

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

A. 510(k) Number:

K061889

B. Purpose for Submission:

For the qualitative detection of Shiga toxins from human fecal specimens, broth cultures and swab sampling of colonies from a culture plate

C. Measurand:

Shiga Toxin 1 & 2

D. Type of Test:

Optical Immunoassay

E. Applicant:

Inverness Medical-BioStar Inc.

F. Proprietary and Established Names:

BioStar OIA SHIGATOX

G. Regulatory Information:

1. Regulation section:

21 CFR Part 866.3255 Escherichia coli serological reagents

2. Classification:

Class 1

3. Product code:

GMZ – Antigens, all types, Escherichia coli

4. Panel:

H. Intended Use:

1. Intended use:

The BioStar OIA SHIGATOX assay is an optical immunoassay (OIA) test for the qualitative, rapid detection of the presence of Shiga Toxins in human diarrheal fecal specimens, broth cultures and swab sampling of colonies from a culture plate. This test is intended for in vitro diagnostic use as an aid in the diagnosis of infection by Shiga toxin producing *Escherichia coli* (STEC) both O157 and all non-O157 Shiga toxin producing strains.

2. Indication(s) for use:

The BioStar OIA SHIGATOX assay is an optical immunoassay (OIA) test for the qualitative, rapid detection of the presence of Shiga Toxins in human diarrheal fecal specimens, broth cultures and swab sampling of colonies from a culture plate. This test is intended for in vitro diagnostic use as an aid in the diagnosis of infection by Shiga toxin producing *Escherichia coli* (STEC) both O157 and all non-O157 Shiga toxin producing strains.

3. Special conditions for use statement:

For prescription use

For Point-of-Care settings

4. Special instrument requirements:

None

I. Device Description:

The OIA SHIGATOX consists of a kit containing the following components: Reagent 1 contains anti-Shiga toxin 1 antibodies (rabbit) conjugated to HRP in a buffered protein solution; Reagent 2 contains anti-Shiga toxin 2 antibodies (rabbit) conjugated to HRP in a buffered protein solution; wash contains buffered saline solution; substrate consists of TMB and hydrogen peroxide; test devices with surfaces coated with anti-Stx 1 and anti-Stx 2 affinity purified rabbit polyclonal antibodies; positive control containing inactivated purified shiga toxin in a buffered protein solution; diluent/negative control consisting of buffered protein solution; reaction tubes; transfer pipettes and rayon swabs.

J. Substantial Equivalence Information:

1. Predicate device name(s):

Premier EHEC – Meridian Diagnostics Inc.

ProSpecT Shiga Toxin Microplate Assay – Alexon Trend Inc.

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

K953362

K980507

3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Intended Use	For the qualitative rapid detection of Shiga toxins from human fecal specimens as an aid in the diagnosis of infection by shiga toxin producing E.coli (STEC)	Same
Specimen type	Liquid or semi-solid fecal or broth culture or swab sampling from colonies	Same plus stools in transport medium
Analyte	Shiga toxins 1&2	Same
Primary comparison method	EIA	Cytotoxicity

Differences		
Item	Device	Predicate
Analytical Sensitivity	Shiga toxin 1 - 1ng/ml Shiga toxin 2 – 1ng/ml	1. Stx 1 – 7pg/well & Stx2 – 15 pg/well 2. Stx1 – 52 pg/ml & Stx2 -126 pg/ml

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

N/A

L. Test Principle:

The OIA ShigaTox test is an optical immunoassay. This technology enables the direct visual detection of a physical change in the optical thickness of molecular thin films. The change is the result of antigen-antibody binding on an optical surface (silicon wafer). In this device, the biological capture film is a combination of affinity –purified polyclonal antibodies to Stx 1 & 2 relative. Samples suspected of containing either or both of the toxins are mixed with reagents containing polyclonal antibodies to Stx 1 & 2 conjugated to HRP. Once a sample containing toxins or either toxin is applied to the surface, the immune complex of toxins and the anti-toxin-HRP conjugate are bound to the surface antibodies. Following a wash step, a precipitating substrate for HRP is added, and a thin film generated by the immobilized immune complex is enhanced by the precipitation of the HRP product. Once washed and dried, a simple color change relative to the gold background color is observed as a purple spot on the gold background indicating the presence of Stx 1 or 2. If antigen is not present in the specimen, no binding takes place, optical thickness remains unchanged and the surface retains the original gold color indicating a negative result.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. *Precision/Reproducibility:*

