

**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:

ImmunoCard 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):

ImmunoCard 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. ImmunoCard 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

| | | | ImmunoCard STAT! EHEC | | |
|----------------|-------|--------------------------|-----------------------|----|----|
| E. coli strain | Broth | ID | 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.

| | | | ImmunoCard STAT! EHEC | | |
|----------------|-------|-----------------------------|-----------------------|----|----|
| E. coli strain | Broth | ID | 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.