ImmunoCard STAT! EHEC

REF **Catalogue number 751630** **IVD** ***In vitro*** **diagnostic medical device**

INTENDED USE

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.

SUMMARY AND EXPLANATION OF THE TEST

Among the *E. coli* human pathogens, Shiga toxin–producing strains of *E. coli* have gained in importance in recent years.[1-10] The group of EHEC, with their highly pathogenic serovars O157:H7, O26, O103, O111, O145, 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. The incidence of food infection caused by Shiga toxin-producing *E. coli* demands reliable and rapid methods of detection. In addition to traditional culture methods, immunological techniques are becoming more useful due to their improved specificity and sensitivity. Immuno*Card* STAT! EHEC is an immunological diagnostic test based on the immunochromatographic lateral flow principle.

BIOLOGICAL PRINCIPLE

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.

DESCRIPTION OF REAGENTS AND MATERIALS PROVIDED

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

1. Immuno*Card* STAT! EHEC Test Devices, containing immobilized monoclonal anti-ST1 and anti-ST2 antibodies. The devices are packaged in individual foil pouches with desiccants. Store at 2-8 C when not in use.
2. Sample Diluent (Negative Control), a buffered diluent containing 0.094% sodium azide as a preservative. The reagent is supplied in a plastic dropper vial. Use as supplied. Store at 2-8 C when not in use.
3. Positive Control, a solution of formalin-treated ST1 and ST2 toxins in a buffered diluent containing 0.094% sodium azide as a preservative. The reagent is supplied in a plastic dropper vial. Use as supplied. Store at 2-8 C when not in use.
4. 50 µL/150 µL disposable plastic transfer pipettes

MATERIALS NOT PROVIDED

All methods:

1. Incubators, 35-39 C
2. Vortex
3. Interval timer
4. Autoclave
5. Disposable latex gloves

SMAC Agar Method:

1. Sorbitol-MacConkey agar plate **without** tellurite or cefixime
2. Polymyxin B solution
3. Small test tube (eg, 10 x 75 mm or 12 x 75 mm)
4. Wooden applicator sticks
5. Distilled or deionized water
6. Disposable plastic transfer pipettes, or micropipettes and disposable tips capable of dispensing 200 µL
7. Disposable inoculation loops

Broth Method:

1. Gram Negative (GN) or MacConkey broth (Broth with neutral red indicator may obscure the readability of the test.)
2. Modified Cary-Blair medium (optional)

PRECAUTIONS

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. Inactivate cultures by autoclaving for a minimum of 15 minutes at 121 C before disposal.
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.

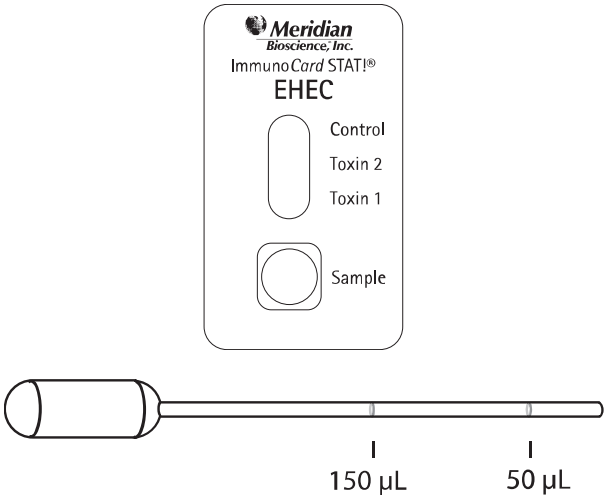
STORAGE AND STABILITY

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.

PROCEDURE NOTES

This test should be performed by qualified personnel per local regulatory requirements.

Diagrams of the Immuno*Card* STAT! EHEC Test Device and the 50 µL/150 µL transfer pipette supplied with the kit are shown below.



PROCEDURE –BROTH METHOD

SPECIMEN COLLECTION – BROTH METHOD

1. Stool specimen storage and handling for the culture of EHEC organisms: The appropriate stool specimen should either be frozen (≤ -70 C) or placed at 2-8 C immediately after collection. 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. Samples in Modified Cary-Blair transport media can be stored at 2-8 C if they are cultured within two to three days. If culturing cannot be performed 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.
2. 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.)
3. **Note**: Stool in transport media (with the exception of Cary-Blair), swabs, or preservatives have not been validated for use by this method.

