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
See Laboratory Testing for Infectious Causes of Diarrhea in Special Instructions.

Special Instructions
- Laboratory Testing for Infectious Causes of Diarrhea

Method Name
Real-Time Polymerase Chain Reaction (PCR) using Fluorescent Resonance Energy Transfer (FRET)

NY State Available
Yes

Specimen

Specimen Type
Fecal

Additional Testing Requirements
In some cases, there may be local public health requirements that impact Mayo Clinic Laboratories (MCL) clients and require additional testing on specimens with positive results for this test. MCL recommends that clients retain an aliquot of each specimen submitted for testing to perform such additional testing, if needed. Alternatively (not preferred), clients who want their specimen returned from MCL should call MCL as soon as possible, at the latest within 96 hours of specimen collection, to request that MCL return an aliquot of the submitted specimen to them. 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:** C and S 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-PakC and S)
Test Definition: STFRP
Shiga Toxin PCR, F

Specimen Volume: Representative portion of feces; 5 mL

Collection Instructions:
1. Collect fresh fecal specimen and submit in container with transport medium.
2. Place feces in preservative within 2 hours of collection.

Specimen Stability Information: Ambient (preferred) <7 days/Refrigerated <7 days/Frozen <7 days

Acceptable:

Specimen Type: Unpreserved feces

Supplies:
- Stool container, Small (Random), 4 oz Random (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 Client Test Request (T728)
- Renal Diagnostics Test Request (T830)

Specimen Minimum Volume
1 mL

Reject Due To

| Formed feces or feces in gel transport medium; EcoFix preservative; formalin or PVA fixative | Reject |

Specimen Stability Information

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<th>Temperature</th>
<th>Time</th>
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<tr>
<td>Fecal</td>
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Clinical and 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. Generally, Shiga toxin-producing organisms cause bloody diarrhea, although this is not universal. Unlike some bacterial gastrointestinal infections, antimicrobial therapy is contraindicated, as antimicrobials 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 as a result of complications.

Several diagnostic methods that are available for the detection of EHEC 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 commonly 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. PCR detection of *stx*, the gene encoding Shiga toxin, directly from fecal specimens is a sensitive and specific technique, providing same-day results. 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 method). 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**


3. Grys TE, Patel R: Update on Shiga toxin-producing *Escherichia coli*. Mayo Clinic, Mayo Medical Laboratories Communiqué


**Performance**

**Method Description**

This method employs a target-specific detection system including polymerase chain reaction (PCR) primers, as well as 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 secondary 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;47:2008-2012)

**PDF Report**

No

**Day(s) and Time(s) Test Performed**

Monday through Sunday

**Analytic Time**

1 day

**Maximum Laboratory Time**

2 days
Test Definition: STFRP
Shiga Toxin PCR, F

Specimen Retention Time
7 days

Performing Laboratory Location
Rochester

Fees and 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 Regional Manager. 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. This test has not been cleared or approved by the U.S. Food and Drug Administration.

CPT Code Information
87798

LOINC® Information

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