AMERICAN SOCIETY FOR MICROBIOLOGY

asm2012

Poster # 114

Evaluation of the New *SHIGA TOXIN CHEK*: An ELISA for the Direct Detection of Stx1 and Stx2 in Human Fecal Specimens, Plate Cultures, and Broth Cultures

D.E. Campbell, J.T. Boone, A.S. Dandro, J.P. Vance, M. Goodykoontz, and J.F. Herbein
TECHLAB, Inc., Blacksburg, Virginia

TECHLAB®, Inc.
2001 Kraft Drive, Blacksburg, VA, 24060
540-953-1664

TECHLAB®

ABSTRACT

Shiga toxin-producing *Escherichia coli* (STEC) were first linked to disease from undercooked beef in 1982. This strain, identified as serotype O157:H7, was associated with hemolytic-uremic syndrome (HUS). In the United States O157:H7 STEC causes approximately 73,000 illnesses while non-O157 serotypes cause approximately 37,000 illnesses per year. STEC produce either one or both Shiga toxins (Stx1 and/or Stx2). STEC producing only Stx2 are more likely to cause HUS than serogroups that only produce Stx1 or both toxins. STEC-related disease and HUS development caused by non-O157 is on the rise, therefore, toxin testing is required to properly diagnose STEC infections and guide therapy. The gold standard for Shiga toxin detection is Vero cell cytotoxicity assay on broth cultures or fecals directly. This assay is labor intensive and time consuming requiring 48 hours for results. Antibiotics and anti-motility drugs can increase the production of toxin increasing the risk for the development of HUS. Early detection aids physicians in diagnosis, allowing for proper treatment and prevention of person-to-person transmission.

The *SHIGA TOXIN CHEK* is an enzyme-linked immunoassay (ELISA) that detects Stx1 and Stx2 from STEC in fecal samples directly, as well as in cultures. *SHIGA TOXIN CHEK* can detect STEC regardless of the serotype or virulence factors present. In this study, *SHIGA TOXIN CHEK* was compared to Vero cell cytotoxicity assay. The test was performed on fecal samples directly (n=913), as well as broth cultures from the same fecal samples (n=789, not all samples grew). The performance data for direct fecals and broth cultures are as follows: sensitivity of 100% and 97.1% , specificity of 99.9% and 99.7%, and correlation of 99.9% and 99.5%, respectively. Overall, the ELISA performed comparable to Vero cell cytotoxicity assay and the study emphasized the benefit of direct fecal testing as not all fecal samples grew when cultured. The test additionally detected Shiga toxin produced by 39 known serotype isolates, including the European O104:H4 outbreak strain.

MATERIALS AND METHODS

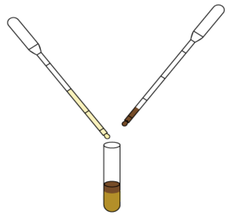
Specimens: For the study, 913 fecal samples submitted to regional reference laboratories were processed. Culturing was performed on all samples and the 789 (86.4%) displaying growth were included. 78 STEC positive samples were tested.

Vero cell cytotoxicity assay: The cytotoxicity assay was performed by adding diluted and filtered fecal or broth culture supernatant to a monolayer of Vero cells in a microtiter plate. Positive results were indicated by cell rounding and confirmed by neutralization with specific antiserum against Stx1 and/or Stx2.

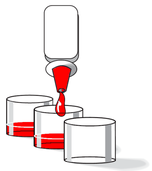
Broth Cultures: 5 mL of MacConkey broth or 8 mL of Gram Negative broth were inoculated with 25 µL of fecal sample (100 µL for samples in transport media). Inoculated tubes were incubated overnight at 37± 2°C before testing. Broth cultures with no growth were not tested. With known serotype isolates, a single colony from a SMAC plate was used for inoculation of the broth.

Davina Campbell, dcampbell@techlab.com

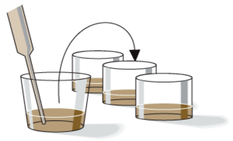
SHIGA TOXIN CHEK



STEP 1: Pipette 200 µL of diluent and 50 µL of sample into a tube. Mix the tube well.



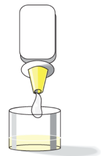
STEP 2: Add 1 drop of conjugate to each well.



STEP 3: Load 100 µL of sample/diluent mixture to wells. Incubate 50 minutes at 37°C or 20 minutes in shaking incubator at 37°C.

STEP 4: Slap out contents of plate. Wash 5X with wash buffer, slapping in between each wash.

STEP 5: Add 2 drops of substrate to each well. Tap gently. Incubate for 10 minutes at room temperature.



STEP 6: Add 1 drop of stop solution. Wait 2 minutes. Read at 450 nm or 450/620 nm with micro plate ELISA reader.

ADVANTAGES

- ❖The first one-step ELISA
- ❖Requires less time compared to competing ELISAs
- ❖Requires only 30 minutes to obtain results
- ❖Direct detection of STEC toxin
- ❖Compatible with broth, plate cultures and specimens in transport media

RESULTS

Serotype testing: Broth cultures from the following known serotype isolates were tested. The results of the *SHIGA TOXIN CHEK* correlated with the Vero cell assay.

- ❖**Stx1 serotypes:** O26:H11, O26, O157:H7, O111:NM, O103:H2, O103:H25, O103:NM, O111:H11, O111:H8, O145:H16, O145:NM, O45:H2, O45:NM, O156:H21, O5:NM, O70:H11, O146:H21, and O125:NM
- ❖**Stx2 serotypes:** O157:H7, O157:NM, O177:NM, O6:H10, O104:H4, O121:H19, O121, O145:H28, O145, O91:H21, O55:H7, and O113:H21
- ❖**Stx1 and 2 serotypes:** O157:H7, O157:NM, O111:NM, O111:H8, O15:H27, and O113:H21

Direct Fecal Results

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 Results

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%**

DISCUSSION

Early detection assists physicians in diagnosis, enabling proper treatment and reducing the risk of complications such as HUS. As STEC often occurs in outbreaks, rapid detection also reduces the risk of person-to-person transmission. The gold standard for Shiga toxin detection is Vero cell cytotoxicity assay. This assay is labor intensive and time consuming requiring 48 hours for results. The new *SHIGA TOXIN CHEK* ELISA provides highly accurate results from direct fecal samples, samples in transport media, broth cultures, or plates. Shiga toxins from O157 and non-O157 serotypes are equally detectable, and results can be obtained in as little as 30 minutes, dramatically improving turn around time for STEC results.

CONCLUSIONS

- ❖Direct testing of fecal samples is beneficial to the patient; enabling early detection and proper treatment.
- ❖Results in as little as 30 minutes and up to 24 hours earlier than other traditional methods.
- ❖The *SHIGA TOXIN CHEK* can be used with direct fecals, broth, plate cultures, and specimens in transport media.
- ❖The *SHIGA TOXIN CHEK* is comparable to Vero cell cytotoxicity assay.