curian®

Shiga Toxin

A Rapid Fluorescent Immunoassay for the Detection of Shiga toxin 1 and Shiga toxin 2 in Cultures Derived from Stool Specimens

REF **760630**

IVD

℞ Only

For Professional Use Only

INTENDED USE

The Curian Shiga Toxin assay, for use with the Curian Analyzer, is a rapid, qualitative, fluorescent immunoassay for the simultaneous detection and differentiation of Shiga toxin 1 (Stx1) and Shiga toxin 2 (Stx2) in a single test device. It is intended for use with cultures derived from human stool specimens to aid in the diagnosis of disease caused by Shiga toxin producing *Escherichia coli* (STEC) infections. Test results are to be used in conjunction with the patient's clinical symptoms and history.

SUMMARY AND EXPLANATION OF TEST

Shiga toxin-producing *Escherichia coli* (STEC) are a diverse group of foodborne pathogens, defined by the presence of the genes that encode for Shiga toxin 1 (Stx1), Shiga toxin 2 (Stx2), or both Stx1 and Stx2.[1, 2] STEC infections continue to be a public health concern because of the potential severity of the gastrointestinal illness and associated complications[3], with symptoms ranging from mild cases of diarrhea to more severe diseases if left undiagnosed, such as acute gastroenteritis, hemorrhagic colitis (HC), and life-threatening hemolytic uremic syndrome (HUS), which is the leading cause of acute renal failure in children.[1, 4]

The majority of STEC infections are caused by ingestion of food or water contaminated with animal feces of bovine origin, however environmental or animal contact and person-to-person transmission are also important sources of infection.[4, 5] Most STEC infection outbreaks and isolated disease cases have been reported from various industrialized countries all over the world, with approximately 265,000 illnesses yearly in the United States.[6, 7] The largest outbreaks of STEC are due to a single *E. coli* serotype, O157:H7, although non-O157 serotypes also cause the same diseases, and awareness of the public health importance of non-O157 serotypes is growing.[4, 8]

Some treatment and therapy methods can negatively impact the prevention of Shiga toxin release and elimination of the pathogen, even increasing the risk and further development of deadly diseases such as HUS[9, 7]. Prompt detection and diagnostic methods are needed for STEC infections to aid clinicians while allowing epidemiologists the opportunity to identify outbreaks and to trace the source of infection[6]. Studies have demonstrated that there is a link between Stx subtype and disease severity, with Stx2 more likely to be associated with severe disease, supporting the need for development and implementation of different case management and public health management of cases based on Stx subtype produced by STEC infection.[2] The Curian Shiga Toxin assay provides rapid, reliable results for the simultaneous detection and differentiation of Stx1 and Stx2 antigens using a fluorescent analyzer to remove subjectivity and assist in the diagnosis of STEC infection.

BIOLOGICAL PRINCIPLES

The Curian Shiga Toxin assay consists of a test strip enclosed in a plastic frame (test card), positive control reagent, and assay-specific Aioprep™ sample preparation device. Curian Shiga Toxin is a lateral flow-based immunoassay for the simultaneous detection and differentiation of Shiga toxin 1 (Stx1) and Shiga toxin 2 (Stx2) in broth or plate cultures derived from human stool specimens. Curian Shiga Toxin utilizes monoclonal antibodies specific to Stx1 and Stx2 as capture and detector antibodies, along with a polyclonal capture antibody.

The Aioprep is pre-filled with blue tinted Sample Diluent/Negative Control and contains a filter and a dropper tip. A sample of the patient's culture enriched stool specimen is transferred from the enrichment media to the Aioprep, using either the included transfer pipettes or a swab (not provided with the kit) to add the sample directly into the Sample Diluent/Negative Control.

The diluted sample is mixed and dispensed drop-wise into the sample port of the Curian Shiga Toxin test card. If Stx1 and/or Stx2 antigens are present in the sample, they bind to the respective monoclonal detector antibodies conjugated to fluorescent particles, forming a complex. As the complex moves through the test strip, the anti-Shiga toxin 1 and/or 2 monoclonal capture antibodies, each bound to the assay membrane at their respective Stx1 and Stx2 test positions of the strip, bind the complexes and yield the respective test lines. When antigen is not present, a complex is not formed, and test lines do not form. As the sample continues to move further up the test strip, the polyclonal capture antibody, bound to the assay membrane at the control position of the strip, binds the conjugated antibodies and yields the control line. A line at the control position of the test strip should be present each time a sample or external control is tested. If the control line is not generated, adequate sample flow has not occurred, and the Curian Analyzer will consider the test invalid.

REAGENTS/MATERIALS PROVIDED

The maximum number of tests obtained from this test kit is listed on the outer box.

