Challenge M251-6

Sample: Stool

Target: No *Salmonella, Shigella, Campylobacter, Yersinia, Aeromonas, Plesiomonas, Vibrio,* STEC, or *Edwardsiella tarda* isolated

HISTORY

A simulated stool sample collected from a 31-year-old traveler with diarrhea was sent to category A laboratories.

SAMPLE STABILITY, HOMOGENEITY AND QUALITY CONTROL

Sample composition: Non-pathogenic *Escherichia coli* – pure culture

Stability: 18 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 is 15% of the total production batch.

RESULTS

Reference laboratories

13/13 (100%) labs reported No *Shigella, Salmonella Campylobacter, Yersinia, Aeromonas*, *Plesiomonas, Vibrio, Edwardsiella tarda* or shiga toxin producing *E. coli* (NSSCYAPVEE) or enteric/gastrointestinal pathogens present/isolated

Participants' results

Table 1. Identification results

Reported	Total	Grade
Absence de pathogene/no enteric/entero/gastrointestinal pathogens ± by NAAT ± by molecular methods	13	4
NSSCYAPVEE ± Présence de flore fécale	19	4
Flore intestinale normale	1	1
NSSCYPE, including ETEC, EAEC, EPEC, EIEC and non-O157 shiga toxin producing *E.coli*	2	4
NSSCYAPV or Shiga toxin producing organisms including Shiga toxin producing *E. coli* isolated.	3	4
NSSCYPV, negative for STEC, ETC, EPEC, EAEC, EIEC, *C.difficile* toxin	1	4
NSSCA, no *Staphylococcus aureus* and *Bacillus* isolated	1	0
NEGATIVE for Shiga toxin stx1 and stx2 genes by PCR. NSSCYAPV	1	4
Shigella species	1	0
refer, snnp	10	ungraded
Total	**52**	

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	
Identification	100	93	Yes

Maximum grade: 4

COMMENTS ON RESULTS

Overall, 93% of participants accurately indicated the absence of enteric bacterial pathogens, consistent with the expected result of "No Salmonella, Shigella, Campylobacter, Yersinia, Aeromonas, Plesiomonas, Vibrio, STEC, or *Edwardsiella tarda* isolated." This high concordance

reflects strong proficiency among laboratories in both interpretation and appropriate reporting for stool culture or multiplex PCR results.

However, one participant incorrectly reported *Shigella* species, which could represent a misidentification of non-pathogenic *E. coli* as *Shigella*, resulting in a grade of "0." This constitutes a clinically significant error, as false identification of a notifiable pathogen could lead to unnecessary patient treatment, inappropriate public health response, and reputational or regulatory implications for the laboratory.

Some ambiguity existed with certain responses such as "Normal Intestinal Flora" and those including organisms not typically reported in routine stool testing (e.g., *Staphylococcus aureus* or *Bacillus*). Indicating the non-presence of significant enteric pathogens would be clinically relevant and align with laboratory best practices.

ISOLATION and IDENTIFICATION

Laboratories must assess the prevalence of specific enteric pathogens in their geographic area to determine the selective media to inoculate routinely as well as the patient's history to determine the specialized media to inoculate upon special request or when clinical circumstances suggest exposure.

At a minimum, routine fecal culture setup should be designed to optimize the recovery of *Salmonella, Shigella, Campylobacter*, and *E. coli* O157. Some laboratories also include other testing to detect STEC or media to allow the recovery of *Aeromonas* species, *Plesiomonas* species, *Vibrio* species, and *Yersinia* species, whereas other laboratories add these tests on request only.[1]

In the current challenge, 17 of the 42 participating laboratories (40%) tested the sample using a molecular method (e.g., Biofire, BD Max); this is significantly higher than the 8% recorded in May 2023 survey. It is anticipated that more laboratories will adopt a molecular approach to routine stool testing over time.

Gastrointestinal infections are commonly caused by viruses (e.g., norovirus, rotavirus), and less commonly by bacteria (approximately 2-6% of bacterial cultures of diarrheal stool identify a pathogen) and parasites (e.g., *Entamoeba histolytica*, *Giardia lamblia*, *Cryptosporidium* spp.).

