**PRINCIPLE:**

Immuno*Card* STAT! EHEC is an immunochromatographic rapid test for the qualitative detection of Shiga toxins 1 and 2 (also called Verotoxins) produced by *E. coli* in cultures derived from clinical stool specimens. Immuno*Card* STAT! EHEC 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.

Immuno*Card* STAT! EHEC is an immunochromatographic rapid test utilizing monoclonal antibodies labeled with red-colored gold particles. The test device has a circular sample port and an oval-shaped test (Toxin 1, Toxin 2) and control (Control) window.

1. The sample is applied to the chromatography paper via the circular sample port (Sample).
2. The sample is absorbed through the pad to the reaction zone containing colloidal, gold-labeled antibodies specific to Shiga toxins.
3. Any Shiga toxin (ST1 and ST2) antigen present complexes with the gold-labeled antibody and migrates through the pad until it encounters the binding zones in the test (Toxin 1, Toxin 2) area.
4. 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.
5. 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). Regardless of whether any Shiga toxin is present or not, a distinct red line should always be formed in the control zone and confirms that the test is working correctly.

**SPECIMEN:**

**Preferred sample types and volumes:**

Specimens in Cary-Blair based media

Raw stool: 200-300uL (100uL for testing and the remaining for retesting if needed)

Collection containers: Airtight transport containers without preservative.

**Undesirable specimens:**

1. Specimens in formalin, PVA, or SAF
2. **Note:** Stool in transport media (with the exception of Cary-Blair), swabs, or preservatives have not been validated for use by this method.

**Interfering substances:**

None known

**Collection:**

1. **Stool specimen storage and handling for the culture of EHEC organisms**:

The appropriate stool specimen should either be placed at 2-8°C immediately after collection orfrozen (<-70°C). The refrigerated specimens should be cultured within 2 hours.

If culturing cannot be performed within 2 hours, the specimen should be placed in a Modified Cary-Blair-based transport media at 2-8ºC. They must be cultured in MacConkey Broth within two to three days

If the Cary-Bair cannot be cultured in MacConkey Broth within this time, the specimens should be frozen at <-70°C immediately upon receipt. Specimens in Modified Cary-Blair transport medium should not be refrigerated then frozen.

1. **Storage of broth showing growth prior to Immuno*Card* STAT! EHEC testing**:

 Broths with growth may be held for up to seven days at 2-8°C before testing with Immuno*Card* STAT! EHEC. If testing is not performed within this time period, the broth should be frozen at ≤ -20°C for up to 21 days. (See section on PRECAUTIONS.)

**Preparation:**

1. Use an applicator stick to mix stool thoroughly regardless of consistency.

2. Using a pipettor or transfer pipette (supplied with the kit):

a. **Unpreserved specimen**: Add 50 µL (first mark from tip of pipette) of unpreserved specimen to a culture tube containing 8 mL of GN broth or 5 mL of MacConkey broth.

b. **Specimen collected in Modified Cary-Blair Medium**: Add 175 µL of the preserved specimen (second mark from tip of pipette) to a culture tube containing 8 mL GN broth or 5 mL MacConkey broth.

c. **Non-pipetable stool**: Use a wooden applicator stick to transfer a 3-4 mm round pellet of stool into either 8 mL of GN or 5 mL of MacConkey broth.

3. Incubate inoculated broth with caps loose at 35-39°C for 16-24 hours. (Visually observe the broth tubes for growth).

4. 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 there is no growth of enteric gram negative flora on stool culture plates cancel the EHEC Shiga Toxin Test.

 Under **Status Change:**

 **Cancelation Condition:** Select – **Test not performed**

 **Specimen Condition:** Select – **2033 – 2 Canceled: no growth Ecoli for EHEC**

5. Using the dropper vial, add five drops (150 µL) of Sample Diluent Buffer to a small test tube.

6. Mix broth culture thoroughly by gently swirling the tube.

7. Using the transfer pipette supplied with the kit, add 175 µL of sample (second mark from tip of pipette) to the tube containing Sample Diluent.

8. Gently mix the contents of the tube with the transfer pipette by squeezing the pipette bulb 3 times. Alternatively, mix using a vortex for 10 seconds. Return the transfer pipette to the tube for later use.