1. **Curian Shiga Toxin Test Card:** A test strip enclosed in a plastic frame which is in a foil pouch with a desiccant. Supplied ready to use.
2. **Curian Shiga Toxin Aioprep Sample Preparation Device/ Negative Control:** A buffered aqueous protein solution containing blue dye and 0.094% sodium azide. The Aioprep device is fitted with a turquoise top cap and a dropper tip with a white cap. Supplied ready to use.
3. **Curian Shiga Toxin Positive Control:** Inactivated Shiga toxins 1 and 2 in an aqueous phosphate buffered solution containing 0.094% sodium azide. Supplied ready to use.
4. **Pipette I:** Transfer pipettes graduated to **50 µL** and **175 µL**.
5. **Pipette II:** Transfer pipettes graduated up to **1.0 mL**.

MATERIALS NOT PROVIDED

All Methods:

1. Disposable gloves, powder free
2. Pipettor and tips

Broth Method:

1. Gram Negative (GN) or MacConkey broth
2. Culture tubes

SMAC Agar Plate Method:

1. Sorbitol-MacConkey agar plate **without** tellurite or cefixime
2. Polymyxin B (20 mg/mL in water)
3. Polyester swabs

EQUIPMENT NOT PROVIDED

1. Vortex mixer
2. Interval timer (Optional)
3. Incubators, 35-39 C
4. Curian Analyzer System, Meridian Bioscience, Inc. Catalog 610190

PRECAUTIONS

1. All reagents are for *in vitro* diagnostic use only.
2. Store the kit at the temperature indicated on labeling when not in use.
3. Follow Good Laboratory Practices[10] (GLPs) when handling the kit, reagents and components.
4. Do not interchange transfer pipettes (PIPETTE I and PIPETTE II) or Aiopreps between assays. Specimens must be sampled and prepared with the transfer pipettes (PIPETTE I and PIPETTE II) and used with the Curian Shiga Toxin Aioprep device provided with the kit. These components are not interchangeable with other assays.
5. Handle and dispose of all human specimens as if they are biologically hazardous.
6. Inspect foil pouch before removing the test card. Do not use test cards that have holes in the foil pouch or where the pouch has not been completely sealed.
7. Do not use test cards where the desiccant indicator has changed from blue to pink.
8. Do not interchange positive control or test cards between kit lots.
9. Do not mark over or near the barcode on the test card.
10. Sample preparation in the Aioprep can be performed inside a laminar flow hood. The Curian Shiga Toxin test card must be incubated outside of a laminar flow hood.

HAZARD and PRECAUTIONARY STATEMENTS

 **Aioprep Sample Preparation Device / Negative Control**	**Signal word** Danger **Hazard statements** H360 - May damage fertility or the unborn child if inhaled **Precautionary Statements - EU (§28, 1272/2008)** P201 – Obtain special instructions before use P202 – Do not handle until all safety precautions have been read and understood P280 - Wear protective gloves/ protective clothing/ eye protection/ face protection P308 + P313 – IF exposed or concerned: Get medical device advice/attention P405 – Store locked up P501 – Dispose of contents/container to an approved waste disposal plant.

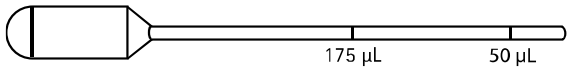
SHELF LIFE AND STORAGE

The expiration date is indicated on the kit label. Store the kit at 2-8 C as indicated on the labeling. Return all kit components to the indicated storage temperature after use.

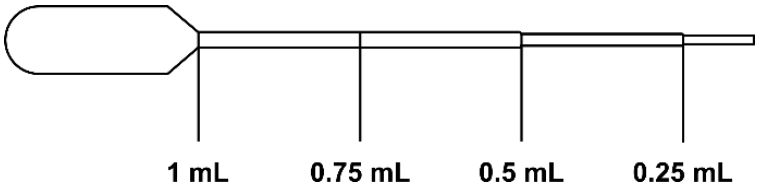
PROCEDURAL NOTES

The Curian Shiga Toxin transfer pipettes (PIPETTE I and PIPETTE II) are diagrammed below:

A. Transfer pipette (**PIPETTE I; 50 µL and 175 µL**) for use in **SPECIMEN PREPARATION / ENRICHMENT** steps of this package insert:



B. Transfer pipette (**PIPETTE II; 1.0 mL**) for use in **TEST PROCEDURE** steps of this package insert:



OPERATION OF THE CURIAN ANALYZER SYSTEM

The Curian Analyzer is an easy to use, menu driven analyzer system. Instructions to complete testing are provided on the analyzer touchscreen and in the Curian Analyzer operator manual.

