Challenge M132-2

August 2013

Stool – *Escherichia coli* O157

HISTORY

The sample was a simulated stool obtained from a 50 year old male with watery diarrhea.

Participants were expected to isolate and identify *Escherichia coli* serotype O157.

CMPT QC

Internal control cultures yielded a pure growth of *E. coli* O157 viable for 22 days.

SURVEY RESULTS

Reference Laboratories: 15/15 (100%) laboratories reported *Escherichia coli* O157 (3 reported H7 positive, 1 of which also reported verotoxin positive). All reference laboratories reported the isolate to Public Health (PH). Consensus was reached for both components therefore, they were graded.

Identification (Table 1)

Participants performed very well in this challenge. All participants processing the sample isolated and reported *E. coli* O157, these labs were graded 4.

Public Health notification (Table 2)

66/72 (92%) laboratories reported the isolate to PH and were graded 4. Two participants indicated they would refer, with no notification to Public Health and was graded 0. One laboratory did not report to PH, did not refer and was graded 0.

Two participants reported results using the incorrect identifier and were graded 0.

MAIN EDUCATIONAL POINTS from M132-2

1. Some strains of Enterohemorrhagic *E. coli* O157 and non O157 may not be detected if using Sorbitol MacConkey (SMAC) agar.
2. Chromogenic agars have been developed which allow for detection of sorbitol and non-sorbitol fermenting isolates.
3. Non O157, verotoxin producing strains, have been involved in outbreaks.
4. Non culture Shiga toxin testing may be a useful alternative to culture methods.
5. Antimicrobial therapy is not recommended and has been linked to an increased risk of HUS in children.

COMMENTS ON RESULTS

Laboratories had no difficulty in isolating or identifying the organism.

Four laboratories used the wrong identifier. This is an ongoing issue. Laboratories are reminded to check that the correct identifier has been used before submitting their final data.

Two laboratories referred the isolate for further testing and did not notify Public Health. Previous CMPT critiques have indicated that all laboratories must report all findings of *E. coli* O157 even is final confirmation is done at a reference laboratory.

As mentioned in other critiques, laboratories are encouraged to indicate if this organism is reportable to PH. Some participants indicated they would refer the organism but it is not clear if they expect the reference laboratory to notify to PH or not .

Grading

Maximum grade: 8

Reporting *E. coli* O157 was graded 4.

Reporting the isolate to PH authorities was graded 4.

Not reporting to PH was graded 0.

Reporting results using the incorrect identifier is always graded 0.

CMPT requires the full identifier in the results report form. Please note that the M is part of the identifier and the sample should be identified as M132-2 and not as 132-2

Table 1. Identification results

Reported results	A Labs	B Labs	Total	Grade
Escherichia coli O157, ± NSSCYAPV ± refer ± verotoxigenic	52	4	56	4
Escherichia coli O157:H7, ± NSSCYAPV, ± refer ± verocytotoxin	13	1	14	4
reported using incorrect identifier	1	1	2	0
does not process	12	4	16	ungraded
Total	**78**	**10**	**88**	

NSSCYAPV: no *Shigella*, *Salmonella, Campylobacter, Yersinia, Aeromonas*, *Plesiomonas*, or *Vibrio*

Table 2. Report to Public Health

Reported to Public Health	Total	Grade
yes	66	4
no report, refer	2	0
no report	1	0
reported using incorrect identifier	2	0
n/a (reporting lab is a Public Health Laboratory)	1	ungraded
does not process	16	ungraded
Total	**88**	

ISOLATION and IDENTIFICATION

Stool specimens submitted for bacterial enteric culture should be screened for Shiga toxin producing *E. coli* O157 (STEC O157). The Sorbitol MacConkey (SMAC) plate is the most commonly used culture screening medium. Most isolates fail to ferment sorbitol and will be recognized as colourless colonies on SMAC.

There are other organisms that can appear as non-sorbitol fermenters, so further testing is required to confirm identification. A sampling of the non-sorbitol fermenting colonies can be tested directly using a latex agglutination test for the presence of the somatic O157 antigen. Species other that *E. coli* O157 may cross react so all positive latex tests must be confirmed to be *E. coli* using biochemical tests [1, 2, 3] .

All presumptive *E. coli* O157 should be tested for the presence of the flagellar (H7) antigen. This test is usually performed at a reference laboratory. Isolates negative for the H7 antigen, or that are non-motile should be tested for the presence of the Shiga toxin or Shiga toxin gene sequences.

Some strains of *E. coli* O157 ferment sorbitol and will not be detected if the SMAC agar is used. Non O157 STEC are also responsible for outbreaks and may not be isolated unless other media or methods for toxin testing are used [1,4]. An alternative may be to perform non-culture Shiga toxin testing either on site or through referral to a reference laboratory.

