MAYO CLINIC LABORATORIES

Test Definition: STFRP

Shiga Toxin, Molecular Detection, PCR, Feces

Overview

Useful For

Sensitive, specific, and rapid detection of the presence of Shiga toxin-producing organisms such as *Escherichia coli* O157:H7 and *Shigella dysenteriae* type 1 in stool

This test is **not recommended** as a test of cure.

Testing Algorithm

For more information see Laboratory Testing for Infectious Causes of Diarrhea.

Special Instructions

- Laboratory Testing for Infectious Causes of Diarrhea

Method Name

Real-Time Polymerase Chain Reaction (PCR)

NY State Available

Yes

Specimen

Specimen Type

Fecal

Additional Testing Requirements

In some cases, local public health requirements may impact Mayo Clinic Laboratories (MCL) clients and require additional testing on specimens with positive test results. MCL recommends clients retain an aliquot of each specimen for potential additional testing. Alternatively, if a specimen needs to be returned, clients must contact MCL within 96 hours of specimen collection. Clients will be responsible for submitting their specimens to appropriate public health departments.

Necessary Information

Specimen source is required.

Specimen Required

The high sensitivity of amplification by polymerase chain reaction requires the specimen to be processed in an environment in which contamination of the specimen by shiga toxin DNA is unlikely.

Submit only 1 of the following specimens:

Preferred:
Specimen Type: Preserved feces
Supplies: Culture and Sensitivity Stool Transport Vial (T058)
Container/Tube: Commercially available transport system specific for recovery of enteric pathogens from fecal specimens (15 mL of nonnutritive transport medium containing phenol red as a pH indicator, either Cary-Blair or Para-Pak C and S)
Specimen Volume: Representative portion of feces; 5 mL
Collection Instructions:
1. Collect fresh fecal specimen and submit in container with transport medium.
2. Within 2 hours of collection, place feces in preservative.
Specimen Stability Information: Ambient (preferred) 7 days/Refrigerated 7 days

Acceptable:
Specimen Type: Unpreserved feces
Supplies:
-Stool container, Small (Random), 4 oz (T288)
-Stool Collection Kit, Random (T635)
Container/Tube: Fecal container
Specimen Volume: Representative portion of feces
Collection Instructions: Collect fresh fecal specimen and submit representative sample in fecal container.
Specimen Stability Information: Refrigerated (preferred) 7 days/Frozen 7 days

Forms

If not ordering electronically, complete, print, and send 1 of the following forms with the specimen:
-Microbiology Test Request (T244)
-Gastroenterology and Hepatology Test Request (T728)
-Renal Diagnostics Test Request (T830)
-Coagulation Test Request (T753)

Specimen Minimum Volume

1 mL

Reject Due To

Formed feces	Reject
Feces in gel transport medium	Reject
Feces in EcoFix preservative	Reject
Feces in formalin	Reject
Feces in PVA fixative	Reject
Preserved feces received	Reject

frozen	

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Fecal	Varies	7 days	

Clinical & Interpretive

Clinical Information

Shiga toxins (also known as Shiga-like toxins, Vero toxins, or Vero-like toxins) are encoded by some strains of *Escherichia coli,* most notably O157:H7. Shiga toxin can also be produced by other serogroups of enterohemorrhagic *E coli* (EHEC), as well as *Shigella dysenteriae* type 1. Although not universal, Shiga toxin-producing organisms generally cause bloody diarrhea. Unlike some bacterial gastrointestinal infections, antimicrobial therapy is contraindicated, as antimicrobial agents may exacerbate disease. Treatment is primarily supportive (eg, hydration). A complication of infection by an organism producing Shiga toxin is hemolytic uremic syndrome (HUS). The percentage of people that develop HUS varies among outbreaks of *E coli* O157:H7 but generally ranges from 3% to 20%. HUS is characterized by a triad of findings: hemolytic anemia, thrombocytopenia, and kidney failure. Most people recover completely; however, some require permanent dialysis, and some die due to complications.

Several diagnostic methods are available for the detection of EHEC but lack sensitivity, are labor intensive, or have a long turnaround time. There are more than 160 serogroups of EHEC; the first serogroup to be associated with HUS was O157:H7. This is also the serogroup that is most frequently implicated in outbreaks. EHEC O157:H7 is detectable as non-fermenting colonies when cultured on sorbitol MacConkey (SMAC) agar, but the majority of non-O157:H7 Shiga toxin-producing *E coli* strains ferment sorbitol and, therefore, are undetectable by this method. The Vero cell line is susceptible to the Shiga toxin, but the assay can take up to 48 hours and is nonspecific. Commercial enzyme-linked immunosorbent assay (ELISA) antigen detection kits have a sensitivity of 90% when compared to culture, but an overnight enrichment step is necessary for adequate sensitivity. Polymerase chain reaction (PCR) detection of *stx*, the gene encoding Shiga toxin, directly from fecal specimens is a sensitive and specific technique, providing same-day results. The PCR assay identifies non-O157:H7 Shiga toxin-producing bacteria, extending the utility beyond strains identifiable on SMAC agar.