1. Power ON the Curian Analyzer by pressing the power button located on the left side of the analyzer. The Curian Analyzer will initialize and perform Self Test.
2. Instrument Checks, Quality Control testing, and Patient Specimen testing are completed by navigating from the Home screen to the test menus. User ID and Sample ID entries are required for Quality Control and specimen testing.
3. Complete use and operation of the Curian Analyzer System by following the instructions in the Curian Analyzer operator manual and on-screen prompts. **Refer to operator manual for detailed instructions for the Curian Analyzer.**

SPECIMEN COLLECTION, HANDLING, AND STORAGE

This procedure is designed to be used with cultures derived from unpreserved stool or stool preserved in Cary-Blair or C&S transport media.

1. **Stool specimen storage and handling for the culture of STEC organisms**: The patient stool specimen should be received in an airtight container and either be frozen (-20 C) or placed at 2-8 C immediately after collection. The refrigerated unpreserved specimens should be cultured within two (2) hours of collection. If culturing cannot be performed within 2 hours, the specimen should be placed in a Cary-Blair or C&S transport media. Samples in Cary-Blair or C&S transport media may be stored at 2-8 C for up to five (5) days prior to preparing cultures. If culturing cannot be performed within this time, the specimens may be frozen at -20 C for up to fourteen (14) days prior to preparing cultures.

Storage Temperatures and Maximum Storage Times for Stool Specimens		
Specimen Conditions	**Storage Temperature**	**Maximum Storage Time**
Unpreserved specimens	2 – 8 C (refrigerated)	2 hours
	-20 C (frozen)	14 days
Preserved specimens in Cary-Blair or C&S media	2 – 8 C (refrigerated)[1]	5 days
	-20 C (frozen)[2]	14 days

[1]Refrigerated storage validated with ThermoScientific Remel Cary-Blair and Meridian Para-Pak C&S media only. If other Cary-Blair or C&S transport media are used, specimens should be stored according to the recommendations in the respective transport media package insert.
[2]Frozen storage validation with ThermoScientific Remel Cary-Blair and Meridian Para-Pak C&S media only and demonstrated that *E. coli* strains can be cultured for testing when spiked at a concentration of 6.0E+07 CFU/mL. If other Cary-Blair or C&S transport media are used, specimens should be stored according to the recommendations in the respective transport media package insert.

2. **Storage of broth showing growth prior to preparation in Aioprep / Sample Diluent for Curian Shiga Toxin testing**: Broths with growth may be held for up to seven (7) days at 2-8 C before testing with Curian Shiga Toxin. If testing is not performed within this time period, the broth should be frozen at ≤ -20 C for up to twenty-one (21) days. Broths may be frozen and thawed twice.

Storage Temperatures and Maximum Storage Times for Broth Cultures		
	Storage Temperature	**Maximum Storage Time**
Broth cultures with growth	2 – 8 C (refrigerated)	7 days
	≤ -20 C (frozen)[1]	21 days

[1]Frozen storage validation studies demonstrated recovery of purified Shiga toxin when spiked up to 5X LoD.

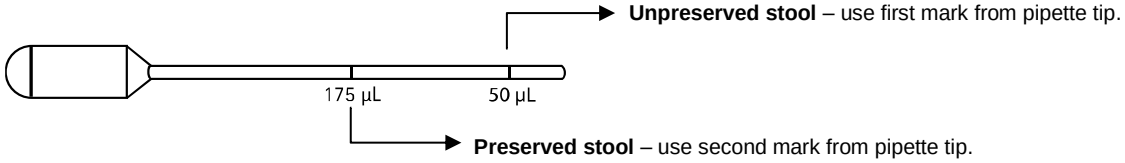
3. **Storage of processed cultures in Aioprep / Sample Diluent prior to Curian Shiga Toxin testing**: Broths with growth that have been prepared in the Aioprep / Sample Diluent may be held at room temperature (19 - 27 C) for up to eight (8) hours or at refrigerated temperature (2-8 C) for up to twenty-four (24) hours prior to testing with the Curian Shiga Toxin assay. SMAC agar plates with growth prepared in the Aioprep / Sample Diluent may be held at room temperature (19 - 27 C) for up to two (2) hours or at refrigerated temperature (2 - 8 C) for up to twenty-four (24) hours prior to testing with the Curian Shiga Toxin assay.

Storage Temperatures and Maximum Storage Times for Processed Cultures in Aioprep / Sample Diluent		
Specimen Conditions	**Storage Temperature**	**Maximum Storage Time**
Prepared broth cultures	19 - 27 C (room temperature)	8 hours
	2 - 8 C (refrigerated)	24 hours
Prepared SMAC agar plate cultures	19 - 27 C (room temperature)	2 hours
	2 - 8 C (refrigerated)	24 hours

SPECIMEN PREPARATION / ENRICHMENT

Note: Power-on the Curian Analyzer and complete Instrument Check (IC) test, and External Quality Testing before starting the sample preparation and test procedure.

