

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION  
DECISION SUMMARY**

**A. 510(k) Number:**

k121411

**B. Purpose for Submission:**

To obtain substantial equivalence for the SHIGA TOXIN CHEK assay

**C. Measurand:**

Shiga toxin 1 (stx1) and Shiga toxin 2 (stx 2)

**D. Type of Test:**

Enzyme immunoassay

**E. Applicant**

TechLab Inc.

**F. Proprietary and Established Names:**

SHIGA TOXIN CHEK

**G. Regulatory Information:**

1. Regulation section:

21 CFR 866.3255, *Escherichia coli* serological reagents

2. Classification:

Class I

3. Product code:

GMZ – Antigens, all types, *Escherichia coli*

4. Panel:

83 - Microbiology

## H. Intended Use:

### 1. Intended use:

The *SHIGA TOXIN CHEK* test is an enzyme immunoassay for the simultaneous qualitative detection of Shiga toxin 1 (Stx1) and Shiga toxin 2 (Stx2) in a single test. It is intended for use with human fecal samples from patients with gastrointestinal symptoms to aid in the diagnosis of disease caused by Shiga Toxin producing *Escherichia coli* (STEC). It may be used directly with human fecal specimens, or broth or plate cultures derived from fecal specimens. The test results should be considered in conjunction with the patient history.

### 2. Indication(s) for use:

The *SHIGA TOXIN CHEK* test is an enzyme immunoassay for the simultaneous qualitative detection of Shiga toxin 1 (Stx1) and Shiga toxin 2 (Stx2) in a single test. It is intended for use with human fecal samples from patients with gastrointestinal symptoms to aid in the diagnosis of disease caused by Shiga Toxin producing *Escherichia coli* (STEC). It may be used directly with human fecal specimens, or broth or plate cultures derived from fecal specimens. The test results should be considered in conjunction with the patient history.

### 3. Special conditions for use statement:

For Prescription Use

### 4. Special instrument requirements:

N/A

## I. Device Description:

The kit consists of:

A Microassay Plate – 12 strips, each strip consisting of 8 wells, coated with monoclonal antibodies specific for Stx1 and Stx2 (stored with desiccant)

Diluent (40 mL) – buffered protein solution containing 0.02% thimerosal

Substrate (14 mL) – solution containing tetramethylbenzidine and peroxide

Wash Buffer Concentrate (50 mL) – 20X concentrate containing phosphate buffered saline, detergent, and 0.2% thimerosal

Stop Solution (7 mL) – 0.6N sulfuric acid

Positive Control (3.5 mL) – inactivated antigen in a buffered protein solution containing

amphotericin B

Conjugate (7 mL) – polyclonal antibodies specific for Stx1 and Stx2 coupled to horseradish peroxidase in a buffered protein solution containing 0.02% thimerosal

Disposable plastic pipettes - graduated at 50 µL, 100 µL, 200 µL and 300 µL

Plastic Adhesive Sheets – 2

Wash Label – 1

J. Substantial Equivalence Information:

1. Predicate device names:

Premier EHEC

Immunocard Stat EHEC

ProspecT Shiga Tox E.coli EHEC

2. Predicate K numbers:

K953362

K062546

K980507

3. Comparison with predicate:

<b>Similarities</b>		
<b>Item</b>	<b>Device</b>	<b>Predicate</b>
Intended Use	Qualitative detection of Shiga toxin	Same
Technology	Immunoassay	Same
Antibody format	Monoclonal/polyclonal	Monoclonal/polyclonal

<b>Differences</b>		
<b>Item</b>	<b>Device</b>	<b>Predicate</b>
Intended Use	Non differentiation of toxins 1 &2	Same except for Immunocard Stat EHEC which differentiates toxin 1 from toxin 2
Technology	Enzyme immunoassay-microwell plate ELISA	Same except for Immunocard Stat EHEC

Differences		
Item	Device	Predicate
		which is an immunochromatographic lateral flow
Specimen types	Direct fecal and broth cultures	Same for ProspecT Shiga Premier EHEC – direct fecal, broth and plate cultures Immunocard – broth & plate cultures
Specimen volume	50µl – broth and direct culture 100 µl – transport media	Same except for ProspecT - 300 µl
Time to result	60 mins	Premier – 25 mins Immunocard – 2 hrs 15 mins ProspecT – 1 hr 50 mins

**K. Standard/Guidance Document Referenced :**

CLSI - EP 17-A: Protocols for determination of Limits of Detection and Limits of Quantitation.

