Posterboard 115

# A New Rapid Immunoassay for the Direct Detection of Shiga Toxin Producing *E. coli* in Feces – Evaluation of the *SHIGA TOXIN QUIK CHEK*

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## ABSTRACT

Shiga toxin producing *Escherichia coli* (STEC) cause diarrheal disease worldwide. If left undiagnosed, the disease can progress to hemorrhagic colitis and/or hemolytic uremic syndrome (HUS). STEC produce either one or both Shiga toxins (Stx1 and/or Stx2), both potent cytotoxins, with isolates producing only Stx2 associated with higher incidence rates of HUS. Because certain treatments and medications can increase the risk of HUS, prompt detection is necessary. Early detection also helps prevent outbreaks and minimizes secondary transmission. According to the most recent estimates, 110,000 STEC cases are diagnosed annually in the United States, with approximately 2/3 of those attributed to STEC strain O157:H7. STEC outbreaks caused by non-O157 strains have become more prevalent in recent years, both in the United States as well as abroad, most notably the O104:H4 outbreak in Europe in the spring of 2011. HUS and mortality rates during the European outbreak were exceptionally high, despite the fact that the O104:H4 strain responsible lacked several key virulence factors generally present in O157:H7 strains, thought to be required for severe disease. The *SHIGA TOXIN QUIK CHEK* is a new rapid immunoassay that detects and differentiates between Stx1 and Stx2 producing STEC in direct testing of fecal samples, as well as in cultures, regardless of serotype or virulence factors present. In studies presented here, the *SHIGA TOXIN QUIK CHEK* was evaluated at 3 geographically distinct sites in the United States and compared to a Vero cell cytotoxicity assay. The test was performed on fecal samples directly (n=887), as well as broth cultures from the same fecal samples (n=770, not all samples grew). Combined performance data are as follows: sensitivity, specificity, PPV, NPV, and correlation for direct fecal testing were 98.7%, 100%, 100%, 99.9%, and 99.9%, respectively; sensitivity, specificity, PPV, NPV, and correlation for testing broth cultures from fecals were 100%, 99.7%, 97.2%, 100%, and 99.7%, respectively. Analytical sensitivity was comparable to the Vero cell assay. The test detected Shiga toxin produced by 37 known serotype isolates, including the O104:H4 European outbreak strain. The *SHIGA TOXIN QUIK CHEK* is the first commercially available rapid immunoassay to simultaneously detect and differentiate between Stx1 and Stx2 in fecal samples without requiring a culture step. The test is accurate and easy to perform, with results in less than 30 minutes.

## MATERIALS AND METHODS

**Specimens**: For this study 887 fecal samples submitted for clinical testing were used. Age and sex information was available for 878 of the patients and is as follows: over 18 (92%), 13-18 (2%), 2-12 (5%), under 2 (1%). 40% of the samples were from males, 60% from females. Sample consistencies were as follows: formed (13%), unformed (87%). Five percent of the samples contained mucous and 1% were visibly bloody.

***SHIGA TOXIN QUIK CHEK***: *Fecal samples:* 750 µL Diluent and 1 drop Conjugate were added to a tube, followed by the addition of 25 µL sample. *Broth cultures and fecal samples in transport media*: 650 µL Diluent and 1 drop Conjugate were added to a tube, followed by the addition of 100 µL sample.

The Diluent/Conjugate/sample mixture was mixed well, and 500 µL was added to the Sample Well. After a 15 minute room temperature incubation, 300 µL of Wash Solution was added to the Reaction Window, allowed to soak in, and 2 drops of Substrate added. Reactions were read 10 minutes after the addition of Substrate.

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

**Broth cultures**: 5 mL tubes of MacConkey broth or 8 mL tubes of Gram negative (GN) broth were inoculated with 25 µL of fecal sample (100 µL for samples received in transport media). Inoculated tubes were incubated overnight at 35-39ºC before testing. Broth cultures with no growth were not tested. When testing known serotype isolates, a single colony from a SMAC plate was used for inoculation of the broth.