Reproducibility studies were performed using a masked method at each of the three Clinical Trial sites and three Point-of-Care sites. A panel of 27 randomly ordered and blinded fecal specimens, spiked with negative, low and moderate levels of Toxin 1 and/or Toxin 2 were provided and tested on 3 successive days by each of the 6 sites. The specimens produced expected results with the OIA SHIGATOX test (486/486) 100% of the time.

b. *Linearity/assay reportable range:*

N/A

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

N/A

d. *Detection limit:*

To determine the analytical sensitivity, 2 fold serial dilutions of purified Stx 1 or Stx2 toxins were prepared. Dilutions were then spiked into buffer and the spiked samples were tested in triplicate. The limit of detection (LOD) was defined as the lowest toxin concentration producing at least 2 positive results of the 3 tests or at least 50% of the total number of samples tested. In liquid stool the LOD for each toxin was determined to be 1ng/ml. 5 samples at each concentration were analyzed on 2 lots of devices.

e. *Analytical specificity:*

Cross Reactivity Study

Bacteria at 1×10^8 orgs/mL were tested with and without spiking with 2.5 ng/mL of Stx1 and 2.5 ng/mL of Stx2. *Cryptosporidium* and *Giardia* were tested at 1×10^6 cysts/mL and *Candida albicans* was tested at 9.3×10^7 cells/mL. At one clinical site, 3 specimens positive for Rota virus were also tested and were negative by OIA. All members of the cross reactivity panel produced the expected negative result without the toxin spike and the expected positive result with the toxin spike. Organisms tested were as follows:

<i>Aeromonas hydrophila</i>	<i>Giardia lamblia</i>
<i>Bacillus cereus</i>	<i>Klebsiella pneumoniae</i>
<i>Bacillus subtilis</i>	<i>Peptostreptococcus anaerobius</i>
<i>Bacteroides fragilis</i>	<i>Porphyromonas asaccharolytica</i>
<i>Bifidobacterium adolescentis</i>	<i>Proteus vulgaris</i>
<i>Campylobacter fetus</i>	<i>Providencia rettgeri</i>
<i>Campylobacter jejuni</i>	<i>Pseudomonas aeruginosa</i>
<i>Candida albicans</i>	<i>Salmonella diarizonae</i>
<i>Citrobacter freundii</i>	<i>Salmonella enteritidis</i>
<i>Clostridium botulinum</i> Type A	<i>Salmonella typhi</i>
<i>Clostridium butyricum</i>	<i>Salmonella typhimurium</i>
<i>Clostridium histolyticum</i>	<i>Serratia liquefaciens</i>
<i>Clostridium innocuum</i>	<i>Serratia marcescens</i>
<i>Clostridium novyi</i>	<i>Shigella flexneri</i> Serotype 1A
<i>Clostridium perfringens</i>	<i>Shigella sonnei</i>
<i>Clostridium septicum</i>	<i>Staphylococcus aureus</i>
<i>Clostridium sordellii</i>	<i>Staphylococcus aureus</i> Cowan I
<i>Clostridium subterminale</i>	<i>Staphylococcus epidermidis</i>
<i>Clostridium tetani</i>	<i>Staphylococcus saprophyticus</i>
<i>Cryptosporidium parvum</i>	<i>Veillonella parvula</i>
<i>Enterobacter aerogenes</i>	<i>Vibrio cholerae</i>
<i>Enterobacter cloacae</i>	<i>Vibrio parahaemolyticus</i>
<i>Enterococcus faecalis</i>	<i>Yersinia enterocolitica</i>
<i>Escherichia coli</i> (non-STEC)	

In addition, one clinical trial site tested three stools in the OIA SHIGATOX assay that were positive in a commercial lateral flow, EIA assay for Rotavirus. All 3 samples were negative in the OIA SHIGATOX assay as expected.

Interfering substances Study

The OIA SHIGATOX assay was tested with whole blood, mucin, liquid Imodium AD, Pepto Bismol, Kaopectate and barium sulfate to determine potential interference. Each interferent was mixed with antigen diluent or a liquid or semisolid stool specimen and tested with the assay. Final

concentrations of Stx 1 & Stx 2 were 2.5 ng/mL. None of the substances tested caused a false positive or false negative result in the assay.

f. Assay cut-off:

N/A

2. Comparison studies:

a. Method comparison with predicate device:

A prospective study was conducted at 3 clinical trial sites in the Eastern, southern and Western regions of the USA to compare performance of the BioStar OIA SHIGATOX to a commercial EIA test. A total of 272 prospective specimens from diarrheal patients were tested. Testing consisted of direct stool testing, Mac Conkey broth culture testing as well as testing from Sorbitol MacConkey plates (SMAC). All positive results from either of the 2 immunoassays were confirmed by cytotoxicity testing CTA.