SPECIMEN PREPARATION / ENRICHMENT – BROTH METHOD

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 of unpreserved specimen (first mark from tip of pipette) 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 150 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 the original broth enrichment was performed with GN broth, GN broth can be used in the second attempt or alternatively, Mac broth can be used instead and vice versa. The sample can also be recultured using the SMAC plate method. Use only broth or agar cultures that exhibit growth in the following steps.
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 150 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.

PROCEDURE –BROTH METHOD
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 150 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.

PROCEDURE – SMAC AGAR PLATE METHOD
SPECIMEN COLLECTION --- SMAC PLATE METHOD
Collect stool specimens without preservative. Specimens may be held at controlled room temperature for up to 4 hours prior to preparing cultures. Stool specimens that cannot be cultured with 4 hours should be placed at 2-8 C and cultured within 24 hours. If the specimens cannot be cultured within 24 hours they should be frozen at ≤-70 C as soon after receipt as possible.

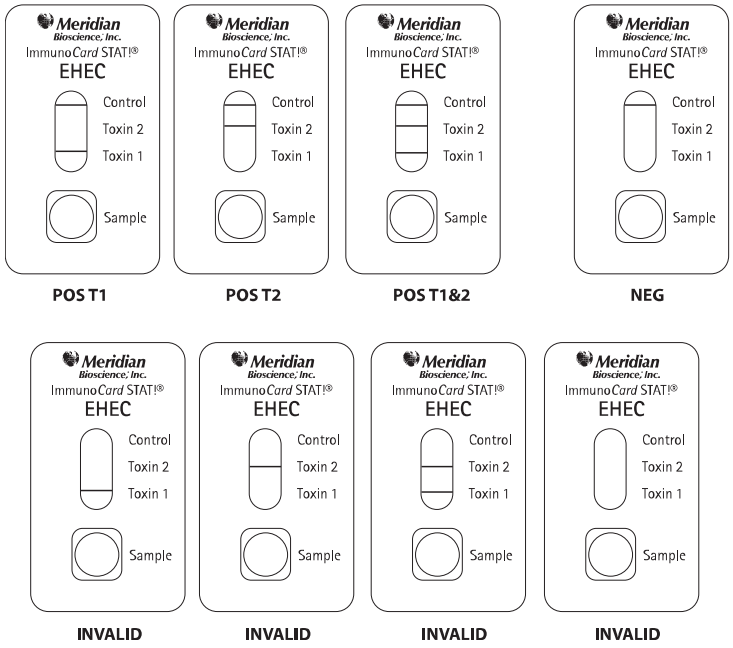
SPECIMEN PREPARATION/ ENRICHMENT – SMAC PLATE METHOD
1. Use an applicator stick to mix stool thoroughly regardless of consistency.
2. Use a Dacron swab to inoculate stool samples onto SMAC agar plates **without** tellurite or cefixime. (NOTE: Tellurite and cefixime inhibit growth of non-O157:H7 *E. coli.)*
3. Incubate the inoculated plate for 18-24 hours at 35-39 C.
4. 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. Alternatively, reculture the sample using the broth enrichment method. Use only agar or broth cultures that exhibit growth in the following steps.
5. Prepare a solution of 50 µg/mL Polymyxin B in distilled water.
6. Dispense 0.5 mL of the 50 µg/mL Polymyxin B solution into a test tube.
7. Using a Dacron swab, sweep a few times across the confluent growth area of the plate, avoiding mucoid colonies. Mucoid colonies may interfere with migration of the sample.
8. Dip the swab carrying the colony sweep in the distilled water/Polymyxin solution and rotate the swab at least 3 times to enhance the release of Shiga toxins from the organisms.
9. Incubate the mixture for 30 minutes at 35 C.