1. **For BROTH METHOD:**
   a. Thoroughly mix stool specimen by vortexing for approximately 5 seconds regardless of consistency.
   b. Using a pipettor or transfer pipette (**PIPETTE I**, supplied with the kit):
      i. **Human stool specimens, unpreserved**: Add **50 µL** of unpreserved specimen (first mark from tip of pipette; refer to image below) to a culture tube containing 8 mL of GN broth or 5 mL of MacConkey broth.
      ii. **Human stool specimens, preserved in Cary-Blair or C&S transport media**: Add **175 µL** of the preserved specimen (second mark from tip of pipette; refer to image below) to a culture tube containing 8 mL GN broth or 5 mL MacConkey broth.



   c. Vortex inoculated broth for approximately 5 seconds.
   d. Incubate inoculated broth **with broth tube cap loose** at 35-39 C for 16-24 hours.
   e. Examine the broth tubes for growth. DO NOT PROCEED WITH TESTING if the broth tube does not exhibit growth after incubation as falsely negative results may occur. Repeat the broth enrichment using the same stool sample or with a new sample collected from the patient. If the original broth enrichment was performed with GN broth, GN broth can be used in the second attempt or alternatively, MacConkey broth can be used instead and vice versa. The sample can also be recultured using the SMAC plate method. If agar cultures are used, proceed with instructions provided in the SMAC PLATE METHOD BELOW. Use only broth cultures that exhibit growth in the following TEST PROCEDURE steps.

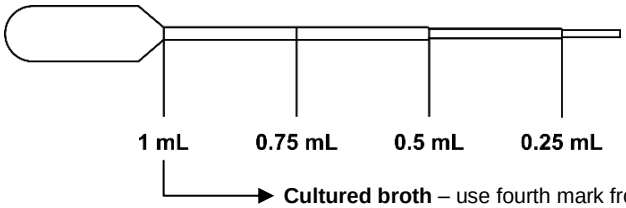
For SMAC AGAR PLATE METHOD:

a. Thoroughly mix stool specimen by vortexing for approximately 5 seconds regardless of consistency.
b. Use a polyester swab to inoculate stool samples onto SMAC agar plates **without** tellurite or cefixime. (**NOTE: Tellurite and cefixime inhibit growth of non-O157:H7 *E. coli*.**)
c. Incubate the inoculated plate for 18-24 hours at 35-39 C.
d. Examine the agar plates for growth. DO NOT PROCEED WITH TESTING if the agar plate does not exhibit growth after incubation as falsely negative results may occur. Repeat the agar enrichment using the same stool sample or with a new sample collected from the patient. The sample can also be recultured using the broth method. If broth cultures are used, proceed with instructions provided in the BROTH METHOD ABOVE. Use only agar cultures that exhibit growth in the following TEST PROCEDURE steps.

TEST PROCEDURE

Bring all test components, reagents, and samples to room temperature (19-27 C) before testing. Use one Curian Shiga Toxin test card for each sample. When ready to perform testing, inspect the pouch for damage. If undamaged, remove test card from its foil pouch. Discard the pouch and desiccant. **Do not use if desiccant is pink. Do not cover or mark on the barcode.**

1. **For BROTH METHOD:**
   a. Use only broth cultures that exhibit growth. Mix broth culture thoroughly by vortexing broth tube for approximately 5 seconds.
   b. Remove the turquoise top cap of the Aioprep device.
   Note: Prepared broth cultures in Aioprep / Sample Diluent may be held at room temperature (19 - 27 C) for up to 8 hours or at refrigerated temperature (2 - 8 C) for up to 24 hours prior to proceeding to step 2 for testing.
   c. Use the provided transfer pipette (**PIPETTE II**) to transfer **1 mL** of cultured broth (fourth mark from tip of pipette) into the Aioprep device. **Ensure that the specimen is completely expelled from the pipette into the Aioprep.**
   d. **Do not invert or shake Aioprep.** Recap the Aioprep tightly and vortex to mix for approximately 5 seconds.



For SMAC AGAR PLATE METHOD:

a. Use only agar cultures that exhibit growth.
b. Remove the turquoise top cap of the Aioprep device.
Note: Prepared SMAC agar plate cultures in Aioprep / Sample Diluent may be held at room temperature (19 - 27 C) for up to 2 hours or at refrigerated temperature (2 - 8 C) for up to 24 hours prior to proceeding to step 2 for testing.
c. Use a pipettor to add **5 µL** of Polymyxin B to the Aioprep device.
d. Using a polyester swab, sweep a few times across the confluent growth area of the plate or several isolated colonies, avoiding mucoid colonies. (**NOTE: Mucoid colonies may interfere with migration of the sample.**)
e. Swirl swab carrying the colony sweep in the Polymyxin B/Sample Diluent Solution and rotate the swab for 5 seconds to enhance the release of Shiga toxins from the organisms.
f. **Do not invert or shake Aioprep.** Recap the Aioprep tightly, vortex for approximately 5 seconds, and incubate for 30 minutes at 35-39 C.
g. Vortex the Polymyxin B/Diluted Sample for approximately 5 seconds and allow to acclimate to 19-27 C.