Swab Sampling of Colonies from a Culture Plate (Colony Sweep Method)

One clinical site evaluated twenty two frozen fecal specimens in a colony sweep procedure. These samples were previously found to contain Shiga toxin producing *E. coli*. All samples were streaked onto XLD (xylose lysine deoxycholate) plates and incubated overnight at 37°C. One sample failed to produce any growth. A sterile rayon swab was used to sweep the first and second quadrants of the growth area and was then immersed into a reaction tube containing 3 drops each of Reagents 1 and 2 and the standard assay protocol followed. The OIA SHIGATOX assay detected 21/21 of the colony sweeps that produced growth for 100% agreement with the previous specimen result.

Direct Stool

A prospective study was conducted at three clinical trial sites in the Eastern, Southern and Western regions of the United States to compare the performance of the BioStar OIA SHIGATOX to a commercial EIA test. Sites analyzed the stool specimens collected for direct testing from the stool sample by both assays and then placed an aliquot of the stool in MacConkey broth within 48 hours of specimen collection. Broth cultures were incubated for 20 – 30 hours and then tested by both immunoassays. A SMAC culture (Sorbitol MacConkey plates) was also plated within 48 hours of the specimen collection for the determination of *E. coli* O157. All positive results from either immunoassay method were confirmed by cytotoxicity testing, CTA.

A total of 272 prospective specimens from diarrheal patients were tested in the OIA SHIGATOX and the EIA method.

Comparison of OIA SHIGATOX to EIA for Direct Stool Samples

		EIA	
		+	-
OIA SHIGATOX	+	12	5
	-	0	255

Positive Agreement: 100% (95%CI: 73.5 – 100%)
 Negative Agreement: 98.1% (95%CI: 95.6 – 99.4%)
 Overall Percent Agreement: 98.2% (95% CI: 95.8 – 99.4%)

Of the five OIA+/EIA - specimens, one was positive by CTA. One of the samples that was negative by direct stool testing in both the EIA and the OIA methods was positive in the OIA broth culture sample and by CTA from the direct stool.

Two of the clinical sites also performed a study in which sixty-two additional frozen specimens were prospectively tested by OIA SHIGATOX and EIA without the operator's knowledge of the original Shiga toxin result.

Comparison of OIA SHIGATOX to EIA for Frozen Direct Stools

		EIA	
		+	-
OIA SHIGATOX	+	21	1
	-	3	37

Positive Agreement: 87.5% (95% CI: 67.6 – 97.3%)
 Negative Agreement: 97.4% (95% CI: 86.2 – 99.9%)
 Overall Percent Agreement: 93.6% (95% CI: 84.3 – 98.2%)

Broth Culture

A total of 269 prospective specimens from diarrheal patients were tested by OIA SHIGATOX and the EIA method from the broth culture. Three fecal specimens failed to produce any growth upon broth culture.

Comparison of OIA SHIGATOX to EIA for Broth Enriched Culture from Fresh Stools

EIA	
+	-

OIA	+	12	1
SHIGATOX	-	0	256

Positive Agreement: 100% (95% CI: 73.5 -100%)
 Negative Agreement: 99.6% (95% CI: 97.9 - 100%),
 Overall Percent Agreement: 99.6% (95% CI: 98.0 – 100%)

The single OIA SHIGATOX +/-EIA – result was confirmed to be a true positive by the CTA analysis of the direct stool.

In the prospective frozen sample study, ten of the frozen specimens were not tested in overnight Sorbitol MacConkey broth culture. Two of the remaining specimens failed to exhibit growth after overnight Sorbitol MacConkey broth culture. The percent positive agreement was 100% and the percent negative agreement was 96.4%. The overall percent agreement in the study was 98%.

