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| Shiga Toxin Detection |
| **Purpose** | This procedure provides instruction for Shiga Toxin Detection. |
| **Principal and Clinical Significance** | Enterohemorrhagic *E. coli* (EHEC) or Shiga toxin producing *E. coli* is the fourth leading cause of diarrhea. EHEC’s produce a toxin which is been implicated to cause hemorrhagic colitis and hemolytic-uremic syndrome (HUS). Shiga Toxins can be classified into two main categories: Shiga Toxin 1 (ST1) and Shiga Toxin 2 (ST2). EHEC strains may produce ST1 or ST2 only or both ST1 and ST2 together. Enterohemorrhagic *E. coli* are capable of initiating life-threatening illness, particularly in young children, the elderly and patients with immune deficiency. The main sources of infection are contaminated, raw or insufficiently heated foods of animal origin. EHEC reservoirs include cattle, sheep and goats and are spread through their feces. ImmunoCard Stat! EHEC is an immunochromatographic rapid test for the qualitative detection of Shiga toxins 1 and 2 produced by *E. coli* in cultures derived from stool specimens. The immunoassay is used in conjunction with the patient’s clinical symptoms and other laboratory tests to aid in the diagnosis of diseases caused by Enterohemorrhagic *E. coli* (EHEC) infections.The EHEC immunoassay utilizes monoclonal antibodies labeled with red-colored gold particles. The test device has a circular sample port and an oval-shaped test and control window. Patient stool sample is added to the sample port window and absorbed through the pad to the reaction zone containing colloidal, gold-labeled antibodies specific to Shiga toxins. Any Shiga toxin (ST1 and ST2) antigen present complexes with the gold-labeled antibody and migrates through the test device until it encounters the binding zones in the test (Toxin 1, Toxin 2) area. The binding zones (Toxin 1 and Toxin 2) contain another anti-ST1 or -ST2 antibody, which immobilizes any Shiga toxin-antibody complex present. Due to the gold labeling, a distinct red line is then formed. The remainder of the sample continues to migrate to another binding reagent zone within the control zone, and also forms a further distinct red line (positive control). The control line is a procedural control to assure that the sample has migrated the appropriate distance along the membrane.  |
| **Policy Statements** | This procedure applies to Microbiologists who perform culture set-up and plate reading. Shiga Toxin testing is performed in conjunction with all stool cultures (with the exception of stool aspirate and rectal swab collections).  |
| **Test Code** | STLC |
| **Materials** |  |  |  |  |
|  | **Reagents** | **Supplies** | **Equipment** | **Media** |
|  | * ImmunoCard STAT! EHEC Kit (Meridian Diagnostics, Inc., Catalog #751630)
* 30 individual test devices
* Positive control

(formalin-treated ST1 and ST2 toxins in buffered diluent containing 0.094% sodium azide)* Sample Diluent/Negative

Control (buffered diluent containing 0.094% sodium azide)* Transfer pipettes
 | * 12 x 75 falcon tubes
* Applicator sticks
 | * Vortex mixer
* Timer
* Incubator

 (35-37°C) | * Gram negative (GN) broth
* Modified Cary-Blair media
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| **Specimen** | * Acceptable specimens include liquid, semi-soft and solid stool. Specimens in Cary-Blair based media are also acceptable.
* Unacceptable specimens include the following:
* Specimens in formalin, PVA or SAF
* Colonoscopy and endoscopy specimens
* Rectal swabs
* Stool aspirates
* For additional information: Refer to [Stool Culture](https://www.childrensmn.org/References/Lab/microbioviral/stool-culture.pdf) (Lab Test Directory) for collection and storage instruction.
* For EHEC testing: Utilize GN broths that have been incubated for 16-24 hours and are showing visible growth.
	+ Broths placed into the refrigerator (2-8°C) after 16-24 hours of incubation can be held for up to 7 days before testing is performed.
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| **Special Safety Precautions** | Microbiologists are subject to occupational risks associated with specimen handling. Refer to the safety policies located in the safety section of the *Microbiology Procedure Manual***.**1. [*Biohazard Containment*](file:///G%3A%5CLab%20Procedures%5CMicrobiology%5C1NEW%20Micro%20Procedure%20Manual.%20%28same%20as%20in%20Starnet%29%5CMCVI%203%20Safety%5CMCVI%203.1%20Biohazard%20Containment.docx)
2. [*Biohazardous Spills*](file:///G%3A%5CLab%20Procedures%5CMicrobiology%5C1NEW%20Micro%20Procedure%20Manual.%20%28same%20as%20in%20Starnet%29%5CMCVI%203%20Safety%5CMCVI%203.4%20Biohazardous%20Spills.docx)
3. [*Safety in the Microbiology Laboratory*](file:///G%3A%5CLab%20Procedures%5CMicrobiology%5C1NEW%20Micro%20Procedure%20Manual.%20%28same%20as%20in%20Starnet%29%5CMCVI%203%20Safety%5CMCVI%203.2%20Safety%20in%20the%20Microbiology%20Lab.docx)

