

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY TEMPLATE**

A. 510(k) Number:

K062546

B. Purpose for Submission:

New device clearance

C. Measurand:

Shiga Toxin 1 and Shiga Toxin 2

D. Type of Test:

Immunochromatographic rapid test based on the lateral flow principle

E. Applicant:

Meridian Bioscience, Inc.

F. Proprietary and Established Names:

Immuno*Card* STAT! EHEC

G. Regulatory Information:

1. Regulation section: 21 CFR 866.3255
2. Classification: Class I
3. Product code: GMZ
4. Panel: Microbiology (83)

H. Intended Use:

1. Intended use(s):

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.

2. Indication(s) for use:

Same as Intended Use

3. Special conditions for use statement(s):

None

4. Special instrument requirements:

None

I. Device Description:

ImmunoCard 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. The maximum number of tests obtained from this test kit is listed on the outer box. The device contents are:

1. ImmunoCard STAT! EHEC Test Devices, containing immobilized monoclonal anti-ST1 and anti-ST2 antibodies. The devices are packaged in individual foil pouches with desiccants.
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.
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.
4. 150 µL disposable plastic transfer pipettes

J. Substantial Equivalence Information:

1. Predicate device name(s):

Premier EHEC and Duopath® Verotoxin GLISA

2. Predicate 510(k) number(s):

K953362 and K031367, respectively.

3. Comparison with predicate:

<i>Characteristics</i>	<i>ImmunoCard STAT! EHEC</i>	<i>Premier EHEC (predicate)</i>	<i>Duopath Verotoxin GLISA (predicate)</i>
<i>Device Type</i>			
Technology	Single use, rapid, lateral flow immunoassay	Microwell-based enzyme-linked immunoassay	Single use, rapid, lateral flow immunoassay
In vitro diagnostic device	Yes	Yes	Yes
Control	Includes external control reagent	Includes external control reagent	No control reagent included
Calibrator	No	No	No
<i>Assay Features</i>			
Human factors	No special equipment	EIA-related equipment	No special equipment
Sterile device	No	No	No
Mechanical safety	Not applicable	Not applicable	Not applicable
Environmental safety	Normal medical waste	Normal medical waste	Normal medical waste
Chemical hazards	None	None	None
Radiation safety	Not applicable	Not applicable	Not applicable
<i>Intended Use</i>			
Detection of Shiga toxins 1 and 2	Yes	Yes	Yes
Differentiation between Shiga toxins 1 and 2	Yes	No	Yes
Screening test	No	No	No
Diagnostic test	Yes	Yes	Yes
Identification test	Yes	No	Yes
Monitoring therapy	No	No	No

<i>Acceptable Samples</i>	<i>ImmunoCard STAT! EHEC</i>	<i>Premier EHEC (predicate)</i>	<i>Duopath Verotoxin GLISA (predicate)</i>
Stool broth culture	Yes	Yes	No
Stool agar culture	Yes	No	Yes
Direct stool	No	Yes	No
<i>Reagents/Components Provided</i>			
Test Medium	Test Device with nitrocellulose strip	Antibody-coated microwell	Test Device with nitrocellulose strip
Conjugate Reagent	In Test Device	Stand alone reagent	In Test Device
Sample Diluent/Negative Control (external)	Yes	Yes	No
Substrate Reagent	No	Yes	No
Stop Solution	No	Yes	No
Procedural/internal control	Yes	No	Yes
External positive control	Yes	Yes	No
External negative control	Yes	Yes	No
<i>Source of Antigens/Antibodies</i>			
Capture ST1 antibodies	Murine monoclonal	Murine monoclonal	Murine monoclonal
Capture ST2 antibodies	Murine monoclonal	Murine monoclonal	Murine monoclonal
Detector ST1 Antibodies	Murine monoclonal	Rabbit polyclonal	Murine monoclonal
Detector ST2 Antibodies	Murine monoclonal	Rabbit polyclonal	Murine monoclonal
Positive Control	Inactivated toxin	Inactivated toxin	None
<i>Comparison of assay steps</i>			
Equipment Required	None	EIA-related	None
Level of skill required	Moderately complex	Moderately complex	Moderately complex
Assay steps	5	15	5
End point	Pink-red band	Yellow color	Pink-red band
Interpretation of test result	Pos = color band, Neg = no color	OD \geq 0.150 (dual wavelength)	Pos = color band, Neg = no color

K. Standard/Guidance Document Referenced (if applicable):

Not applicable.

L. Test Principle:

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.