2. Remove the white tip cap from the bottom of the Aioprep and discard. While holding the Aioprep vertically, squeeze in the center of the barrel to add 3 free-falling drops into the 'SAMPLE' port of the test card. **(NOTE: Failure to add 3 free-falling drops may lead to invalid test results.)** Discard the Aioprep device immediately.

3. Start the Test Read by navigating to the 'TEST' Menu, selecting 'Analyze Now' or 'Incubate and Analyze', and then following the on-screen instructions.
   For Analyze Now:
   a. Incubate the test card at 19 - 27 C on the benchtop for 20 minutes. **(NOTE: Over incubation of tests may lead to false-positive or invalid test results.)**
   b. Enter Sample ID and press Ok.
   c. Within 2 minutes of the end of incubation, insert the test card into the drawer of the Curian Analyzer and close the drawer. Analysis of the reaction will be initiated by the Curian Analyzer.
   d. The Curian will analyze the test card and automatically report the test result.
   For Incubate and Analyze:
   a. Enter Sample ID and press Ok.
   b. Immediately insert the test card into the drawer of the Curian Analyzer and close the drawer. The analyzer will time the incubation for 20 minutes.
   c. After the end of the incubation, the Curian will analyze the test card and automatically report the test result.

ASSAY EXTERNAL QUALITY CONTROL (QC) TEST PROCEDURE

Note: Good Laboratory Practice (GLP) guidelines[10] recommend the use of control material. Users should follow the appropriate federal, state, and local guidelines concerning the running of external quality controls.

Bring all test components and reagents to 19-27 C before testing. Use one Curian test card for each external QC test (Negative and Positive Controls). When ready to perform testing, remove the test card from its foil pouch. Discard the pouch and desiccant. **Do not use if desiccant is pink. Do not mark over the barcode on the test card.**

Start the Test Read by navigating to the 'TEST' Menu, selecting the 'QC TEST', and then selecting 'ANALYZE NOW' or 'INCUBATE AND ANALYZE'. Follow the on-screen instructions.

<u>Negative Control</u>

1. Remove the top turquoise cap from Aioprep device completely, then recap the Aioprep tightly. Remove the white tip cap from the bottom of the Aioprep and discard. While holding the Aioprep vertically, squeeze in the center of the barrel to add 3 free-falling drops of Negative Control reagent (Sample Diluent) into the 'SAMPLE' port of the test card. Discard the Aioprep device immediately.

2. **For Analyze Now**:
   a. Incubate the test card at 19-27 C on the benchtop for 20 minutes.
   b. Enter Sample ID and press Ok.
   c. Within 2 minutes of the end of incubation, insert the test card into the drawer of the Curian Analyzer and close the drawer. Analysis of the reaction will be initiated by the Curian Analyzer.
   d. Select 'Curian Shiga Toxin QC Negative'.
   e. The Curian will analyze the test card and automatically report the test result.

   For Incubate and Analyze:
   a. Enter Sample ID and press Ok.
   b. Insert test card immediately into the drawer of the Curian Analyzer and close the drawer.
   c. Select 'Curian Shiga Toxin QC Negative'.
   d. The Curian will time the incubation for 20 minutes, then read the test card and automatically report the test result.

Positive Control

1. Invert the positive control bottle to mix. Remove the Positive Control tip cap. Holding vertically, squeeze 3 drops of Positive Control into the 'SAMPLE' port of the test card.
2. **For Analyze Now:**
   a. Incubate the test card at 19-27 C on the benchtop for 20 minutes.
   b. Enter Sample ID and press Ok.
   c. Within 2 minutes of the end of incubation, insert the test card into the drawer of the Curian Analyzer and close the drawer. Analysis of the reaction will be initiated by the Curian Analyzer.
   d. Select 'Curian Shiga Toxin QC Positive'.
   e. The Curian will analyze the test card and automatically report the test result.

   For Incubate and Analyze:
   a. Enter Sample ID and press Ok.
   b. Insert the test card immediately into the drawer of the Curian Analyzer and close the drawer.
   c. Select 'Curian Shiga Toxin QC Positive'.
   d. The Curian will time the incubation for 20 minutes, then read the test card and automatically report the test result.

If the expected control reactions are not observed, repeat the control tests as the first step in determining the root cause of the failure. If control failures are repeated, please contact Meridian's Technical Services Department at 1-800-343-3858 (US) or your local distributor.