9. The diluted stool broth culture can be stored for up to 30 minutes at 20-25°C before testing.

**MATERIALS AND EQUIPMENT:**

**Materials:**

1. Immuno*Card* STAT! EHEC Test Devices. Store at 2-8°C when not in use.
2. Sample Diluent (Negative Control). Store at 2-8°C when not in use.
3. Positive Control, a solution of formalin-treated ST1 and ST2 toxins. Store at 2-8°C when not in use.
4. 50 µL/175 µL disposable plastic transfer pipettes
5. MacConkey broth (Broth with neutral red indicator may obscure the readability of the test.)
6. Modified Cary-Blair medium (optional)

**Equipment:**

1. Incubators, 33-39°C
2. Vortex
3. Interval timer
4. Autoclave
5. Disposable latex gloves

**Preparation:**

1. Bring the entire kit to 20- 25°C before use.

**Performance Considerations:**

1. For in vitro diagnostic use.
2. The Positive Control reagent contains formalin-treated (inactivated) shiga toxins ST1 and ST2. It should be handled, however, as a potentially hazardous material.
3. Some reagents contain the preservative sodium azide which is a skin irritant. Avoid contact with reagents. Disposal of reagents containing sodium azide into drains consisting of lead or copper plumbing can result in the formation of explosive metal oxides. Eliminate the build-up of oxides by flushing drains with large volumes of water during disposal.
4. Do not use kit or components beyond their assigned expiration dates.
5. Test devices are packaged in foil pouches that exclude moisture during storage. Inspect each foil pouch before opening. Do not use test devices from pouches that have holes in the foil or where the pouch has not been completely sealed. False negative reactions may result if test components and reagents are improperly stored.
6. Do not use the Sample Diluent or Positive Control if it is discolored or turbid. Discoloration or turbidity may be a sign of microbial contamination.
7. Directions should be read and followed carefully.
8. This is not a direct stool test. Stool specimens must be cultured on SMAC agar plates or in GN or MacConkey broth before testing. Failure to enrich samples before testing will cause erroneous results.
9. Low levels of toxins may deteriorate and become undetectable in broth samples that are stored frozen before testing. Best results will be obtained if broth samples are tested fresh. Multiple freeze-thaw cycles should be avoided.

10. Dispose of stool specimens as potentially biohazardous materials.

11. Pipettes that are supplied with the kit should be marked at the intervals shown in the pipette diagram in PROCEDURE NOTES. When instructed to use the pipette supplied with the kit, do not use transfer pipettes with markings that differ from the diagram.

**Storage Requirements:**

Immuno*Card* STAT! EHEC is stable until the expiration date printed on the box when stored at 2 to 8°C. The Test Device should be used within 15 minutes after removal from the sealed foil pouch.

**CALIBRATION:**

Not applicable to this assay.

**QUALITY CONTROL:**

At the time of each use, kit components should be visually examined for obvious signs of microbial contamination, freezing, or leakage. Do not use contaminated or suspect reagents.

Quality control is run on each new shipment/lot upon receipt using the positive and negative controls from the last shipment for new lot/old lot comparison.

External controls are run monthly for each lot number.

**Internal procedural controls: Internal controls are contained within the test strip and therefore are evaluated with each test.**

1. A PINK-RED band appearing at the Control line serves as a procedural control and indicates the test has been performed correctly, that proper flow occurred and that the test reagents were active at the time of use.
2. A clean background around the Control or Test lines also serves as a procedural control. Control or test lines that are obscured by heavy background color may invalidate the test and may be an indication of reagent deterioration, use of an inappropriate sample or improper test performance.

**External controls**

The results expected with the controls are described in the INTERPRETATION OF RESULTS.

**External positive and negative controls are run monthly.** The external controls are used to monitor reagent reactivity and test performance. Failure of the controls to produce the expected results can mean that one of the reagents or components is no longer reactive at the time of use, the test was not performed correctly or that reagents or samples were not added. Repeat the control tests as the first step in determining the root cause of the failure. The kit should not be used if control tests do not produce the correct results after repeat testing. Contact the manufacturer and the lead technologist.

**TEST PROCEDURE:**

1. Bring all Test Devices, reagents and samples to room temperature (20-25°C) before testing.
2. Use one Immuno*Card* STAT! EHEC Test Device for each patient sample.
3. Remove the Immuno*Card* STAT! EHEC Test Device from its foil pouch. Label the device with the patient’s identification.
4. Using the transfer pipette provided in the kit, slowly add 175 µL of the diluted specimen (second mark from tip of pipette) to the sample port of the device.
5. Incubate the test at 20-25°C for 20 minutes.
6. Read the results within 1 minute after the end of incubation.

**CALCULATIONS:**

There are no calculations associated with this procedure.

**INTERPRETATION OF RESULTS;**

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**POS T1 POS T2 POS T1&2 NEG**

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**INVALID INVALID INVALID INVALID**

**Negative test:** A PINK-RED band at the Control Line position. No other bands are present.

**Positive test for Shiga toxin 1:** PINK-RED bands 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 I. The intensity of the test line can be less that 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 toxins 1 and 2:** PINK-RED bands at the Control, Toxin 2, and Toxin 1 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:**

1. No band at the designated position for the Control line. The test is invalid since the absence of a control band indicates the test procedure was performed improperly or that deterioration of reagents has occurred.
2. A PINK-RED band appearing at either the Toxin 1 or Toxin 2 Test Line position of the device after the defined incubation limit or a band of any color other than PINK-RED. Falsely positive results may occur if tests are incubated too long. Bands with colors other than PINK-RED may indicate reagent deterioration.