The positive control reagent contains formalin-treated (inactivated) shiga toxins ST1 and ST1. It should be handled as a potentially hazardous material.The positive control and Sample Diluent contain the preservative sodium azide, which is a skin irritant. Avoid contact with reagents. |
| **Storage** | Store kit at 2-8°C until the expiration date printed on the box |
| **Quality Control** | 1. Daily Quality Control
* The ImmunoCard Stat! EHEC has internal controls contained within the test strip and are evaluated with each test. The control line serves as a procedural control and indicates that the test has been performed correctly, that proper flow occurred and that the test reagents were active at the time of use. A clean background around the Control or Test lines also serves as a procedural control. Record daily quality control in the culture workup in Sunquest.
1. New Lot Control
* External positive and negative controls should be performed with each new lot or shipment of kits before putting into service. In conjunction with new lot controls, perform parallel kit testing with old lot controls from a previous “in use” lot. The external controls are used to monitor reagent reactivity and test performance. Record results in the New Lot Inventory and Quality Control binder.
	+ Positive Control: Add 5 drops of the Positive Control to the sample port of a test device.
	+ Negative Control: Add 5 drops of the Sample Diluent to the sample port of a test device.
	+ Run according to procedure instructions. Refer to the Interpretation section of the procedure for acceptable/unacceptable results.
1. QC failures
	* + If there is a QC failure, the patient result is invalid and will not be reported. Document the observation, record corrective action and notify the microbiology supervisor.
		+ For failures that cannot be resolved, call Meridian’s Technical Services Department at 1-800-343-3858.
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| **Procedure** | 1. **Stool Specimen Inoculation**
	1. Use an applicator stick to mix stool thoroughly regardless of consistency.
	2. Add stool specimen to GN broth. Use the following guidelines:
		* Formed stool: Use a wooden applicator stick to transfer a 3-4 mm round pellet of stool to a culture tube containing 8ml of GN broth.

 3mm 4mm* + - Liquid stool: Add 50 μL (first mark from the tip of the pipette) of unpreserved specimen to a culture tube containing 8 mL of GN broth.

 * + - Stool in Modified Cary-Blair Medium: Ensure that the stool has been mixed thoroughly in the preservative. Add 175 μL of the preserved specimen (second mark from tip of pipette) to a culture tube containing 8 mL of GN broth.
	1. Incubate inoculated broth with the caps loosened in ambient air at 33-35°C for 16-24 hours.
		+ Refrigerate GN broth after 24 hours of incubation if testing cannot be done at that time.
		+ If the STLC set-up time is AFTER 23:00, SLT testing cannot be performed during the following day shift. The evening/night shift should be notified to refrigerate these GN broths after 24 hours of incubation for next day testing.
1. **Shiga Toxin Immunoassay**
	* + 1. Bring all Test Devices, reagents and samples to room temperature before use.
			2. All reagents should be gently mixed prior to use.
			3. After incubation, before proceeding with the EHEC assay, visually observe the GN broth tubes for growth. DO NOT PROCEED WITH TESTING if the broth tube does not exhibit growth as falsely negative results may occur. Check the routine stool culture plates for growth. If there is pinpoint growth, re-incubate the broth for up to a total of 24 hours from time of culture set up.

 * + - 1. If the GN broth shows no growth, the EHEC test will not be performed. Issue credits using the following credit codes at the STLC billing tab: **CSLT1 and CSLT2.**
			2. If the GN broth was not setup (i.e. rectal swab or stool aspirate collections), the EHEC test will not be performed. Issue credits using the following credit codes at the STLC billing tab: **CSLT1, CSLT2, and CSLTC**.
			3. Label a 12x75 Falcon tube for each GN to be tested.
			4. Add 5 drops (150μL) of the Sample Diluent (red top vial) to the tube(s).
			5. Mix the GN broth thoroughly by swirling the tube.
			6. Using the transfer pipette supplied with the kit, add 175μL of broth specimen (second mark from tip of pipette) to the tube containing Sample Diluent.
			7. Gently mix the contents of the tube with the pipette by squeezing the pipette bulb 3 times or by using a vortex mixer for 10 seconds. Return the transfer pipette to the tube for later use.
			8. The diluted stool broth culture can be stored for up to 30 minutes at room temperature (20-25°C).
1. **Test procedure**
2. Remove the appropriate number of Test Devices from their pouches and label with the patient’s identification. The Test Device must be used within 15 minutes after removal from the sealed pouch.
3. Using the original specimen transfer pipette, remix if necessary. Slowly add 175 μL of the diluted specimen (second mark from the tip of pipette) to the sample port of the device.
4. Incubate the test at room temperature for 20 minutes.
5. Read the results within 1 minute after the end of incubation.
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| **Interpretation** | * **Negative test**: A pink/red band at the Control line position. No other bands present.
* **Positive test for Shiga toxin 1**: Pink/red band at the Control and Toxin 1 line positions. No bands at the Toxin 2 test line. The appearance of a Toxin 1 test line, even if very weak, indicates the presence of Shiga toxin 1. The intensity of the test line can be less than that of the Control line.
* **Positive test for Shiga toxin 2**: Pink/red bands at the Control and Toxin 2 line positions. No bands at the Toxin 1 test line. The appearance of a Toxin 2 test line, even if very weak, indicates the presence of Shiga toxin 2. The intensity of the test line can be less than that of the Control line.
* **Positive test for Shiga toxin 1 and 2:** Pink/red bands at the Control, Toxin1 and Toxin 2 line positions. The appearance of Toxin 2 and Toxin 1 test lines, even if very weak, indicates the presence of Shiga toxins 1 and 2. The intensity of the test lines can be less than that of the Control line.
* **Invalid Test Results:**
	+ No band at the designated position for the Control line. This indicates the test procedure was performed improperly or that deterioration of reagents has occurred.
	+ A pink/red band appearing at either the Toxin 1 or Toxin 2 Test Line position after the defined incubation limit. False positive results may occur if the tests are incubated too long.
	+ A band of color other than pink/red may indicate reagent deterioration.
	+ Invalid and difficult to interpret test results should be repeated with the same sample.

