

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

A. 510(k) Number:

k121364

B. Purpose for Submission:

To obtain substantial equivalence for the Shiga Toxin Quik Chek

C. Measurand:

Shiga Toxin 1 (STX1) and Shiga Toxin 2 (STX2)

D. Type of Test:

Rapid membrane enzyme immunoassay

E. Applicant

TechLab Inc.

F. Proprietary and Established Names:

SHIGA TOXIN QUIK 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 QUIK CHEK* test is a rapid membrane enzyme immunoassay for the simultaneous qualitative detection and differentiation of Shiga toxin 1 (Stx1) and Shiga toxin 2 (Stx2) in a single test device. 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 with fecal specimens, or broth or plate cultures derived from fecal specimens. The test results should be considered in conjunction with the patient history.

2. Indications for use:

The *SHIGA TOXIN QUIK CHEK* test is a rapid membrane enzyme immunoassay for the simultaneous qualitative detection and differentiation of Shiga toxin 1 (Stx1) and Shiga toxin 2 (Stx2) in a single test device. 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 with 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 the following components

25 Membrane Devices in each kit – each pouch contains 1 device

Diluent (22 mL per bottle) – Buffered protein solution with graduated dropper assembly

Wash Buffer (12 mL per bottle) – Buffered solution with graduated dropper assembly

Substrate (3.5 mL per bottle) – Solution containing tetramethylbenzidine

Conjugate (2.5 mL per bottle) – Antibodies specific for Stx1 and Stx2 coupled to

horseradish peroxidase in a buffered protein solution

Positive Control (1 mL per bottle) – Antigen in a buffered protein solution

Disposable plastic transfer pipettes – graduated at 25 µL, 400 µL and 500 µL

J. Substantial Equivalence Information:

1. Predicate device names:

ImmunoCard STAT! EHEC

Premier EHEC

ProSpecT Shiga Toxin E. coli

2. Predicate K number(s):

k062546

k953362

k980507

3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Intended Use	Qualitative detection of Shiga toxins 1 & 2	Same
Antibody format	Monoclonal/polyclonal	Same

Differences		
Item	Device	Predicate
Intended Use	Differentiation of Shiga toxins 1 & 2	No differentiation of toxins
Technology	Rapid membrane enzyme immunoassay	ImmunoCard is an immunochromatographic (lateral flow) Premier & ProSpecT are microwell plate ELISAs
Specimen types	Direct human fecal specimens Broth cultures Plate cultures	Immunocard – broth and plate cultures only Premier – same as Shiga Toxin Quik Chek

Differences		
Item	Device	Predicate
		ProspecT – Direct fecal human specimens and broth cultures
Specimen volume	25 µl but 100 µl – for transport media or broth culture	ImmunoCard & Premier - 50 µl ProspecT - 300 µl
Assay duration	30 minutes	Immunocard – 25 minutes Premier – 2 hrs 15 minutes ProspecT – 1 hr 50 minutes

K. Standard/Guidance Document Referenced:

CLSI - EP17A - “Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline”.

L. Test Principle:

The *SHIGA TOXIN QUIK CHEK* test utilizes specific antibodies against Stx1 and Stx2. The membrane device contains a reaction window with three vertical lines of immobilized antibodies. The “1” test line contains monoclonal antibodies against Stx1. The control line (“C”) is a dotted line that contains anti-horseradish peroxidase (HRP) antibodies. The “2” test line contains monoclonal antibodies against Stx2. The conjugate consists of antibodies to Stx1 and Stx2 coupled to horseradish peroxidase. To perform the test, the sample is added to a tube containing a mixture of diluent and conjugate. The diluted sample-conjugate mixture is added to the sample well and the device is allowed to incubate at room temperature for 15 minutes. During the incubation, any Stx1 and/or Stx2 present in the sample binds to the antibody-peroxidase conjugates. The toxin-antibody-peroxidase complexes migrate through a filter pad to a membrane where they are captured by the immobilized Stx1 and Stx2 specific monoclonal antibodies in the test lines. The reaction window is subsequently washed with wash buffer, followed by the addition of substrate. After a 10 minute incubation period, the reaction window is examined visually for the appearance of vertical blue lines on the “1” and “2” sides of the reaction window. A blue line on the “1” side of the reaction window is a positive result indicating the presence of Stx1. A blue line on the “2” side of the reaction window is a positive result indicating the presence of Stx2. A positive “C” reaction, indicated by a vertical dotted blue line under the “C” portion of the reaction window, confirms that the test is working properly, the procedure was followed, and the results are valid.