**Analytical sensitivity comparison:** Overnight broth cultures from either Stx1 or Stx2 producing STEC were diluted 1/10 in a negative fecal matrix. These 1/10 dilutions were further serial diluted 1:1 in negative fecal matrix to a maximum dilution of 1/327,680. These dilutions were treated as fecal samples and tested by both Vero cell assay and the *SHIGA TOXIN QUIK CHEK* .

## RESULTS

**Interpretation of the *SHIGA TOXIN QUIK CHEK***: 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 vertical dotted blue line under the "C" portion of the Reaction Window confirms that the test is working properly.

**Negative result**

**Positive for Stx1**

**Positive for Stx2**

**Positive for Stx1 and Stx2**

## RESULTS

| Stx1 Performance Fecal Samples | | |
|---|---|---|
| N=887 | Vero Assay | |
| | Pos | Neg |
| **QUIK CHEK Stx1 Pos** | 48 | 2 |
| **QUIK CHEK Stx1 Neg** | 1 | 836 |
| Sensitivity | 98.0% | (95% CI 90.8-99.9%) |
| Specificity | 99.8% | (95% CI 99.3-99.9%) |
| PPV | 96.0% | (95% CI 89.0-97.9%) |
| NPV | 99.9% | (95% CI 99.5-100%) |
| Correlation | 99.7% | (95% CI 98.9-99.9%) |

| Stx1 Performance Broth Cultures | | |
|---|---|---|
| N=770 | Vero Assay | |
| | Pos | Neg |
| **QUIK CHEK Stx1 Pos** | 42 | 4 |
| **QUIK CHEK Stx1 Neg** | 0 | 724 |
| Sensitivity | 100% | (95% CI 91.9-100%) |
| Specificity | 99.5% | (95% CI 99.0-99.5%) |
| PPV | 91.3% | (95% CI 83.9-91.3%) |
| NPV | 100% | (95% CI 99.5-100%) |
| Correlation | 99.5% | (95% CI 98.6-99.5%) |

| Stx2 Performance Fecal Samples | | |
|---|---|---|
| N=887 | Vero Assay | |
| | Pos | Neg |
| **QUIK CHEK Stx2 Pos** | 48 | 0 |
| **QUIK CHEK Stx2 Neg** | 1 | 838 |
| Sensitivity | 98.0% | (95% CI 91.8-98.0%) |
| Specificity | 100% | (95% CI 99.6-100%) |
| PPV | 100% | (95% CI 93.7-100%) |
| NPV | 99.9% | (95% CI 99.5-99.9%) |
| Correlation | 99.9% | (95% CI 99.2-99.9%) |

| Stx2 Performance Broth Cultures | | |
|---|---|---|
| N=770 | Vero Assay | |
| | Pos | Neg |
| **QUIK CHEK Stx2 Pos** | 45 | 1 |
| **QUIK CHEK Stx2 Neg** | 2 | 722 |
| Sensitivity | 95.7% | (95% CI 88.3-97.8%) |
| Specificity | 99.9% | (95% CI 99.4-100%) |
| PPV | 97.8% | (95% CI 90.2-99.9%) |
| NPV | 99.7% | (95% CI 99.2-99.9%) |
| Correlation | 99.6% | (95% CI 98.7-99.9%) |

| Combined Stx1&2 Performance Fecal Samples | | |
|---|---|---|
| N=887 | Vero Assay | |
| | Pos | Neg |
| **QUIK CHEK Stx1/2 Pos** | 77 | 0 |
| **QUIK CHEK Stx1/2 Neg** | 1 | 809 |
| Sensitivity | 98.7% | (95% CI 94.8-98.7%) |
| Specificity | 100% | (95% CI 99.6-100%) |
| PPV | 100% | (95% CI 96.0-100%) |
| NPV | 99.9% | (95% CI 99.5-99.9%) |
| Correlation | 99.9% | (95% CI 99.2-99.9%) |

| Combined Stx1&2 Performance Broth Cultures | | |
|---|---|---|
| N=770 | Vero Assay | |
| | Pos | Neg |
| **QUIK CHEK Stx1/2 Pos** | 69 | 2 |
| **QUIK CHEK Stx1/2 Neg** | 0 | 699 |
| Sensitivity | 100% | (95% CI 95.3-100%) |
| Specificity | 99.7% | (95% CI 99.3-99.7%) |
| PPV | 97.2% | (95% CI 92.6-97.2%) |
| NPV | 100% | (95% CI 99.5-100%) |
| Correlation | 99.7% | (95% CI 98.9-99.7%) |

**Analytical sensitivity compared to Vero cell cytotoxicity:** The Vero cell assay detected Stx1 at a maximum dilution of 1/160 and Stx2 at a maximum dilution of 1/20,480. The *SHIGA TOXIN QUIK CHEK* detected Stx1 at a maximum dilution of 1/160 and Stx2 at a maximum dilution of 1/10,240.