M. Performance Characteristics:

1. Analytical performance:

a. Precision/Reproducibility:

Assay precision, intra-assay variability and inter-assay variability were assessed with a reference panel prepared from broths inoculated with ST1 and ST2. Of the 11 samples in the reproducibility panel, 2 were prepared as high positive (HP) samples, four as low positive (LP) samples near the assay limit of detection, 4 as high negative (HN) samples just below the assay limit of detection, and one as a low negative sample (LN). Each clinical site tested the panel twice per day for three consecutive days. The expected results were obtained at each test interval at each site resulting in an assay precision of 100% with no variability.

b. Linearity/assay reportable range:

Not applicable – this is a qualitative test with no numerical output.

c. Traceability, Stability, Expected values (controls, calibrators, or methods):

Not applicable

d. *Detection limit:*

Method: Diluted broth media was inoculated with defined quantities of Shiga Toxin 1 (ST1) or Shiga Toxin 2 (ST2) toxin, then tested. Results and

Conclusions: The results given in Table 12-12 show the analytical sensitivity of the assay is 1.25 ng of toxin per mL for both ST 1 and ST 2.

Toxin	Concentration	Results		
		C	T1	T2
Shiga Toxin 1	20 ng	+	+	-
	10 ng	+	+	-
	5 ng	+	+	-
	2.5 ng	+	+	-
	1.25 ng	+	+	-
	0.625 ng	+	-	-
Shiga Toxin 2	20 ng	+	-	+
	10 ng	+	-	+
	5 ng	+	-	+
	2.5 ng	+	-	+
	1.25 ng	+	-	+
	0.625 ng	+	-	-

Legend: C = Control Line, T1 = Shiga toxin 1 test line, T2 = Shiga toxin 2 test line

e. *Analytical specificity:*

Method: Broth media was inoculated with different Shiga toxin-producing strains of *E. coli*, then tested. Results and Conclusions: The following 39 STEC stock cultures were cultivated in GN or MacConkey broth. O157:H7 (32 strains), O157:NM (1), O111:NM (2), O111:H21 (1), O121:H19 (1), O126:H27 (1), O45:H2 (1). All of the isolates produced positive reactions on ImmunoCard STAT! EHEC.

Legend: GN= Gram negative broth, Mac = MacConkey broth, C = Control Line, T1 = Shiga toxin 1 test line, T2 = Shiga toxin 2 test line

E. coli strain	Broth	ID	ImmunoCard STAT! EHEC		
			C	T1	T2
O157:NM	GN	Microbank 95 ATCC 700277	+	—	+
O157:NM	Mac	Microbank 95 ATCC 700277	+	—	+
O157:H7	GN	Microbank 70 EMDI-67	+	+	+
O157:H7	Mac	Microbank 70 EMDI-67	+	+	+

Table is continued on the next page.

E. coli strain	Broth	ID	ImmunoCard STAT! EHEC		
			C	T1	T2
O157:H7	GN	Microbank 92	+	+	+
O157:H7	Mac	Microbank 92	+	+	+
O45:H2	GN	Microbank 81	+	+	—
O45:H2	Mac	Microbank 81	+	+	—
O157:H7	GN	Microbank 22 EMDI-11	+	+	—
O157:H7	Mac	Microbank 22 EMDI-11	+	+	—
O157:H7	GN	Microbank 14 EMDI-46	+	—	+
O157:H7	Mac	Microbank 14 EMDI-46	+	—	+
O121:H19	GN	Microbank 23 EMDI-17	+	—	+
O121:H19	Mac	Microbank 23 EMDI-17	+	—	+
O157:H7	GN	Microbank 28 Dupont 1450	+	—	+
O157:H7	Mac	Microbank 28 Dupont 1450	+	—	+
O157:H7	GN	Microbank 65 EMDI-15	+	+	+
O157:H7	Mac	Microbank 65 EMDI-15	+	+	+
O157:H7	GN	Microbank 77	+	+	+
O126:H27	Mac	Microbank 25 EMDI-32	+	+	—
O157:H7	GN	Microbank 31 EMDI-55	+	—	+
O157:H7	Mac	Microbank 31 EMDI-55	+	—	+
O157:H7	GN	Microbank 34 EMDI-121	+	—	+
O157:H7	Mac	Microbank 34 EMDI-121	+	—	+
O111:NM	Mac	Microbank 8 EMDI-48	+	+	+
O157:H7	GN	Microbank 24 EMDI-26	+	—	+
O157:H7	Mac	Microbank 24 EMDI-26	+	—	+
O157:H7	GN	Microbank 33 EMDI-52	+	+	+
O157:H7	Mac	Microbank 33 EMDI-52	+	+	+

Table is continued on the next page.