Non Culture Methods

STEC harbors and expresses the genes for Shiga toxins type 1 (Stx 1) and type 2 (Stx2), the virulence factors that lead to Hemolytic Uremic Syndrome (HUS). Single STEC may express either Stx1, Stx2, or both. Non-culture methods are aimed at the detection of the toxins or toxin genes. They have the advantage of detecting any of the STEC serotypes.

Toxins are detected by cytotoxicity or immunoassays while genes are detected by nucleic acid amplification tests.

CLINICAL RELEVANCE

The natural reservoir is the gastrointestinal tract of cattle. STEC transmission occurs through the consumption of a wide variety of contaminated foods, raw milk, and raw produce, through contact with animals or their environment, and directly from person to person[1,7, 8].

Shiga toxin producing strains are capable of causing mild non bloody diarrhea, severe bloody diarrhea and HUS. Symptoms are mediated by toxins which are biochemically and genetically similar to the Shiga toxin produced by *Shigella dysenteriae* type 1.

Patients infected with STEC present at first with abdominal cramps and watery diarrhea; this may progress within 1 or 2 days to hemorrhagic colitis and about 5 to 15% of patients typically develop HUS [5, 9].

HUS is characterized by thrombocytopenia, hemolytic anemia and kidney injury. Some patients present neurological symptoms including severe headache, and encephalopathy [5].

Bacteremia is rare as STEC are not invasive but they secrete ribosome inactivating toxins which are responsible for the organ damage [10].

Shiga toxins are compound toxins composed of a catalytic A subunit and a multimeric B subunit (AB_5) which binds to the cell surface of the target cells [11]. Once bound, the toxin is incorpo-

Non Culture Methods

Cell Cytotoxicity assays

These assays use Vero or HeLa cell lines to detect the presence of biologically active Shiga toxins in stools. These cell lines are very sensitive to Shiga toxin because they have high concentrations of receptors for the toxin [2].

Sterile fecal or enrichment broth filtrates are inoculated onto the cell monolayer and observed for typical cytopathic effect. Confirmation that the cytopathic effect is caused by Shiga toxin is performed by neutralization using anti-Stx 1 and anti-Stx 2 antibodies [5,6].

Shiga Toxin Immunoassays

There are few immunoassays commercially available for the detection of Shiga toxin. Most assays recommend the use of enrichment broth cultures rather than direct testing of stool specimens because of the low amount of free toxin in stools. Some assays can differentiate between Stx1 and Stx2 [6].

Nucleic Acid Amplification Tests (NAAT)

Most NAAT assays are designed and validated for testing isolated colonies taken from plated media while some assays have been validated for testing on stool specimens after incubation in an enrichment broth [2]. Depending on the primers used, these assays can distinguish between stx1 and stx2 genes [6].

rated into the cells by endocytosis and reaches the endoplasmic reticulum by retrograde transport via the Golgi apparatus.

The A subunit is then enzymatically activated and released; the active A subunit has RNA N-glycosidase activity it cleaves a specific N-glycosidic bond in the 28S rRNA, inhibiting protein synthesis and ultimately causing cell death[12].

Although O157:H7 is the serotype most frequently isolated from humans, a recent CDC surveillance report showed that O157 serotypes comprised 41.1% of all the STEC isolated. Non-O157:H7 STECs have been emerging in Canada and are common in Australia, Germany, and Austria [4,13].

In 2011 a large outbreak of gastroenteritis with bloody diarrhea and HUS was reported in Germany. The strain involved in the outbreak was an enteroaggregative STEC O104:H4. This strain was notable for its high HUS rate of (22% of cases -88% of those were adults), and because it expressed an extended-spectrum β-lactamase (ESBL) [14].

ANTIMICROBIAL SUSCEPTIBILITY

Reporting susceptibility test results is not recommended. Treatment of STEC infection is supportive. Generally, patients with HUS are managed for symptoms of renal failure, anemia, bleeding and intestinal injury.

The use of antibiotics is contraindicated as it has been shown to be neither useful nor safe. Some studies have shown that antibiotic usage may induce the development and release of the Shiga toxins. Other studies have shown that antibiotic usage in children with O157 STEC infections can increase the risk of HUS.

Susceptibility testing may be done for epidemiological purposes. Once susceptible to most antimicrobial agents, *E coli* O157 and other STEC are showing increasing resistance [15, 16].

REFERENCES

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CMPT Shiga Toxin program

In May 2012 CMPT launched a new PT program: "Shiga Toxin program" to test proficiency of laboratories performing tests to detect Shiga Toxin production or Shiga Toxin genes in fecal isolates.

The sample provided by CMPT is suitable for culture and non-culture methods.

To learn more about the program visit our website.

www.cmpt.ca/programs_shigatoxin/shiga_program.html