M. Performance Characteristics:

1. Analytical performance:

a. *Precision/Reproducibility:*

The reproducibility of the *SHIGA TOXIN QUIK CHEK* test was determined using 12 masked fecal specimens. Testing was performed at 2 independent laboratories and on-site at TECHLAB[®], 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[®], 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.

Precision – Intra-Assay

For the determination of intra-assay performance, 6 positive fecal specimens (two positive for Stx1, two positive for Stx2, two positive for both Stx1 and Stx2) and six negative fecal specimens were analyzed. Each specimen was assayed on 5 cassettes. All positives remained positive and all negatives remained negative.

Precision – Inter-Assay

The inter-assay precision of the *SHIGA TOXIN QUIK 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 on 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 cutoff point 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 cutoff point 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 0.042 ng/mL. A concentration of 0.025 ng/mL was positive 50% of the time, and a concentration of 0.022 ng/mL was negative 95% of the time.

The cutoff point 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 cutoff point 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 0.039 ng/mL. A concentration of 0.025 ng/mL was positive 50% of the time, and a concentration of 0.013 ng/mL was negative 95% of the time.

From broth cultures

The cutoff point 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 cutoff point 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 0.042 ng/mL. A concentration of 0.025 ng/mL was positive 50% of the time, and a concentration of 0.010 ng/mL was negative 95% of the time.

The cutoff point 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 cutoff point 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 0.039 ng/mL. A concentration of 0.025 ng/mL was positive 50% of the time, and a concentration of 0.013 ng/mL was negative 95% of the time.

e. *Analytical specificity:*

Cross reactivity

The *SHIGA TOXIN QUIK 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 QUIK CHEK* test.

<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-toxigenic)	<i>Escherichia coli</i> (non-toxigenic)	<i>Escherichia coli</i> O157:H7
<i>Escherichia coli</i> EIEC (enteroinvasive) (enteropathogenic)		<i>Escherichia coli</i> EPEC

<i>Escherichia coli</i> ETEC (enterotoxic)		
<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 typhimurium</i>	<i>Salmonella enteric serovar minnesota</i>	<i>Salmonella</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,</i>
<i>B1</i>	<i>Human Enterovirus 69</i>	
<i>Feline calicivirus</i>	<i>Human rotavirus</i>	

Additional strains tested for inclusivity are listed below:

Various *E. coli* Shiga toxin-producing strains and serotypes were tested in the *SHIGA TOXIN QUIK 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), O111a:NM, 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

Shiga Toxin Type Stx2: Strain Types - O26:H11, O157:H7 (4 strains), O157:NM, O8:H19 (2 strains), O8:H10, ORU:H29, O177:NM, O6:H10, O104:H4 (European 2011 outbreak strain), O121:H19 (3 strains), O121, O145:H28, O145, O113:H21, O104:H21, O55:H7, O91:H21

Shiga Toxin Type Stx1 and Stx2: Strain Types - O157:H7 (7 strains), O157:NM (2 strains), O111:H8, O111, O111:NM, O113:H21

Interference Studies

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/Palmitic Acid (40% w/v), Metronidazole (0.25% w/v), Vancomycin (0.25% w/v), Priolsec OTC® (5 µg/mL), TUMS (50 µg/mL), Tagamet® (5 µg/mL), Leukocytes (0.05% v/v), Ciprofloxacin (0.25% w/v).

