

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

A. 510(k) Number:

K071799

B. Purpose for Submission:

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

C. Measurand:

Shiga Toxins 1 & 2

D. Type of Test:

Optical Immunoassay

E. Applicant:

Inverness Medical - BioStar Inc.

F. Proprietary and Established Names:

BioStar OIA SHIGA TOX

G. Regulatory Information:

Product Code	Classification	Regulation Section	Panel
GMZ- Antigens, all types, <u>Escherichia coli.</u>	<u>Class 1</u>	<u>21 CFR Part 866.3255</u> <u>Escherichia coli</u> <u>serological reagents</u>	<u>83 Microbiology</u>

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, fecal specimens in Cary Blair Transport Media or 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. Indications 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, fecal specimens in Cary Blair Transport Media or 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:

The predicate device is BioStar OIA SHIGATOX – K061889 from Inverness Medical BioStar Inc. The tests have similar Indications for Use but unlike the predicate, this device allows the use of specimens in Cary Blair Transport media and use of an additional broth enrichment media namely Gram Negative (GN) broth. The devices use the same technology but slight differences in performance may result from using transport media due to the additional dilution and additional time between collection and analysis. The device operation, sample type and immunochemistry are similar to other marketed products.

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:

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:*

The analytical sensitivity was determined by adding known concentrations of Shiga toxin 1 or 2 into the assay. Assays were done in triplicate with the OIA SHIGA TOX test. The limit of detection was 1ng/ml for diluent, liquid and semi-solid stool for both Toxin 1 & 2.

e. *Analytical specificity:*

Cross Reactivity Study

Bacteria at 1×10^7 or greater cfu/mL were tested with and without spiking with 5.0 ng/mL of Stx1 and 5.0 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:

Aeromonas hydrophila

Giardia lamblia

Bacillus cereus

Klebsiella pneumoniae

Bacillus subtilis

Peptostreptococcus anaerobius

Bacteroides fragilis

Porphyromonas asaccharolytica

Bifidobacterium adolescentis

Proteus vulgaris

Campylobacter fetus

Providencia rettgeri

Campylobacter jejuni

Pseudomonas aeruginosa

Candida albicans

Salmonella diarizonae

Citrobacter freundii

Salmonella enteritidis

Clostridium botulinum Type A

Salmonella typhi

Clostridium butyricum

Salmonella typhimurium

Clostridium histolyticum

Serratia liquefaciens

Clostridium innocuum

Serratia marcescens

Clostridium novyi

Shigella flexneri Serotype 1A

Clostridium perfringens

Shigella sonnei

Clostridium septicum

Staphylococcus aureus

Clostridium sordellii

Staphylococcus aureus Cowan I

Clostridium subterminale

Staphylococcus epidermidis

Clostridium tetani

Staphylococcus saprophyticus

Cryptosporidium parvum

Veillonella parvula

Enterobacter aerogenes
Enterobacter cloacae
Enterococcus faecalis
Escherichia coli (non-STEC)

Vibrio cholerae
Vibrio parahaemolyticus
Yersinia enterocolitica

Interfering substances Study

The OIA SHIGATOX assay was tested with 50% whole blood, 12.5 mg/g mucin, 50% liquid Imodium AD, 50% Pepto Bismol, 50% Kaopectate and 29% 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. None of the substances interfered with the generation of a positive signal or produced a false positive result.

f. Assay cut-off:

N/A

2. Comparison studies:

a. Method comparison with predicate device:

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%)

Ninety eight frozen fecal specimens were collected from 2 lab sites. Fecal specimens were thawed and tested directly in the BioStar OIA SHIGA TOX Assay and used to inoculate Cary Blair transport media. Testing of the Cary Blair specimens was performed within 1 hour and 24 hours of inoculation. Of the 32 positive and 66 negative specimens, 28 were positive and 70 negative after 1 hr of Cary Blair inoculation while 29 were positive and 69 negative after 24 hrs. At 1 hr. positive agreement was 88% and negative agreement 100% while at 24 hours positive agreement was 91% and negative agreement 100%.

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 SHIGATOX	+	12	1
	-	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 SHIGATOX	+	22	1
	-	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%)

Ninety eight fecal specimens were thawed and inoculated into MacConkey (MAC) broth and Gram Negative (GN) broth. The tubes were incubated for 24 hrs at 37°C and then each sample was tested in the OIA Shiga TOX Assay. In addition, the Cary Blair samples held for 24 hrs. at room temperature were used to inoculate both GN and MAC broths. All samples were incubated for 24 hrs at 37°C and tested in the OIA Shiga TOX method. From MAC broth 26 samples were positive and 44

negative and from GN broth 28 were positive and 42 negative. Positive agreement was 100% and negative agreement 95%. From the Cary Blair samples in GN broth, 27 were positive and 41 negative and from direct MAC broth 26 were positive and 42 negative. Positive agreement was 100% and negative agreement 98%. From Cary Blair samples in MAC broth 29 were positive and 42 negative while from the direct MAC broth 28 were positive and 43 negative. Positive agreement was 93% and negative agreement was 93%.

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 SHIGATOX	+	9	8
	-	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 SHIGATOX	+	9	13
	-	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

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

Stool and Broth Culture Samples

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 have further confounded prevalence estimates.

N. Proposed Labeling:

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

O. Conclusion:

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