**Broth culture testing:** Broth cultures from the following known serotype isolates were tested. The results of the *SHIGA TOXIN QUIK CHEK* matched those of the Vero cell assay: O26:H11 (Stx1), O26:H11 (Stx2), O157:H7 (Stx1), O157:H7 (Stx2), O157:H7 (Stx1&2), O157:NM (Stx2), O157:NM (Stx1&2), O8:H19 (Stx2), O111:NM (Stx1), O8:H10 (Stx2), ORU:H29 (Stx2), O177:NM (Stx2), O6:H10 (Stx2), O104:H4 (Stx2, European 2011 outbreak strain), O103:H2 (Stx1), O103:H25 (Stx1), O103:H6 (Stx1), O103:N (Stx1), O111:H11 (Stx1), O111:H8 (Stx1&2), O111:H8 (Stx1), O121:H19 (Stx2), O121 (Stx2), O145:H28 (Stx2), O145:H16 (Stx1), O145:NM (Stx1), O145 (Stx2), O45:H2 (Stx1), O45:NM (Stx1), O104:H21 (Stx2), O111 (Stx1&2), O111:NM (Stx1&2), O111a:NM (Stx1), O113:H21 (Stx2), O113:H21 (Stx1&2), O125:NM (Stx1), O146:H21 (Stx1), O156:H21 (Stx1), O26 (Stx1), O5:N (Stx1), O55:H7 (Stx2), O70:H11 (Stx1), O91:H21 (Stx2).

## DISCUSSION

Reported incidence rates for STEC range from 0% to 4.1% and vary depending upon the season, geographical location, and patient population. Due to the low prevalence rate of the disease, specificity is of the utmost importance. Current commercial microwell ELISAs have reported specificities ranging from 95.8-98.6% for direct fecal specimen testing. With a low incidence rate, such as the 0.8% STEC rate seen at our facility in recent years, these specificities result in positive predictive values of less than 50%. The high specificity of the *SHIGA TOXIN QUIK CHEK* ensures the accuracy of positive results with a high PPV.

The gold standard Vero cell cytotoxicity assay is too time-consuming and labor intensive for regular clinical use. Current CDC guidelines for STEC recommend simultaneously testing broth cultures from fecal samples for Shiga toxin and selective plate cultures for O157 colonies. However, the presence of samples with detectable amounts of Shiga toxin in the feces that tested negative when cultured raises the question of the efficacy of only testing cultured samples. True positives may be missed if only broth cultures are tested, and as non-O157 strains can produce toxin and cause disease, selective plating for O157 is of limited diagnostic use.

Based on its performance, our results show that the *SHIGA TOXIN QUIK CHEK* is suitable for use as a stand-alone test for the detection of STEC. As it can be used for testing fecal samples directly, it will provide both cost and time savings to laboratories currently using STEC diagnostics that require overnight culturing of fecal samples.

## CONCLUSIONS

- The *SHIGA TOXIN QUIK CHEK* is a new, rapid, easy to perform immunoassay for the independent, simultaneous detection and differentiation of Shiga toxin *E. coli* (STEC) toxins 1 and 2 (Stx1 and Stx2). Results are obtained in less than 30 minutes.
- Identification of Stx2-only producing strains allows physicians to identify patients at increased risk of developing HUS.
- The test detects both O157 and non-O157 strains of STEC, including the European O104:H4 outbreak strain.
- The test can be used to test fecal samples directly (including samples in C&S or Cary Blair transport media), as well as cultures.
- The test exhibits performance comparable to the Vero cell cytotoxicity assay.

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