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| **Limitations** | 1. The performance of ImmunoCard Stat! EHEC has not been evaluated with direct stool samples.
2. Stool in transport media (with the exception of Cary-Blair), swabs or preservatives have not been validated for use by this method.
3. This test is qualitative and no quantitative interpretation should be made with respect to the intensity of the positive line when reporting the result.
4. False negative results:
	* + May occur with the addition of more than 5 drops of Sample Diluent.
		+ May occur if tests are incubated at reduced temperatures or times.
		+ May occur with failure to add 175 μl of broth culture to the Sample Diluent.
5. False positive results:
	* + May occur if tests are over incubated.
6. NOTE: Shiga toxin 1 produced by *E. coli* and the toxin produced by *Shigella dysenteriae* type 1 strains are nearly identical. Therefore, this test may give a positive result with toxins from *S. dysenteriae* type 1 strains. Plating on selective growth media coupled with biochemical analysis can differentiate the two organisms.
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| **Method Performance Specifications** | The lower limits of detection are at 1.25 ng/mL for both ST1 and ST2 using purified toxin. |
| **Result Reporting** | 1. Record results in Sunquest MRE in the Culture Entry tab and Workup section.

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| **RESULT** | **KEY** (lowercase) | **CODE** |
| Positive for SLT1 | q |  SLT1: POSITIVE: SLT-1 (Shiga toxin 1) antigen detected by EIA |
| Positive for SLT2 | w |  SLT2: POSITIVE: SLT-2 (Shiga toxin 2) antigen detected by EIA |
| Negative for SLT1 | e | SLT1N: NEGATIVE: No SLT-1 (Shiga toxin 1) detected by EIA |
| Negative for SLT2 | r | SLT2N: NEGATIVE: No SLT-2 (Shiga toxin 2) detected by EIA |

1. Refer to the following Sunquest GUI examples below for entering EHEC test results:
* **Workup field #20:**

* **Observation Field:**

1. Report positive results by telephone to the physician or patient’s nurse. Document in the computer: the person called, credentials and the date/time of the call.
2. Send positive GN broths to MDH (packaged in a Category “A” Infectious Shipper). Refrigerate sample while waiting to be sent to MDH.
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| **References** | 1. ImmunoCard STAT! EHEC [Package Insert]. Cincinnati, OH.Meridian Diagnostics, Inc.; January 2017.
2. Centers for Disease Control and Prevention (CDC), Recommendations for Diagnosis of Shiga Toxin-Producing *Escherichia coli* Infections by Clinical Laboratories. MMWR 2009:58:No.RR-12
3. Leber, Amy. *Clinical Microbiology Procedures Handbook*, 4th edition. Vol. 1-3 (Section 3.8). 2016. American Society for Microbiology, Washington D.C., 20036.
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| **Training Plan/ Competency Assessment** | **Training Plan** | **Initial Competency Assessment** |
| 1. Employee must read the procedure.
2. Employee will observe trainer performing the procedure.
3. Employee will demonstrate the ability to perform procedure, record results and document corrective action after instruction by the trainer.
 | 1. Direct observation.
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| **Historical Record** |  |  |  |  |
|  | **Version** | **Written/Revised by:** | **Effective Date:** | **Summary of Revisions** |
| 1.0 | Tina Blankenheim | 06/27/2011 | Initial Version |
| 2.0 | Becky Carlson | 4/19/2015 | Re-numbered from MC 930 for CMS load. |
| 3.0 | Susan DeMeyere/ Andrew Fangel | 7/30/2018 | Added exceptions STO-ASP & RS in policy statement.  |
|  | 4.0 | Susan DeMeyere | 5/11/2021 | Adjusted credit codes for EHEC |  |  |
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