**L. Test Principle:**

The *SHIGA TOXIN CHEK* test uses antibodies to Stx1 and Stx2. The microassay wells supplied with the kit contain immobilized monoclonal antibodies against Stx1 and Stx2. The detecting antibody consists of a mixture of anti-Stx1 and anti-Stx2 polyclonal antibodies conjugated to horseradish peroxidase. In the assay, an aliquot of a fecal specimen or culture is emulsified in the *Diluent* and the diluted specimen is then transferred to the microassay well containing the detecting antibody. If Stx1 and/or Stx2 are present in the specimen, they will bind to the detecting antibody and to the immobilized monoclonal antibodies during the incubation phase. Any unbound material is removed during the washing steps. Following the addition of substrate, a color is detected due to the enzyme-antibody-antigen complexes that form in the presence of toxin.

## M. Performance Characteristics:

### 1. Analytical performance:

#### a. *Precision/Reproducibility:*

The reproducibility of the *SHIGA TOXIN CHEK* test was determined using 11 fecal specimens that were coded to prevent their identification during testing. Testing was performed at 2 independent laboratories and on-site at TECHLAB<sup>®</sup>, Inc. The samples were tested, twice a day over a 5-day period by multiple technicians at each site using 2 different kit lots. A positive and negative control was run with each panel of the masked samples. The results from each laboratory were submitted to TECHLAB<sup>®</sup>, Inc. and compared with in-house results. The results were consistent among the different locations, and exhibited a correlation of 100%. The samples produced the expected results 100% of the time.

For the determination of intra-assay performance, 6 positive fecal specimens and 6 negative fecal specimens were analyzed. Each specimen was assayed in replicates of eight. All positives remained positive and all negatives remained negative.

The inter-assay precision of the *SHIGA TOXIN CHEK* test was determined using 12 fecal specimens (six negative, two positive for Stx1, two positive for Stx2, and two positive for both Stx1 and Stx2). The samples were tested, twice a day over a 5-day period using 2 different kit lots. A positive and negative control was run each day. All positives remained positive and all negatives remained negative.

#### b. *Linearity/assay reportable range:*

N/A

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

N/A

#### d. *Detection limit:*

From direct fecal testing

The limit of detection (LoD) for Stx1 was determined by using highly purified Stx1, and was defined as the concentration of toxin which yielded positive results 95% of the time, and negative results 5% of the time. The LoD was determined empirically by testing dilutions of Stx1 in a negative fecal pool, in replicates of 20. Using this method, the cutoff was found to be 0.280 ng/mL.

A concentration of 0.275 ng/mL was positive 50% of the time, and a concentration of 0.260 ng/mL was negative 95% of the time.

The LoD for Stx2 was determined by using highly purified Stx2, and was defined as the concentration of toxin which yielded positive results 95% of the time, and negative results 5% of the time. The LoD was determined empirically by testing dilutions of Stx2 in a negative fecal pool, in replicates of 20. Using this method, the cutoff was found to be 0.230 ng/mL. A concentration of 0.200 ng/mL was positive 50% of the time, and a concentration of 0.150 ng/mL was negative 95% of the time.

#### From broth culture

The limit of detection (LoD) for Stx1 was determined by using highly purified Stx1, and was defined as the concentration of toxin which yielded positive results 95% of the time, and negative results 5% of the time. The LoD was determined empirically by testing dilutions of Stx1 in overnight GN broth culture of non-toxin producing *E. Coli* O157 (ATCC 043888), in replicates of 20. Using this method, the cutoff was found to be 0.180 ng/mL. A concentration of 0.120 ng/mL was positive 50% of the time, and a concentration of 0.110 ng/mL was negative 95% of the time.