E. coli strain	Broth	ID	ImmunoCard STAT! EHEC		
			C	T1	T2
O157:H7	Mac	Microbank 7 EMDI-70	+	+	+
O157:H7	GN	Microbank 40 EMDI-115	+	+	+
O157:H7	Mac	Microbank 40 EMDI-115	+	+	+
O157:H7	GN	Microbank 10 EMDI-19	+	—	+
O157:H7	Mac	Microbank 10 EMDI-19	+	—	+
O157:H7	GN	Microbank 18 EMDI-2	+	+	+
O157:H7	Mac	Microbank 18 EMDI-2	+	+	+
O126:H27	GN	Microbank 25 EMDI-32	+	+	—
O157:H7	GN	Microbank 35 EMDI-120	+	+	+
O157:H7	Mac	Microbank 35 EMDI-120	+	+	+
O111:H21	GN	Microbank 48 EMDI-82	+	+	+
O111:H21	Mac	Microbank 48 EMDI-82	+	+	+
O157:H7	GN	Microbank 62 EMDI-116	+	+	+
O157:H7	Mac	Microbank 62 EMDI-116	+	+	+
O111:NM	GN	Microbank 52 3007-85	+	+	+
O111:NM	Mac	Microbank 52 3007-85	+	+	+
O157:H7	GN	Microbank 61 EMDI-50	+	+	+
O157:H7	Mac	Microbank 61 EMDI-50	+	+	+
O157:H7	GN	Microbank 39 EMDI-54	+	+	+
O157:H7	Mac	Microbank 39 EMDI-54	+	+	+
O157:H7	GN	Microbank 51 EMDI-81	+	+	+
O157:H7	Mac	Microbank 51 EMDI-81	+	+	+
O157:H7	GN	Microbank 55 EMDI-118	+	+	+

Table is continued on the next page.

E. coli strain	Broth	ID	ImmunoCard STAT! EHEC		
			C	T1	T2
O157:H7	Mac	Microbank 55 EMDI-118	+	+	+
O157:H7	GN	Microbank 60 EMDI-76	+	+	—
O157:H7	Mac	Microbank 60 EMDI-76	+	+	—
O157:H7	Mac	Microbank 77	+	+	+
O157:H7	GN	Microbank 89	+	—	+
O157:H7	Mac	Microbank 89	+	—	+
O157:H7	GN	Microbank 94 ATCC 43890	+	—	+
O157:H7	Mac	Microbank 94 ATCC 43890	+	—	+
O157:NM	GN	Microbank 96 ATCC 700376	+	+	—
O157:NM	Mac	Microbank 96 ATCC 700376	+	+	—
O157:H7	GN	Microbank 63 EMDI-4	+	+	+
O157:H7	Mac	Microbank 63 EMDI-4	+	+	+
O157:H7	GN	Microbank 73 EMDI-71	+	—	+
O157:H7	Mac	Microbank 73 EMDI-71	+	—	+
O157:H7	GN	Microbank 88	+	+	+
O157:H7	Mac	Microbank 88	+	+	+
O157:H7	GN	Microbank 93 ATCC 43885	+	—	+
O157:H7	Mac	Microbank 93 ATCC 43885	+	—	+
O157:H7	Mac	Microbank 27 EMDI-46	+	—	+
O157:H7	GN	Microbank 91	+	+	+
O157:H7	Mac	Microbank 91	+	+	+

Cross reactivity:

Method: Stool broth cultures containing low levels of Toxin 1 or Toxin 2 were spiked with bacterial, viral and yeast strains that might be expected to be present in human stool samples either as part of normal flora or from a disease state (TEST). The final concentration of bacteria or yeast in each sample was $\geq 7.5 \times 10^7$ organisms/mL. The final concentration of viruses in each sample was $\geq 1.6 \times 10^5$

TCID₅₀/mL. Unspiked samples were tested in parallel to provide a reference against which the reactions with spiked samples could be compared (CONTROL). An organism that diminished a positive reaction by 4 or more grades, that caused a positive to become negative, or that caused the appearance of a positive reaction in a formerly negative sample was considered to be an interfering organism. Results: None of the potential co-contaminants of stool samples affected positive or negative test results. No crossreactivity has been observed with this assay for any of the following organisms crossreacted with the ImmunoCard 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 (nontoxigenic strain), *Escherichia hermannii*, *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*, *Staphylococcus 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.

f. *Assay cut-off:*

Not applicable

2. Comparison studies:

a. *Method comparison with predicate device:*

See Clinical Studies

b. *Matrix comparison:*

The results of each broth method (Gram Negative (GN) or MacConkey (Mac) broth) 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

3. Clinical studies:

a. *Clinical Sensitivity and Specificity:*

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. There were 448/469 samples which 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 and 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)			
<i>Mac Broth Method</i>			
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)			
<i>GN Broth Method</i>			
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.

- c. Other clinical supportive data (when a. and b. are not applicable):

Not applicable

4. Clinical cut-off:

Not applicable

5. Expected values/Reference range:

Not applicable - this is a qualitative test with no numerical output.

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

O. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.