TEST PROCEDURE --- SMAC PLATE METHOD
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 and control.
3. Remove the Immuno*Card* STAT! EHEC Test Device from its foil pouch and discard the pouch.
4. Place the Test Device on a flat surface and label with the name of the patient or control to be tested.
5. Mix the inoculated water/Polymyxin sample prepared in step 6 above (SPECIMEN PREPARATION) by gently swirling the tube.
6. Using a pipettor or transfer pipette (**NOT** supplied with kit), add 200 µL into the circular sample port on the Test Device.
7. Incubate the test at 20-25 C for 10 minutes.
8. Read the results within 1 minute after the end of incubation.

EXTERNAL CONTROL TESTS – SMAC AGAR OR BROTH METHOD

1. Bring all Test Devices and reagents to (20-25 C) before testing.
2. Use one Immuno*Card* STAT! EHEC Test Device each for a Positive and Negative Control.
3. Remove the Immuno*Card* STAT! EHEC Test Device from its foil pouch. Label the device with the control to be tested.
4. Add exactly 5 drops of the Positive Control reagent to the sample port of a device marked for the positive control.
5. Add exactly 5 drops of the Sample Diluent to the sample port of a device marked for the negative control.
6. Incubate the test at 20-25 C for 20 minutes.
7. Read the results within 1 minute after the end of incubation.

INTERPRETATION OF RESULTS



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.

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.

Internal 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: External control reagents should be tested according to the requirements of the laboratory or those of applicable local, state or accrediting agencies.

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

External positive and negative controls should be run on opening each kit. However, the number of additional tests performed with the external controls will be determined by the requirements of local, state or federal regulations or accrediting agencies. 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.

EXPECTED VALUES

The positive rate for each laboratory will be dependent on several factors including the method of specimen collection, the handling and transportation of the specimen, the time of year or the presence of EHEC infection at the time of testing. A negative test should be expected in the absence of EHEC infection.

LIMITATIONS

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 150 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.**

SPECIFIC PERFORMANCE CHARACTERISTICS
SMAC PLATE METHOD
Test Devices were evaluated in the United States using the SMAC method and 249 fresh stools and 41 Shiga-toxin positive frozen stools.

	COMPARATIVE METHOD (Premier EHEC)		
SMAC Agar Method	Positive	Negative	Total
Positive	46	1*	47
Negative	0	243	243
Total	46	244	290
			CI
Positive agreement	46/46	100%	92.3% - 100%
Negative agreement	243/244	99.6%	97.7% - 100%
Overall agreement	289/290	99.7%	98.7% - 100%

* *E. coli* O157:H7 was recovered from the culture but was not detected by the reference method.

BROTH METHOD
This study was conducted with samples tested fresh or following frozen storage. Samples were obtained from patients in the United States, Canada and Argentina. Five US laboratories evaluated 469 samples using Mac and GN broths. (120 of the samples were solid stool specimens obtained from patients assumed to have gastroenteritis.) 448/469 samples produced growth in GN broth, while 449 produced growth in Mac broth. Three of the GN broth samples were excluded from evaluation with the comparative device due to insufficient volume. Seven of the 469 samples grew in Mac broth only while another 3 samples grew in GN broth only. 14 failed to grow in either broth. Stool specimens were collected from male and female patients of all ages. Samples producing discrepant results between Premier EHEC and ICS EHEC were further analyzed using cytotoxin assay. These samples generally produced weak reactions (<0.300) in Premier EHEC.

COMPARATIVE METHOD (Premier EHEC)			
Mac Broth Method			
ICS EHEC			
	Positive	Negative	Total
Positive	60	1	61
Negative	4*	384	388
Total	64	385	449
			CI
Positive agreement	60/64	93.8%	84.8% - 98.3%
Negative agreement	384/385	99.7%	98.6% - 100%
Overall agreement	444/449	98.9%	97.4% - 99.6%

* Two ICS EHEC -, Premier EHEC + samples were negative by a reference cytotoxin method.

COMPARATIVE METHOD (Premier EHEC)			
GN Broth Method			
ICS EHEC			
	Positive	Negative	Total
Positive	57	1	58
Negative	7*	380	387
Total	64	381	445
			CI
Positive agreement	57/64	89.1%	78.8% - 95.5%
Negative agreement	380/381	99.7%	98.5% - 100%
Overall agreement	437/445	98.2%	96.5% - 99.2%

* Four ICS EHEC -, Premier EHEC + sample were negative by a reference cytotoxin method.