The LoD for Stx2 was determined by using highly purified Stx2, and was defined as the concentration of toxin which yielded positive results 95% of the time, and negative results 5% of the time. The LoD was determined empirically by testing dilutions of Stx2 in overnight GN broth culture of non-toxin producing *E. Coli* O157 (ATCC 043888), in replicates of 20. Using this method, the cutoff was found to be 0.300 ng/mL. A concentration of 0.200 ng/mL was positive 50% of the time, and a concentration of 0.170 ng/mL was negative 95% of the time.

#### e. *Analytical specificity:*

The *SHIGA TOXIN CHEK* test was evaluated for cross-reactivity with the bacterial and viral strains listed below. None of the strains were shown to interfere with the performance of the *SHIGA TOXIN CHEK* test.

#### Crossreactivity

<i>Aeromonas hydrophila</i>	<i>Campylobacter coli</i>	<i>Campylobacter fetus</i>
<i>Campylobacter jejuni</i>	<i>Candida albicans</i>	<i>Citrobacter freundii</i>
<i>Clostridium difficile</i>	<i>Clostridium perfringens</i>	<i>Enterobacter cloacae</i>
<i>Enterococcus faecalis</i> (non-toxicogenic)	<i>Escherichia coli</i> (non-toxicogenic)	<i>Escherichia coli</i> O157:H7
<i>Escherichia coli</i> EIEC (enteroinvasive)	<i>Escherichia coli</i> EPEC (enteropathogenic)	
<i>Escherichia coli</i> ETEC (enterotoxigenic)		
<i>Escherichia fergusonii</i>	<i>Escherichia hermannii</i>	<i>Gardnerella vaginalis</i>

<i>Helicobacter pylori</i>	<i>Klebsiella pneumoniae</i>	<i>Lactobacillus acidophilus</i>
<i>Proteus vulgaris</i>	<i>Providencia stuartii</i>	<i>Pseudomonas aeruginosa</i>
<i>Pseudomonas fluorescens</i>	<i>Salmonella enteric serovar minnesota</i>	
<i>Salmonella typhimurium</i>		
<i>Serratia liquefacians</i>	<i>Shigella flexneri</i>	<i>Shigella sonnei</i>
<i>Staphylococcus aureus</i>	<i>Staphylococcus aureus</i> (Cowan)	<i>Staphylococcus epidermidis</i>
<i>Yersinia enterocolitica</i>		
<i>Human Adenovirus, Type 2, 14, 40 and 41</i>		
<i>Human Coxsackievirus A9, B1</i>	<i>Human Enterovirus 69</i>	
<i>Feline calicivirus</i>	<i>Human rotavirus</i>	

### Strains/Serotypes

Various *E. coli* Shiga toxin-producing strains and serotypes were tested in the *SHIGA TOXIN CHEK* test by both the Sorbitol MacConkey Agar (SMAC) plate and MacConkey broth culture methods. *Escherichia coli* O157 strains were also tested using CT-SMAC and ChromAgar O157 plate cultures. Each strain is a clinical isolate and each was tested by a cytotoxin assay and by a polymerase chain reaction (PCR) to confirm the presence of the Shiga toxin gene(s). All organisms generated positive results for the appropriate toxin(s) when tested.

Following is a list of the serotypes tested, the number of strains tested in that group type and the type of toxin produced by each strain.

**Shiga Toxin Type Stx1: Strain Types** - O26:H11 (5 strains), O157:H7, O111:NM (2 strains), O103:H2, O103:H25, O103:H6, O103:N, O111:H11, O111:H8, O145:H16, O145:NM, O45:H2 (4 strains), O45:NM, O125:NM, O146:H21, O156:H21, O26, O5:N, O70:H11, O111a:NM

**Shiga Toxin Type Stx2: Strain Types** - 157:H7 (6 strains), O104:H4 (European 2011 outbreak strain), O177:NM, O6:H10, O121:H19 (3 strains), O121, O145:H28, O145, O113:H21, O104:H21, O55:H7, O91:H21, O6:H10

**Shiga Toxin Type Stx1 and Stx2: Strain Types** - O157:H7 (8 strains), O157:NM (2 strains), O111:H8, O111, O111:NM (2 strains), O113:H21, O15:H27