Interference from High Analyte Concentrations

A study was performed to ensure that a high concentration of Stx1 toxin does not interfere with the detection of Stx2 toxin, or high concentrations of Stx2 toxin do not interfere with the detection of Stx1 toxin. Low and high concentrations were based on either 100x (high) or 3x (low) concentrations of Stx1 and Stx2 toxin. Low samples were prepared by spiking a negative fecal pool with 3x the 95% cutoff (LOD) for either toxin. High samples were prepared by spiking a negative fecal pool with 100x the 95% cutoff for either toxin. Testing was performed in triplicate according to the Package Insert instructions for direct testing. The results demonstrated that elevated levels of one analyte did not affect the detection of the other analyte.

f. Assay cut-off:

The cutoff for the *SHIGA TOXIN QUIK CHEK* test was established at concentrations of 0.04 ng/mL Stx1 and 0.04 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 QUIK 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 QUIK CHEK* (STQC) test was compared to the Vero Cell Cytotoxin Assay (with neutralization), considered the clinical reference standard (gold standard) and included 873 fresh and 14 frozen samples. Age and sex information was available for 878 patients. Of the 878 patients, 8% were ≤ 18 years and 59.8% were females and 40.2% were males. The following tables show a summary of the clinical performance of the Stx1 portion and the Stx2 portion of the *SHIGA TOXIN QUIK CHEK* test at all 3 sites. The results show that the Stx1 portion exhibited a sensitivity of 98.0%, a specificity of 99.8%, and an overall correlation of 99.7% with

cytotoxin assay. The Stx2 portion exhibited a sensitivity of 98.0%, a specificity of 100%, and an overall correlation of 99.9% with cytotoxin assay.

Direct Fecal Testing Results

n = 887	Vero Cell Cytotoxin Assay	
	Stx 1 +	Stx 1 -
STQC Stx1 +	48	2
STQC Stx1 -	1	836

n = 887	Vero Cell Cytotoxin Assay	
	Stx 2 +	Stx 2 -
STQC Stx2 +	48	0
STQC Stx2 -	1	838

		95% Confidence Interval
Sensitivity	98.0%	87.8 – 99.9%
Specificity	99.8%	99.0 – 99.9%
Correlation	99.7%	99.7 – 99.7%

		95% Confidence Interval
Sensitivity	98.0%	87.8 – 99.9%
Specificity	100%	99.4 – 99.9%
Correlation	99.9%	100 – 100%

Broth Cultures

The performance of the *SHIGA TOXIN QUIK CHEK* test using overnight broth cultures (GN or MacConkey broth) from fecal specimens was compared to the Vero Cell Cytotoxin Assay (with neutralization). The following tables show a summary of the clinical performance of the Stx1 portion and the Stx2 portion of the *SHIGA TOXIN QUIK CHEK* test. The results show that the Stx1 portion exhibited a sensitivity of 100%, a specificity of 99.5%, and an overall correlation of 99.5% with cytotoxin assay. The Stx2 portion exhibited a sensitivity of 95.7%, a specificity of 99.9%, and an overall correlation of 99.6% with cytotoxin assay.

Broth Culture Testing Results

n = 770	Vero Cell Cytotoxin Assay	
	Stx 1 +	Stx 1 -
STQC Stx1 +	42	4
STQC Stx1 -	0	724

n = 770	Vero Cell Cytotoxin Assay	
	Stx 2 +	Stx 2 -
STQC Stx2 +	45	1
STQC Stx2 -	2	722

		95% Confidence Interval
Sensitivity	100%	89.6 – 100%
Specificity	99.5%	98.5 – 99.8%
Correlation	99.5%	99.5 – 99.5%

		95% Confidence Interval
Sensitivity	95.7%	84.3 – 99.3%
Specificity	99.9%	99.1 – 100%
Correlation	99.6%	99.6 – 99.6%

b. Clinical specificity:

See 3.a 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:

N/A

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.