The results of each broth method are compared to each other in following table.

	Mac Positive	Mac Negative	Mac No Growth	Total
GN Positive	54	3	1	58
GN Negative	3	382	5	390
GN No growth	4	3	14	21
Total	61	388	20	469

ANALYTICAL SENSITIVITY
SMAC AGAR TEST
The SMAC agar method is capable of detecting toxin in one colony-forming unit or 25 ng/mL and 62.5 ng/mL for unpurified Shiga toxins 1 and 2, respectively.
GN or MAC BROTH TEST
The lower limits of detection are at 1.25 ng/mL for both ST1 and ST2 using purified toxin.

REPRODUCIBILITY
SM AGAR TEST
Three independent laboratories tested three samples in triplicate, on each of three different times in one day (intra-assay variability) and on three different days (inter-assay variability). Samples consisted of three negative, three low positive and three strong positive. The Immuno*Card* STAT! EHEC test produced 100% reproducibility including control lines.

GN or MAC BROTH TEST
Three independent laboratories tested 12 samples (n = 2 strong positive, n = 4 weak positive, n = 4 weak negative, n = 2 strong negative) in duplicate on one day (intra-assay variability) and on three different days (inter-assay variability). The Immuno*Card* STAT! EHEC test produced 100% reproducibility including control lines.

ASSAY REACTIVITY
SMAC AGAR TEST: The following 40 STEC stock cultures were cultivated on SMAC plates and followed by the polymyxin extraction. All of the isolates produced positive reactions on Immuno*Card* STAT! EHEC.
O157:H7 (32 strains), O96:H9 (1), O111:NM (1), O26:H11 (2), O103:H2 (1), O145:NM (1), O45:H2 (1), O45:NM (1).
GN or MAC BROTH TEST: The following 39 STEC stock cultures were cultivated in GN or MacConkey broth. All of the isolates produced positive reactions on Immuno*Card* STAT! EHEC.
O157:H7 (32 strains), O157:NM (1), O111:NM (2), O111:H21 (1), O121:H19 (1), O126:H27 (1), O45:H2 (1),

CROSSREACTIVITY STUDIES
SMAC AGAR TEST: None of the following organisms reacted with the Immuno*Card* STAT! EHEC. *(*Microorganisms and number of strains tested): *Pseudomonas aeruginosa* (10), *Klebsiella pneumoniae* (10), Enterobacter species (10), Proteus species (10), Non-ST-producing *E. coli* (10), Aeromonas species (3), *Serratia marcescens* (5), Shigella species (3)
GN OR MAC BROTH TEST: None of the following organisms crossreacted with the Immuno*Card* STAT! EHEC: *Aeromonas hydrophila Campylobacter coli, Campylobacter jejuni, Candida albicans, Citrobacter freundii, Clostridium difficile, Clostridium perfringens, Enterobacter cloacae, Enterococcus faecalis, Escherichia coli* (2 nontoxigenic strains)*, Escherichia coli O157:H7* (nontoxingenic strain), *Escherichia hermanii, Escherichia fergusonii, Helicobacter pylori, Klebsiella pneumoniae, Proteus vulgaris, Pseudomonas aeruginosa, Pseudomonas fluorescens, Salmonella Group B, Salmonella hiversum, Salmonella minnesota, Salmonella typhimurium, Serratia liquifaciens* (2 strains), *Shigella boydii, Shigella flexeri, Shigella sonnei, Staphyloccocus aureus, Staphylococcus aureus* (Cowan), *Staphylococcus epidermidis, Yersinia enterocolitica* (2 strains), Adenovirus Type 14, Adenovirus Type 2, Adenovirus Type 41, Feline calicivirus, Coxsackie A9, Coxsackie B1, Enterovirus Type 69, Herpes Simplex Virus II, Parainfluenza Type 3, Rotavirus.

TESTS FOR INTERFERING SUBSTANCES (BROTH TESTS ONLY)
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).