter coli</i> , ± refer	32	4
<i>Campylobacter jejuni/coli</i> , ± presumptive, refer	3	4
<i>Campylobacter</i> species, refer ± presumptive	10	4
Gram negative bacilli, curved/gull winged, suggestive of <i>Campylobacter</i> species, refer	1	4
<i>Moraxella (Neisseria) ovis</i> , refer	1	0
contaminant-like growth	1	0
sample not normally processed	4	ungraded
Total	52	

CULTURE - COMMENTS ON RESULTS

Like the Gram smear results, almost all the laboratories identified either *C. coli*, *C. jejuni/coli*, or identified *Campylobacter* species with suggested/presumptive identification and referred for final identification. These test results readily permit attending physician and allied personnel to consider the isolate as a *Campylobacter* species and to initiate prompt antimicrobial treatment as well as investigate epidemiology, infection control, and surveillance activities.

The two laboratories (one that indicated a *Moraxella ovis* and one a contaminant) were graded as 0. They should review their procedures for identification of blood culture isolates that Gram smear as curved, rod-shaped gram-negative bacilli.

Four laboratories do not process blood culture and were ungraded.

Grading

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.

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**. No further statistical analysis is performed on the results.

Table 2. Suitability for grading

Component	% Acceptable responses		Graded
	Reference labs	Participants	
Gram - Cells	n/a	n/a	n/a
Gram - Organisms	100	89	Yes
Culture - Identification	100	94	Yes

Maximum grade: Gram stain: 4 Culture: 4

ISOLATION and IDENTIFICATION

Campylobacter species are gram negative, non-spore forming rods that are curved, or spiral-shaped. There are over 20 species that cause clinical disease in humans, the most common being *C. jejuni*, *C. coli*, *C. lari*, *C. upsaliensis*. Organisms are usually motile, species are generally microaerophilic; however, some strains grow aerobically or anaerobically. Most *Campylobacter* species require a microaerobic atmosphere containing approximately 5% O₂, 10% CO₂, and 85% N₂ for optimal recovery. Some species of *Campylobacter* also require increased hydrogen for primary isolation and growth. ²

Campylobacter colonies may have different appearances depending on the medium they are grown. In general, *Campylobacter* species produce gray, flat, irregular, and spreading colonies. Hemolysis on blood agar is not observed.

The typical Gram stain appearance of *Campylobacter* species are gram negative, curved to spiral-shaped rods. Oxidase positive colonies grown on selective media incubated at 42°C with typical Gram stain appearance can be reliably reported as *Campylobacter* species. The major differentiating feature of *C. jejuni* compared to other *Campylobacter* species is hippurate hydrolysis. Some strains of *C. jejuni* are hippurate negative, making it difficult to distinguish from *C. coli*. ² Biochemical flowcharts to identify *Campylobacter* species please refer to Lastovica et al.(2006) ³ and Nachamkin (2011). ⁴

Species-specific PCR have been described for *C. jejuni*, *C. coli*, *C. lari*, *C. upsaliensis* and *C. fetus*. ² For laboratories with MALDI-TOF available, this technology appears to be a reliable method of identifying *Campylobacter* to the species level. A study of 1007 strains comparing conventional

methods, MALDI-TOF and real-time PCR/sequencing (gold standard in this study), found that MALDI-TOF had only a 0.4% discrepancy rate, compared to 4.5% via conventional phenotypic methods.⁵

Commonly used blood culture systems should support the growth of *Campylobacter* and give appropriate signals if positive however, some authors recommend to subculture after 24 to 48hs if *Campylobacter* infection is suspected.²

In addition, detection of *Campylobacter* bacteremia may require a median growth rate exceeding the typical incubation time of 5 days and thus, it is conceivable that many episodes of *Campylobacter*-related bacteremia go unnoticed. It has therefore been also recommended by some authors that the contents of blood culture bottles should be sub-cultured at the end of the standard 5-day incubation period.²

A study comparing the performance of automated blood culture systems for the isolation of *Campylobacter* species from blood specimens reported poor performance of some systems with the median growth rate for culture bottles being 5–10 days for *C. jejuni* and 3–5 days for *C. fetus*.⁶ It has therefore been recommended by some authors that the contents of blood culture bottles should be sub-cultured onto solid media at the end of the standard 5-day incubation period.⁷

ANTIMICROBIAL SUSCEPTIBILITY

The methods of choice for *Campylobacter* recommended by the Clinical and Laboratory Standards Institute (CLSI) are agar dilution and broth microdilution.⁸

Susceptibility testing continues to play a critical role in guiding therapy and epidemiological monitoring of resistance. Macrolides, quinolones, and tetracyclines are among the common antimicrobials recommended for testing.

High levels of resistance to tetracycline and ciprofloxacin are frequently reported by many national surveillance programs, but resistance to erythromycin and gentamicin in *Campylobacter jejuni* remains low.⁹

Antibiotics are employed in immunocompromised patients, patients whose symptoms are severe or persistent, and those with extra-intestinal infections.¹⁰

CLINICAL RELEVANCE

Campylobacter infection in humans has been associated with a range of gastrointestinal conditions; they have also been reported to be involved in extra-gastrointestinal manifestations, including bacteremia, lung infections, brain abscesses, meningitis, cellulitis and septic arthritis, in individual cases and small cohorts of patients.^{11,12}

Risk factors for campylobacteriosis include international travelling, followed by consumption of undercooked chicken, environmental exposure, and direct contact with farm animals.⁸ Cattle were the attributed source for 16 to 19% of the total cases of campylobacteriosis in Denmark and

Switzerland.^{11,13,14}

Campylobacter bacteremia is uncommon, but can be seen in association with *C. jejuni*, *C. coli*, and *C. fetus*.¹⁰ *C. fetus* is an important cause of systemic campylobacteriosis, which may be in part due to its surface layer protein (SLP) which acts as a capsule and protects the bacterium from the normal bactericidal action of serum.¹⁵ The incidence of *Campylobacter* bacteremia has been shown to be <1% of the total incidence of *Campylobacter* infections.¹⁶ However, the true incidence may be underreported as blood cultures are often not drawn in patients with gastroenteritis in the community, and due to difficulties in isolation and identification due to the fastidious nature of the bacterium.

Although many studies have found that most cases of *Campylobacter* bacteremia occur in elderly or immunocompromised patients^{10,12,17} a study in Finland found that the majority of patients (70%) did not have any significant underlying diseases.¹⁶

MAIN EDUCATIONAL POINTS from G251 and M251-4

1. The appearance of thin, curved, gram-negative bacilli from a Gram smear and/ or from growth of a clinical specimen (e.g. blood culture, faeces, or occasionally tissue) should prompt investigations for the presence of *Campylobacter* species by the laboratory.
2. The laboratory has an additional responsibility to notify public health authorities of the isolation of *Campylobacter* from any clinical source, so that prompt investigation into the potential for outbreaks, and possible source controls can be put in place.
3. Most septic episodes of *Campylobacter* species can be managed well with appropriate antimicrobial agents, with quite low morbidity and rare mortality.

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