If any result is difficult to interpret, the test should be repeated with the same sample to eliminate the potential for error. Obtain a new sample and retest when the original sample repeatedly produces unreadable results.

**REPORTING OF RESULTS:**

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| **Result Obtained** |  **Report** |
| Positive Shiga toxin 1 and shiga toxin 2 | **Shiga toxin 1: Positive****Shiga toxin 2**: **Positive** |
|  Positive Shiga Toxin 1 and negative Shiga toxin 2 | **Shiga toxin 1: Positive****Shiga toxin 2: Negative** |
| Negative Shiga toxin 1 and positive Shiga  toxin 2 | **Shiga toxin 1: Negative****Shiga toxin 2: Positive** |
| Negative shiga toxin 1 and negative shiga  toxin 2 | **Shiga toxin 1: Negative****Shiga toxin 2: Negative** |

**Positive Shiga toxin 1 and/or 2 results**

All positive Shiga toxin tests must be referred to the Minnesota Department of Health for additional testing and typing.

If E.coli O157 has not been isolated from culture, the MacConkey broth can be sent to MDH for EIP. Fill out the referral form.

**PROCEDURE NOTES:**

Among the *E. coli* human pathogens, Shiga toxin–producing strains of *E. coli* have gained in importance in recent years. The group of EHEC, with their highly pathogenic serovars O157:H7, 026, 0103, 0111, 0145, and other strains are of particular concern. Production of Shiga toxins is the most common criteria for the detection of this group of bacteria. 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 simultaneously. EHEC are capable of initiating life-threatening illnesses, particularly in young children, the elderly or patients with immune deficiency. The main sources of infection are contaminated, raw or insufficiently heated foods of animal origin, eg, meat and dairy products. The reservoirs for EHEC are cattle, sheep and goats and is spread through their feces. These microorganisms can enter food during the processing of meat and dairy products if hygienic conditions are inadequate. Immuno*Card* STAT! EHEC is an immunological diagnostic test based on the immunochromatographic lateral flow principle.

## Analytical Sensitivity

The lower limits of detection are at 1.25 ng/mL for both ST1 and ST2 using purified toxin.

Interfering Substances

The following substances when introduced directly into stool samples, do not interfere with testing at the concentrations identified: Barium Sulfate (0.05 mg/mL), Prilosec OTC (5 ug/mL), Pepto-Bismol (1:2100),Tums (0.05 mg/mL), Tagamet (5 ug/mL), Mylanta (1:2100), Leukocytes (0.05% v/v), Mucin (0.03 mg/mL), Stearic/palmitic acid (0.04 mg/mL, whole blood (0.05% v/v).

**LIMITATIONS OF THE PROCEDURE:**

1. The test is qualitative and no quantitative interpretation should be made with respect to the intensity of the positive line when reporting the result.
2. Test results are to be used in conjunction with information available from the patient clinical evaluation and other diagnostic procedures.
3. Failure to add 175 µL of broth culture to the 5 drops of Sample Diluent (step 7 of SPECIMEN PREPARATION/ENRICHMENT – BROTH CULTURE) can lead to falsely negative results. As a visual aid it may be helpful to verify that the broth culture was added. Mark the side of the test tube with a marking pen to indicate the top of the Sample Diluent volume. Once broth culture is added, this line should appear in the middle of the diluted sample volume. The addition of more than 5 drops of Sample Diluent can also lead to falsely negative test results.
4. Over incubation of tests may lead to false-positive test results. Incubating tests at reduced temperatures or times may lead to falsely negative results.
5. The performance of Immuno*Card* STAT! EHEC HAS NOT BEEN EVALUATED with direct stool samples. It has only been evaluated in SMAC plate culture or GN and MacConkey liquid cultures.
6. **NOTE: Shiga toxin 1 produced by *E*. *coli* and the toxin produced by *Shigella dysenteriae* type 1 strains are nearly identical. Therefore, Immuno*Card* STAT! EHEC may give a positive result with toxins from *S. dysenteriae* type 1 strains. The two organisms can be differentiated by plating on selective growth media coupled with biochemical analysis.**

**BACK-UP PROCEDURE:**

Send to reference laboratory for Shiga toxin

**REFERENCES:**

ImmunoCard STAT EHEC Broth Method, SN11169 CLSI 04/10, Meridian Bioscience, Inc. Cincinnati, OH 45244

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