Other causes of gastroenteritis include food poisoning, allergic reactions, or reactions to certain medications or foods. *Clostridiodes difficile* is most commonly associated with healthcare–acquired gastrointestinal infections.

The main testing options for gastrointestinal infections include: 1) bacterial culture (which facilitates antimicrobial susceptibility testing, if indicated); 2) enzyme immunoassays (EIAs) to test for the presence of pathogen proteins; and 3) molecular diagnostics, such as polymerase chain reaction (PCR), to test for the presence of pathogen DNA or RNA. Multiplex PCR assays can test for a wide range of pathogens associated with diarrhea; can replace a set of pathogen-specific cultures, antigen tests, and stool microscopy exams; and promote a timelier (hours versus days) and more accurate diagnosis of gastroenteritis. Multiplex PCR assays, have shown

higher sensitivity, specificity, and positive predictive value than culture. [1-3]

However, multiplex molecular gastrointestinal tests do not yield a bacterial isolate; instead, they simply provide a result. These results should be reported to public health departments in accordance with provincial mandates. However, for specific pathogens, public health laboratories also require the actual bacterial isolate in order to properly conduct outbreak investigations. Therefore, clinical laboratories absolutely must reach out to their public health laboratory well in advance of adopting a multiplex molecular gastrointestinal test in order to determine how specimens will be cultured for outbreak investigations, who will perform the cultures, and how the specimens will be collected and transported.

REPORTING

The laboratory report should reflect results for each organism routinely included in testing (both positive and negative results must be reported for each organism tested for). For example, a negative routine fecal culture should be reported as "no *Salmonella*, *Shigella, Campylobacter,* or STEC isolated." [4]

ANTIMICROBIAL SUSCEPTIBILITY

Not applicable to this challenge

CLINICAL RELEVANCE

Diarrheal illness is a problem worldwide, with substantial regional variation in the prevalence of specific pathogens. Although stool cultures are commonly requested, their usefulness in otherwise healthy patients has been questioned and the yield of such cultures is often quite low.[5] However, specific diagnosis may be important for a variety of reasons.

While many enteric infections are self-limiting and do not require antimicrobial treatment, appropriate antimicrobial therapy can shorten illness in some bacterial infections.[6] Empiric therapy may result in courses of unnecessary antibiotics, with harm to patients as a result of side effects or unwanted consequences (e.g., *C. difficile* infection), the potential for generation of resistant organisms, and inappropriate use of scarce resources. The outcome of some bacterial diarrheal illnesses, for example, *Salmonella* infection in young healthy adults, may be worsened or prolonged using antibiotics. [7]

Lack of specific diagnosis can also impede disease surveillance, outbreak detection, and other critical measures that protect the public health. Unfortunately, only approximately 2-6% of stool specimens submitted are positive for enteric pathogens. This translates into a very high price for positive stool cultures. Cultures, however, are useful not only for individual patient care, but also for public health surveillance and control programs. [5] There may also be diagnostic value in negative stool culture results.

MAIN EDUCATIONAL POINTS from M251-6

1. Accurate reporting of negative stool results is clinically and epidemiologically valuable, even in the absence of enteric pathogens; a standardized reporting (e.g., "No Salmonella, Shigella, Campylobacter, STEC isolated") supports clinical decision-making, avoids unnecessary treatment, and contributes to public health surveillance.
2. Molecular testing is increasing but requires strategic integration; Multiplex PCR assays provide rapid and sensitive detection of gastrointestinal pathogens but do not generate bacterial isolates needed for outbreak investigations or antimicrobial susceptibility testing. Laboratories must establish workflows to obtain isolates when indicated.
3. Misidentification of enteric pathogens has significant consequences; false-positive identification of notifiable organisms (e.g., *Shigella* species) may trigger unnecessary patient isolation, inappropriate antibiotic use, and unwarranted public health responses.
4. Diagnostic yield in stool cultures is low but still important; although most diarrheal illnesses are viral and self-limited, identifying a bacterial cause has implications for patient management and infection control, especially in vulnerable populations.
5. Use of standardized result language improves clarity and prevents miscommunication. Vague or non-specific terms (e.g., "normal fecal flora") should be avoided when reporting on targeted pathogen testing.

REFERENCES

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