Reference Values

Not applicable

Interpretation

A positive polymerase chain reaction (PCR) result indicates the likely presence of Shiga toxin-producing *Escherichia coli* in the specimen. Although *Shigella dysenteriae* serotype 1 may produce a positive result, it is extremely rare in the United States.

A negative result indicates the absence of detectable Shiga toxin DNA in the specimen but does not rule out the presence of Shiga toxin-producing *E coli* and may occur due to inhibition of PCR, sequence variability underlying primers or probes, or the presence of Shiga toxin DNA in quantities less than the limit of detection of the assay. Shiga toxins are encoded on mobile genetic elements and can theoretically be lost by their bacterial host.

Cautions

Interfering substances in the fecal specimen may affect the accuracy of this assay; results should always be interpreted in conjunction with clinical and epidemiological findings.

This assay detects *stx* subtypes *stx1, stx2, stx2c*, and *stx2d*. It does not detect *stx2e* or *stx2f,* which are seldom associated with human disease.

Repeat testing **should not be performed** on specimens collected less than 7 days apart.

Supportive Data

This assay was prospectively clinically validated using 204 stool specimens submitted for the antigen test (enzyme immunoassay: EIA). In addition, the assay was used to test 85 archived fecal specimens previously tested for either *Escherichia coli* O157:H7 or Shiga toxin by EIA, with results compared to the prior results. Discordant results on the archived specimens were resolved by submission to the Minnesota Department of Health (MDH) for polymerase chain reaction (PCR) using different primers. Compared to a combined gold standard (ie, positive by EIA, culture, or MDH PCR) the Mayo PCR assay had 100% sensitivity and specificity; in total, 46 positive and 243 negative specimens were evaluated. No cross-reactivity was observed when tested on a panel of more than 50 organisms commonly found in stool. The analytical sensitivity was 2 targets/mcL.

Clinical Reference

1. Gould LH, Bopp C, Strockbine N, et al. Recommendations for diagnosis of shiga toxin--producing Escherichia coli infections by clinical laboratories. MMWR Recomm Rep. 2009;58(RR-12):1-14
2. Nyre LM, Kiemele DL, Zomok CD, et al: Clinical experience with rapid PCR for detection of Shiga toxin in stool. Abstract of the Annual Meeting of the American Society for Microbiology, 2010 General Meeting, San Diego, CA, May 23-27, 2010
3. Procop GW, Church DL, Hall GS, et al: The Enterobacteriaceae. In: Koneman's Color Atlas and Textbook of Diagnostic Microbiology. 7th ed. Wolters Kluwer; 2017:213-315

Performance

Method Description

This method employs a target-specific detection system, including polymerase chain reaction (PCR) primers and fluorescent resonance energy transfer (FRET) hybridization probes designed for the *stx1* and *stx2* genes. The LightCycler instrument amplifies and monitors target nucleic acid sequences by fluorescence during PCR cycling. This is an automated PCR system that can rapidly detect amplified product development. The detection of amplified products is based on the FRET principle. For FRET product detection, a hybridization probe with a donor fluorophore, fluorescein, on the 3' end is excited by an external light source, which emits light that is absorbed by a second hybridization probe with an acceptor fluorophore, LC-Red 640, at the 5' end. The acceptor fluorophore then emits a light of a different wavelength that is measured with a signal that is proportional to the amount of specific PCR product. The process is completed in a closed tube system.(Grys TE, Sloan LM, Rosenblatt JE, Patel R: Rapid and sensitive detection of Shiga toxin-producing *Escherichia coli* from nonenriched stool specimens by real-time PCR in comparison to enzyme immunoassay and culture. J Clin Microbiol. 2009;47[7]:2008-2012)

PDF Report

No

Day(s) Performed
Monday through Sunday

Report Available
1 to 2 days

Specimen Retention Time
7 days

Performing Laboratory Location
Mayo Clinic Laboratories - Rochester Main Campus

Fees & Codes

Fees
- Authorized users can sign in to Test Prices for detailed fee information.
- Clients without access to Test Prices can contact Customer Service 24 hours a day, seven days a week.
- Prospective clients should contact their account representative. For assistance, contact Customer Service.

Test Classification
This test was developed and its performance characteristics determined by Mayo Clinic in a manner consistent with CLIA requirements. It has not been cleared or approved by the US Food and Drug Administration.

CPT Code Information
87798

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
STFRP	Shiga Toxin PCR, F	80679-4

Result ID	Test Result Name	Result LOINC® Value
SRC59	Specimen Source	31208-2
56052	Result	80679-4