### Interfering Substances (U.S. Formulations)

The following substances had no effect on positive or negative test results analyzed at the concentrations indicated: Hog gastric mucin (3.5% w/v), Human blood (40% v/v), Barium sulfate (5% w/v), Imodium® (5% v/v), Kaopectate® (5% v/v), Pepto-Bismol® (5% v/v), Maalox® Advanced (5% v/v), Steric Acid (40% w/v), Metronidazole (0.25% w/v), Vancomycin (0.25% w/v), Prilosec OTC® (5 µg/mL), TUMS (50 µg/mL), Tagamet® (5 µg/mL), Leukocytes (0.05% v/v), Ciprofloxacin (0.25% w/v).

f. *Assay cut-off:*

The cutoff for the *SHIGA TOXIN CHEK* test for direct fecal specimens was established at concentrations of 0.28 ng/mL Stx1 and 0.23 ng/mL Stx2, and for broth cultures at concentrations of 0.18 ng/mL Stx1 and 0.30 ng/mL Stx2.

2. Comparison studies:

a. *Method comparison with predicate device:*

N/A

b. *Matrix comparison:*

N/A

3. Clinical studies:

a. *Clinical Sensitivity:*

The performance of the *SHIGA TOXIN CHEK* test was evaluated at 3 independent sites. A summary of overall performance at the 3 sites follows.

**Direct Fecal Testing**

The performance of the *SHIGA TOXIN CHEK* test was compared to the Vero Cell Cytotoxin Assay (with neutralization) and included 899 fresh and 14 frozen specimens. The following table shows a summary of the clinical performance of the *SHIGA TOXIN CHEK* test. The results show that the *SHIGA TOXIN CHEK* test exhibited a sensitivity of 100%, a specificity of 99.9%, and an overall correlation of 99.9% with the cytotoxin assay.

***SHIGA TOXIN CHEK* Test Versus the Vero Cell Cytotoxicity Assay**

N = 913	Vero Cell Cytotoxicity Assay Positive	Vero Cell Cytotoxicity Assay Negative
<i>SHIGA TOXIN CHEK</i> Positive	78	1
<i>SHIGA TOXIN CHEK</i> Negative	0	834

		95% Confidence Limits
<b>Sensitivity</b>	100%	94.2 – 100%
<b>Specificity</b>	99.9%	99.2 – 100%
<b>Correlation</b>	99.9%	100 – 100%

### Broth Cultures

The performance of the *SHIGA TOXIN CHEK* test using overnight broth cultures (GN or MacConkey broth) from fecal specimens was compared to the Vero Cell Cytotoxin Assay. The following table shows a summary of the clinical performance of the *SHIGA TOXIN CHEK* test. The results show that the *SHIGA TOXIN CHEK* test exhibited a sensitivity of 97.1%, a specificity of 99.7%, and an overall correlation of 99.5% with the cytotoxin assay.

### *SHIGA TOXIN CHEK* Test Versus the Vero Cell Cytotoxicity Assay

N = 789	Vero Cell Cytotoxicity Assay Positive	Vero Cell Cytotoxicity Assay Negative
<i>SHIGA TOXIN CHEK</i> Positive	67	2
<i>SHIGA TOXIN CHEK</i> Negative	2	718

		95% Confidence Limits
<b>Sensitivity</b>	97.1%	89.0 – 99.5%
<b>Specificity</b>	99.7%	98.9 – 99.9%
<b>Correlation</b>	99.5%	99.5 – 99.5%

b. *Clinical specificity:*

See 3a. above.

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:

The *SHIGA TOXIN CHEK* test detects the presence of Stx1 and Stx2. Expected values for a particular population should be established by each laboratory. The positivity rate may be dependent upon a number of factors including geography, process of specimen collection, handling and transport, patient age.

Shiga toxin *E. coli* is the source of an estimated 110,000 cases (0.04% of the population) of foodborne illness annually in the United States (11). Reported incidence rates in fecal samples submitted for testing range from 0% - 4.1% and vary depending upon the season, geographical location, and patient population, with higher incidence rates seen in the summer months and in preschool-aged children and the elderly (28). A positive result in the *SHIGA TOXIN CHEK* test confirms the presence of Shiga toxin in the sample; a negative result indicates the absence of toxin or insufficient levels of toxin for detection.

**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.