INSTRUMENT CHECK (IC) TEST PROCEDURE

Note: The analyzer's External Control (IC Test) has a default schedule of 30 days. The IC Test will be required to be performed prior to running a QC test or patient test if this has not been performed within this timeframe. The user will be locked-out of running a QC test or patient test if this test is overdue.

1. Power ON the Curian by pressing the power button located on the side of the Curian. The analyzer will initialize and perform SELF TEST (automatic) checks.
2. From the HOME screen, select 'TEST', and then select 'INSTRUMENT CHECK'.
3. Insert the Fluorescent IC Card into the drawer. A 'PASS' or 'FAIL' result will be displayed once the analysis is complete.

If the expected result is not observed, repeat the IC Test as the first step in determining the root cause of the failure. Refer to the Curian Operator's Manual for detailed instructions for the analyzer.

INTERPRETATION OF RESULTS

Result interpretation is completed automatically by the Curian Analyzer system. The result will be shown on screen. Results can be retrieved from analyzer storage, printed and/or exported.

Patient Test Results:

One of the following result interpretations will be generated by the Curian Analyzer.

Curian Analyzer Result	Result Interpretation
Stx 1: Positive **Stx 2: Negative**	Shiga toxin 1 is present in sample.
Stx 1: Negative **Stx 2: Positive**	Shiga toxin 2 is present in sample.
Stx 1: Positive **Stx 2: Positive**	Shiga toxin 1 and Shiga toxin 2 are both present in sample.
Stx 1: Negative **Stx 2: Negative**	Shiga toxin 1 and Shiga toxin 2 are not present in sample.
Invalid	Result indicates inadequate flow of sample. Repeat the test. If repeat testing yields the same results, contact Meridian's Technical Services Department at 1-800-343-3858 (US) or your local distributor for additional assistance.

External Quality Controls:

One of the following result interpretations will be generated by the Curian Analyzer.

1. **Pass:** This indicates the test card and reagents are performing as intended.
2. **Fail**: This result indicates the test card and/ or reagents are not performing as intended or a user error has occurred. Test should be repeated to assist in trouble-shooting the error. If repeat testing results in a failed output, please contact your Meridian Bioscience Representative.
3. **Control Invalid**: This result indicates inadequate flow of sample. Repeat the test. If repeat testing yields the same results, contact Meridian's Technical Services Department at 1-800-343-3858 (US) or your local distributor for additional assistance.

Instrument Check:

One of the following result interpretations will be generated by the Curian Analyzer.

1. **Pass:** This indicates that the essential and critical components of the Curian Analyzer are working correctly.
2. **Fail**: This indicates that there may be an issue with the Curian Analyzer. Refer to the Curian operator manual if an error message has occurred. Repeat testing to assist in trouble-shooting the error. If repeat testing results in a failed output, please contact Meridian's Technical Services Department at 1-800-343-3858 (US) or your local distributor for additional assistance.

QUALITY CONTROL

This test should be performed per applicable local, state, or federal regulations or accrediting agencies.

There are four controls for the Curian Shiga Toxin test system. Two internal controls, one for the analyzer and one for the assay, and two external controls, one for the analyzer and one for the assay.

1. Analyzer Internal Control: Self Test (automatic)
2. Assay Internal Control: Control Line
3. Analyzer External Control: Instrument Check (IC) Test
4. Assay External Controls: Positive and Negative Controls (QC test)

EXPECTED VALUES

The prevalence of Shiga toxin-producing *E. coli* (STEC) infection observed during the 2022 study period for prospectively collected specimens was 0.3% for both Shiga toxin 1 (5/1538) and Shiga toxin 2 (4/1538). The prevalence of STEC infection by sex assigned at birth for the tested population was 0.3% for Stx1 and 0.2% for Stx2 in males and 0.3% for both Stx1 and Stx2 in females. The age demographic for the tested population ranged from less than 2 years to 60 years and older, with the mean age of 43 years old.

LIMITATIONS OF THE PROCEDURE:

1. The Curian Shiga Toxin assay must only be used with the Curian Analyzer.
2. Curian Shiga Toxin is a qualitative, *in vitro* diagnostic test. The Curian Analyzer will only provide qualitative results. This test is not intended to provide quantitative results.

SPECIFIC PERFORMANCE CHARACTERISTICS

CLINICAL PERFORMANCE

Prospective Study

The Curian Shiga Toxin assay was evaluated from September 2021 to June 2022 at five clinical study sites representing geographically distinct regions throughout the United States. There were 1,627 stool specimens from patients suspected of having a Shiga toxin-producing *Escherichia coli* (STEC) infection for whom a diagnostic *E. coli* test had been ordered by a practicing physician. Specimens were prospectively collected and tested with the Curian Shiga Toxin assay using stool specimens inoculated into appropriate broth. Of those 1,627, evaluable reference data was available for 1,538, all of which were evaluable prospective specimens. All specimens were tested at the study sites with the Curian Shiga Toxin assay and in a central laboratory with the reference method, the Vero cell Cytotoxin Assay (with neutralization) assay performed on the broth culture obtained from the stool specimens. Clinical performance (sensitivity and specificity) for prospective specimens against the reference method (Vero cell Cytotoxin Assay) for both Shiga toxin 1 (Stx1) and Shiga toxin 2 (Stx2) are presented in the following tables. There were no observable differences in performance of the Curian Shiga Toxin assay with respect to study site, broth type, kit lot, or patient gender or age.