Comparison of OIA SHIGATOX to EIA for Broth Enriched Culture from Frozen Stools

		EIA	
		+	-
OIA	+	22	1
SHIGATOX	-	0	27

Positive Agreement: 100% (95% CI: 84.6 -100%)
 Negative Agreement: 95.6% (95% CI: 81.7 - 99.9%)
 Overall Percent Agreement: 98% (95% CI: 89.4 – 100%)

SMAC Culture

Two hundred and sixty nine (269) of the direct stool samples were analyzed by SMAC culture. The OIA SHIGATOX and EIA assays were compared to the SMAC culture results. Interpretation of the comparison between the OIA SHIGATOX or the EIA test and SMAC is confounded by the fact that, as a metabolic test, SMAC is specific for *E. coli* O157:H7, while OIA SHIGATOX reacts with all Shiga toxin-producing *E. coli* (STEC). Also, SMAC requires the presence of live cells in the sample, while the OIA SHIGATOX test does not have that limitation. Based on these differences, it was anticipated that a number of samples could be SMAC-negative and OIA SHIGATOX positive.

OIA SHIGATOX Direct Fresh Stool Results compared to SMAC Culture of Direct Stools

SMAC	
+	-

OIA	+	9	8
SHIGATOX	-	1	251

Positive Agreement: 90% (95% CI: 55.5 – 99.8%)
 Negative Agreement: 96.9% (95% CI: 94.0 – 98.7%)
 Overall Percent Agreement: 96.7% (95% CI: 93.7 – 98.5%)

EIA Direct Fresh Stool Results compared to SMAC Culture of Direct Stools

		SMAC	
		+	-
EIA	+	8	4
	-	2	255

Positive Agreement: 80% (95% CI: 44.4 - 97.5%)
 Negative Agreement: 98.5% (95% CI: 96.1 – 99.6%)
 Overall Percent Agreement: 97.8% (95% CI: 95.2 – 99.2%)

The one apparent OIA false negative result compared to the SMAC result was not confirmed by CTA and was not positive by EIA. Of the 8 apparent false positives by the OIA method, 4 of the samples were positive by CTA. The second EIA false negative result was positive by CTA and the OIA method. One of the OIA +/SMAC + samples was negative by CTA. The 2 EIA -/SMAC + samples were negative by CTA and one of the samples was negative by the OIA method as well. Three of the EIA +/SMAC – samples were positive by CTA and the OIA method. The remaining EIA +/SMAC – sample was negative by CTA but positive by the OIA method.

In addition, two of the clinical sites conducted a prospective comparison of the OIA method to SMAC culture using frozen samples. Thawed aliquots of all samples were tested in the OIA and SMAC methods for this comparison.

Frozen Stool samples comparing OIA SHIGATOX to SMAC

		SMAC	
		+	-
OIA	+	9	13
SHIGATOX	-	0	40

Positive Agreement: 100% (95% CI: 66.4 - 100%)
 Negative Agreement: 75.5% (95% CI: 61.7 – 86.2%)
 Overall Percent Agreement: 79% (95% CI: 66.8 – 88.3%)

All thirteen of the OIA+/SMAC- samples were positive for STEC in previous testing.

CTA

In the clinical study there were 19 specimens positive by OIA, EIA, or both methods. An aliquot of the stool specimen for each of these 19 specimens was submitted for CTA along with an aliquot of the broth culture media. One sample produced an inconclusive result and was excluded from this analysis. Thirteen of the samples were positive by CTA. The OIA SHIGATOX detected 12 of these 13 samples while the EIA method detected 11. Twelve of the broth aliquots were positive by CTA. The OIA SHIGATOX assay detected all 12 of these samples as did the EIA method. Of the 13 CTA positives, SMAC was positive for only 8 samples.

Comparison of OIA SHIGATOX, EIA, and SMAC to CTA for Direct Stool and Broth Culture Samples

	CTA Direct Stool	CTA Broth Culture
OIA SHIGATOX	12/13	12/12
EIA	11/13	12/12
SMAC	8/13	N/A

b. Matrix comparison:

N/A

3. Clinical studies:

a. Clinical Sensitivity:

N/A

b. Clinical specificity:

N/A

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

N/A

4. Clinical cut-off:

N/A

5. Expected values/Reference range:

Major outbreaks of enterohemorrhagic E. coli (EHEC) are usually considered local, appearing in a specific area and requiring concentrated investigation by public health personnel. Prevalence rates may therefore vary greatly based on a number of factors, including geographic location, patient demographics, sampling and testing methodology. The increasing role of non-O157:H7 strains has further confounded prevalence estimates.

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.

