TECHLAB®

K121411

OCT 2 2012

SHIGA TOXIN CHEK 510(k) SUMMARY

This summary of 510(k) safety and effectiveness is being submitted in accordance with the requirements of 21 CFR 807.92.

Applicant/Contact Information:

Date Prepared:	August 30, 2012
Name:	TECHLAB®, Inc.
Address:	2001 Kraft Drive Corporate Research Center Blacksburg, VA 24060
Contact Person:	Donna T. Link
Phone Number:	540-953-1664
Email:	dlink@techlab.com
Signature:	

1.1 Manufacturing Facility Address

TECHLAB®, Inc.
2001 Kraft Drive
Blacksburg, VA 24060-6358

1.2 Product and Trade Name of the Device

SHIGA TOXIN CHEK

1.3 Common Name or Classification Name

E. coli toxins detection test

1.4 Classification and Regulation

Class I
21 CFR 866.3255; *Escherichia coli* serological reagents

1.5 Product Code(s)

GMZ – Antigens, all types, *Escherichia coli*

1.6 Panel

83 Microbiology

2001 Kraft Drive, Blacksburg, VA 24060-6358 - PH: (540) 953-1664 - FAX: (540)953-1665 - e-mail: techlab@techlab.com

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.

Explanation

Shiga toxin producing *Escherichia coli* (STEC) were first described by O' Brien, *et al.* after discovering that *E. coli* culture supernatant, which was cytotoxic to HeLa and Vero cells, could be neutralized by rabbit anti-Shiga toxin antibodies. STEC cause foodborne and waterborne diarrheal disease worldwide which, if left undiagnosed, can progress to hemorrhagic colitis and/or hemolytic uremic syndrome (HUS). Since certain treatments and medications can increase the risk of HUS, prompt detection is necessary to prevent outbreaks and secondary transmission. STEC strain O157:H7 has historically been the focus of attention in the United States since first isolated from undercooked hamburgers, causing an estimated 73,000 illnesses annually. However, STEC infections caused by non-O157 strains have become more prevalent in recent years, both in the United States as well as abroad. O157:H7 infections are routinely diagnosed by culture of fecal samples on selective media, but this methodology allows non-O157 STEC strains to go undetected. STEC produce either one or both Shiga toxins (Stx1 and/or Stx2), both potent cytotoxins. Isolates producing only Stx2 have been attributed to higher incidence rates of HUS. Shiga toxins can be detected by tissue culture assay, but this method is both time consuming and labor intensive. By detecting the toxins, the *SHIGA TOXIN CHEK* test can detect STEC present in fecal samples or culture, regardless of the serotype or other virulence factors.

Device Description

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.

Materials Provided

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 – Quantity 2
Wash Label – Quantity 1
IVD *In Vitro* Diagnostic Medical Device

Comparative Information of Predicate Devices

Kit Name	510(k) Numbers	Intended Use	Format	Target Population
Vero Cell Cytotoxin Assay (with neutralization)*	Clinical Reference Standard (gold standard)	Detection of Shiga toxins 1 and 2 from fecal specimens, broth cultures, individual colonies or colony sweeps of agar plates	Cell culture cytotoxicity and neutralization	Persons suspected of having STEC infection
Premier™ EHEC	K953362	Detection of Shiga toxins 1 and 2 from direct fecal samples, broth cultures of fecal specimens, individual colonies or colony sweeps of agar plates	Microwell ELISA	Persons suspected of having STEC infection
ImmunoCard Stat! EHEC	K062546	Detection of Shiga toxins 1 and 2 in cultures derived from clinical stool specimens	Immuno-chromatographic rapid test	Persons suspected of having STEC infection
ProSpecT Shiga Toxin *E. coli* (EHEC) Microplate ELISA	K980507	Detection of Shiga toxins (Stx1 and Stx2) in aqueous extracts of fecal specimens and broth enriched fecal cultures	Microplate ELISA	Persons suspected of having STEC infection

***Comparative device used to establish equivalency.**

Similarities				
Item	***SHIGA TOXIN CHEK***	***ImmunoCard* STAT! EHEC** **K062546**	**PREMIER EHEC** **K953362**	**ProSpecT Shiga Toxin *E. coli* (STEC)** **K980507**
Intended Use	Qualitative Detection of Shiga toxins 1 and 2	Qualitative Detection of Shiga toxins 1 and 2	Qualitative Detection of Shiga toxins 1 and 2	Qualitative Detection of Shiga toxins 1 and 2
Technology	Immunoassay	Immunoassay	Immunoassay	Immunoassay
Antibody Format	Monoclonal/Polyclonal	Monoclonal/Polyclonal	Monoclonal/Polyclonal	Monoclonal/Polyclonal

Differences				
Item	***SHIGA TOXIN CHEK***	***ImmunoCard* STAT! EHEC**	**PREMIER EHEC**	**ProSpecT Shiga Toxin *E. coli* (STEC)**
Intended Use	Non-differentiation	Differentiation	Non-differentiation	Non-differentiation
Technology	Enzyme Immunoassay – Microwell Plate ELISA	Immunochromatographic (lateral flow)	Enzyme Immunoassay – Microwell Plate ELISA	Enzyme Immunoassay – Microwell Plate ELISA
Specimen Types	Direct Human Fecal Specimens Broth Cultures Plate cultures	Broth and Plate Cultures only	Direct Human Fecal Specimens Broth cultures Plate cultures	Direct Human Fecal Specimens Broth cultures
Amount of Specimen required	50 µL – fecal or broth culture 100 µL – transport media	50 µL – fecal	50 µL – fecal	300 µL - fecal
Time to Result	60 minutes or alternate rapid 30 minutes	25 minutes **after** the 16-24 hr. enrichment procedure	2 hour 15 minutes	1 hour 50 minutes