Curian Shiga Toxin Overall Performance for Prospective Specimens versus Vero Cell Cytotoxin Assay

		Reference Method: Vero cell Cytotoxin Assay					
		Stx1 Positive	Stx1 Negative	Total	Parameter	Estimate	95% CI
Curian Shiga Toxin Assay	Stx1 Positive	5	9	14	Sensitivity	100.0%	[56.6% - 100.0%]
	Stx1 Negative	0	1524	1524	Specificity	99.4%	[98.9% - 99.7%]
	Total	5	1533	1538			

		Reference Method: Vero cell Cytotoxin Assay					
		Stx2 Positive	Stx2 Negative	Total	Parameter	Estimate	95% CI
Curian Shiga Toxin Assay	Stx2 Positive	4	7	11	Sensitivity	100.0%	[51.0% - 100.0%]
	Stx2 Negative	0	1527	1527	Specificity	99.5%	[99.1% - 99.8%]
	Total	4	1534	1538			

Archived Study

To further estimate sensitivity and specificity of the Curian Shiga Toxin assay, 140 archived stool samples were retrospectively tested for Stx1 and Stx2 using the Curian Shiga Toxin assay at all five study sites. The clinical performance (sensitivity and specificity) for archived samples against the reference method (Vero cell Cytotoxin Assay) are presented in the table below. Of the 140 eligible samples enrolled, 5 were excluded due to inconclusive Vero cell Cytotoxin results, leaving a total of 135 evaluable archived specimens. There were no observable differences in performance of the Curian Shiga Toxin assay with respect to study site, broth type, kit lot, or patient gender or age.

Curian Shiga Toxin Overall Performance for Archived Samples versus Vero Cell Cytotoxin Assay

		Reference Method: Vero cell Cytotoxin Assay					
		Stx1 Positive	Stx1 Negative	Total	Parameter	Estimate	95% CI
Curian Shiga Toxin Assay	Stx1 Positive	46	2	48	Sensitivity	100.0%	[92.3% - 100.0%]
	Stx1 Negative	0	87	87	Specificity	97.8%	[92.2% - 99.4%]
	Total	46	89	135			

		Reference Method: Vero cell Cytotoxin Assay					
		Stx2 Positive	Stx2 Negative	Total	Parameter	Estimate	95% CI
Curian Shiga Toxin Assay	Stx2 Positive	32	2	34	Sensitivity	97.0%	[84.7% - 99.5%]
	Stx2 Negative	1	100	101	Specificity	98.0%	[93.1% - 99.5%]
	Total	33	102	135			

ANALYTICAL SENSITIVITY

Analytical sensitivity studies were performed to determine the analytical limit of detection (LoD) of quantified Shiga toxin 1 (Stx1) and Shiga toxin 2 (Stx2) in cultures derived from human stool specimens for the Curian Shiga Toxin assay. The LoD is defined as the lowest concentration of the target analyte that produces positive results ≥ 95% of the time.

The LoD values determined for the Curian Shiga Toxin assay in cultured stool matrix is 0.185 ng/mL for Stx1 and 0.125 ng/mL for Stx2.

ASSAY REACTIVITY / INCLUSIVITY
Various Shiga toxin-producing *Escherichia coli* strains (STEC) were evaluated in the Curian Shiga Toxin assay by the Sorbitol MacConkey Agar (SMAC) plate, Gram Negative (GN) broth, and MacConkey (MAC) broth culture methods. Each strain was a clinical isolate tested by an FDA-cleared commercial assay and a cytotoxin assay to confirm the presence of Shiga toxin(s). All strains representing various serotypes and toxin combinations tested showed reactivity with the Curian Shiga Toxin assay, detailed below:

STX1 Type Strains: O26:H11 (6), O111:H8 (3), O45:H2 (3), O103:H25 (2), O145 (2), O103:H11 (2), O157:H7, O103:H2, O111

STX2 Type Strains: O121:H19 (4), O145 (3), O104:H21 (2), O113:H21 (2), O157:H7, O157:NM, O145:H25, O145:H28, O91:H21

STX1+2 Type Strains: O111:H8 (3), O157:H7 (2), O111 (2), O145, O113:H21, O26:H11

REPRODUCIBILITY
The reproducibility of the Curian Shiga Toxin assay was determined by testing contrived cultured broth samples across three independent laboratories. Samples were created with Shiga toxin 1 (Stx1) and/or Shiga toxin 2 (Stx2) spiked into pooled negative cultured broth matrix at high negative, low positive, and moderate positive concentrations, along with a true negative cultured broth sample. Ten panels consisting of 30 blinded samples comprising various combinations of Stx1 and Stx2 concentrations were provided to each of the three laboratories for a total of 900 samples. Testing was conducted at each laboratory over 5 different days. Each day two separate operators tested each one a separate panel while alternating between kit lots. Testing included three different kit lots (2 lots per site). In addition, positive and negative controls were run daily.

For Stx1, the overall agreement between the Curian Shiga Toxin assay result and the expected assay result was 99.6% (95% CI: 98.9% - 99.8%) with 100% for all sample types except for combined Stx1 and Stx2 high negative samples, which showed an agreement of 95.6% (95% CI: 89.1% - 98.3%). For Stx2, the overall agreement between the Curian Shiga Toxin assay result and the expected assay result was 99.7% (95% CI: 99.0% - 99.9%) with 100% for all sample types except for Stx2 high negative samples, which showed an agreement of 97.8% (95% CI: 92.3% - 99.4%), and combined Stx1 and Stx2 high negative samples, which showed an agreement of 98.9% (95% CI: 94.0% - 99.8%).

CROSSREACTIVITY / MICROBIAL INTERFERENCE
The Curian Shiga Toxin assay was evaluated for cross-reactivity and microbial interference with the organisms listed below. Unless otherwise indicated, each organism was tested at minimum concentrations of 1.2×10^7 CFU/mL for bacteria/fungi or 1.0×10^5 $TCID_{50}$/mL for viruses. None of the organisms showed cross-reactivity or microbial interference in the Curian Shiga Toxin assay.

Aeromonas hydrophila
Bacillus subtilis
Bacteroides fragilis
Campylobacter coli
Campylobacter concisus
Campylobacter fetus
Campylobacter hyointestinalis
Campylobacter jejuni
Candida albicans
Citrobacter freundii
Clostridium difficile
Clostridium perfringens
Enterobacter cloacae
Enterococcus faecalis
Escherichia coli (non-toxigenic)
Escherichia coli EIEC
Escherichia coli EPEC
Escherichia coli ETEC
Escherichia coli O157:H7 (non-toxigenic)
Escherichia fergusonii
Escherichia hermanii
Gardnerella vaginalis
Helicobacter pylori
Klebsiella pneumoniae
Lactobacillus acidophilus
Proteus vulgaris
Providencia stuartii
Pseudomonas aeruginosa
Pseudomonas fluorescens
Salmonella enterica susb. enterica serovar Hilversum
Salmonella enterica susb. enterica serovar Typhimurium
Salmonella minnesota
Serratia liquefaciens
Serratia marcescens
Shigella boydii
Shigella flexneri
Shigella sonnei
Staphylococcus aureus
Staphylococcus aureus (Cowan's)
Staphylococcus epidermidis
Streptococcus equisimilis subsp. dysgalactiae
Yersinia enterocolitica

Human Adenovirus 2
Human Adenovirus 14
Human Adenovirus 40
Human Adenovirus 41
Human Coxsackievirus A9
Human Coxsackievirus B1
Human Enterovirus 69
Human Rotavirus
Feline calicivirus

Shigella species shown to be reactive with the Curian Shiga Toxin assay:
S. dysenteriae (strain ATCC 9361) was found to be Shiga toxin 1 (Stx1) positive at concentrations greater than 1.25×10^6 CFU/mL in the Curian Shiga Toxin assay.

TESTS FOR INTERFERING SUBSTANCES
The chemical and biological substances listed below were evaluated at the indicated concentrations for interference in the Curian Shiga Toxin assay. None of the substances showed interference with the Curian Shiga Toxin assay performance.

Barium Sulfate (5% w/v)
Ciprofloxacin (0.25% w/v)
Hog gastric mucin (3.5% w/v)
Human blood (40% v/v)
Human hemoglobin (10.0% w/v)
Human urine (5% v/v)
Imodium® A-D (5% v/v)
Kaopectate® (5% v/v)

Leukocytes (0.05% v/v)
Mylanta® (8.400 mg/mL)
Palmitic Acid/Fecal Fat (40% w/v)
Pepto-Bismol® (5% v/v)
Prilosec OTC® (5 µg/mL)
Stearic Acid/Fecal Fat (40% w/v)
Tagamet® (5 µg/mL)
TUMS® (50 µg/mL)
Naproxen sodium (0.5% w/v)
Metronidazole (0.25% w/v)
Vancomycin (0.25% w/v)

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	Temperature limitation	CONJ ENZ	Enzyme Conjugate
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