Summary of Performance Data

Predicate Device Method Comparison
N/A

Other Method Comparison – Clinical Reference Standard (Gold Standard)
Vero Cell Cytotoxin Assay with neutralization

Clinical Performance
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
SHIGA TOXIN CHEK Positive	78	1
SHIGA TOXIN CHEK Negative	0	834

		95% Confidence Limits
Sensitivity	100%	94.2 – 100%
Specificity	99.9%	99.2 – 100%
Correlation	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
SHIGA TOXIN CHEK Positive	67	2
SHIGA TOXIN CHEK Negative	2	718

		95% Confidence Limits
Sensitivity	97.1%	89.0 – 99.5%
Specificity	99.7%	98.9 – 99.9%
Correlation	99.5%	99.5 – 99.5%

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®, 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.

Analytical Sensitivity

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.

Determination of Limitation of Detection (LOD) - cutoff points for Stx1 and Stx2 directly from fecal specimens:

The results were determined following EP17A - "Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline".

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 0.280 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 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 0.230 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.

Determination of Limitation of Detection (LOD) - cutoff points for Stx1 and Stx2 from broth cultures:

The results were determined following EP17A - "Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline".

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 0.180 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 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 0.300 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.

In conclusion, the data generated for Determination of Limitation of Detection (LOD), support Package Insert claims of analytical sensitivity 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.

Analytical Specificity (Cross Reactivity)

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 *SHIGA TOXIN CHEK* test.

Aeromonas hydrophila	*Campylobacter coli*	*Campylobacter fetus*
Campylobacter jejuni	*Candida albicans*	*Citrobacter freundii*
Clostridium difficile	*Clostridium perfringens*	*Enterobacter cloacae*
Enterococcus faecalis	*Escherichia coli* (non-toxigenic)	*Escherichia coli* O157:H7 (non-toxigenic)
Escherichia coli EIEC (enteroinvasive)	*Escherichia coli* EPEC (enteropathogenic)	*Escherichia coli* ETEC (enterotoxic)
Escherichia fergusonii	*Escherichia hermannii*	*Gardnerella vaginalis*
Helicobacter pylori	*Klebsiella pneumoniae*	*Lactobacillus acidophilus*
Proteus vulgaris	*Providencia stuartii*	*Pseudomonas aeruginosa*
Pseudomonas fluorescens	*Salmonella enteric serovar minnesota*	*Salmonella typhimurium*
Serratia liquefacians	*Shigella flexneri*	*Shigella sonnei*
Staphylococcus aureus	*Staphylococcus aureus* (Cowan)	*Staphylococcus epidermidis*
Yersinia enterocolitica		
Human Adenovirus, Type 2, 14, 40 and 41	Human Coxsackievirus A9, B1	Human Enterovirus 69
Feline calicvirus	Human rotavirus	

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

Precision – Intra-Assay

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.

Precision – Inter-Assay

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 on each day. All positives remained positive and all negatives remained negative.

Conclusion

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

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
10903 New Hampshire Avenue
Silver Spring, MD 20993

TECHLAB®, Inc.
c/o Donna T. Link
Director of QA, Regulatory & Compliance
2001 Kraft Drive
Blacksburg, VA 24060-6358

OCT 2 2012

Re: K121411
Trade/Device Name: SHIGA TOXIN CHEK
Regulation Number: 21 CFR 866.3255
Regulation Name: *Escherichia coli* serological reagents
Regulatory Class: Class I
Product Code: GMZ
Dated: September 26, 2012
Received: September 26, 2012

Dear Ms. Link:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration.

If your device is classified (see above) into class II (Special Controls), it may be subject to such additional controls. Existing major regulations affecting your device can be found in Title 21, Code of Federal Regulations (CFR), Parts 800 to 895. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Parts 801 and 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR 803); and good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820). This letter will allow you to begin marketing your device as described in your Section 510(k) premarket notification. The FDA finding of substantial equivalence of your device to a legally marketed

predicate device results in a classification for your device and thus, permits your device to proceed to the market.

If you desire specific advice for your device on our labeling regulation (21 CFR Parts 801 and 809), please contact the Office of *In Vitro* Diagnostic Device Evaluation and Safety at (301) 796-5450. Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to http://www.fda.gov/MedicalDevices/Safety/ReportaProblem/default.htm for the CDRH's Office of Surveillance and Biometrics/Division of Postmarket Surveillance.

You may obtain other general information on your responsibilities under the Act from the Division of Small Manufacturers, International and Consumer Assistance at its toll-free number (800) 638-2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/cdrh/industry/support/index.html.

Sincerely yours,

Sally A. Hojvat, M.Sc., Ph.D.
Director
Division of Microbiology Devices
Office of *In Vitro* Diagnostics and Radiological Health
Center for Devices and Radiological Health

Enclosure

2. INDICATIONS FOR USE

510(k) Number: K 121411

Device Name: *SHIGA TOXIN CHEK*

Indications 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.

FOR *IN VITRO* DIAGNOSTIC USE.

Prescription Use ___√___ (Part 21 CFR 801 Subpart D) AND/OR Over-The-Counter Use _____ (21 CFR 807 Subpart C)

(PLEASE DO NOT WRITE BELOW THIS LINE-CONTINUE ON ANOTHER PAGE IF NEEDED)

Concurrence of CDRH, Office of In Vitro Diagnostic Devices (OIVD)

Ruth Shewar

Division Sign-off
Office of In Vitro Diagnostic Device
Evaluation and Safety

510(k) K121411