Performance of the Shiga Toxin Quik Chek Immunoassay, Enzyme Immunoassay, and *E. coli* 0157 Culture in Children

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Abstract

Background:
Shiga toxin-producing Enterohemorrhagic *E. coli* (EHEC) can cause serious clinical sequelae, particularly in children. In North America, outbreaks are frequent and rapid diagnosis is beneficial. Also, EHEC is a documented laboratory-acquired infection. Rapid, direct, Shiga toxin detection methods may reduce laboratory handling of infected stool, broth, and organism growing in culture. Our objective was to evaluate the performance of the Shiga Toxin Quik Chek test when performed directly on stool and after specimen incubation in GN broth

Methods:
891 stool specimens (fresh and in Cary Blair medium) from patients ≤18 years of age were enrolled prospectively during two "high incidence" EHEC periods: July 1-Nov. 30, 2013, and May 1-Nov. 30, 2014. Duplicate specimens were removed from analysis. Shiga Toxin Quik Chek (STQC), a rapid enzyme immunoassay that detects STX1 and STX2, was performed directly on stool, and after specimen incubation on Remel GN broth after specimen incubation. The Premier EHEC enzyme immunoassay (EIA) and culture on CHROMagar 0157 were also performed after incubation in GN broth. A "true positive" (TP) was defined as positivity by 2 of 3 testing method (STQC, EIA, or culture) OR by one testing method plus positive results by an adjudicating duplex *stx1/stx2* PCR.

Results:
During the "high prevalence" testing periods, 891 specimens qualified for inclusion and 13 tested positive by at least one test. 10 were TP, giving an incidence of 1.1%. STQC exhibited a sensitivity of 80% with direct testing, and 100% sensitivity and specificity with GN broth. The Premier EIA had a sensitivity of 70% and *E. coli* 0157 culture 70%. There were also 3 false-positive EIA results.

Conclusion:
The Shiga Toxin Quik Chek test was rapid and user-friendly. Sensitivity on direct stool was comparable to that with EIA performed on broth. Performance of STQC following incubation in GN broth was excellent. While use of STQC on broth has longer TAT than with direct stool testing, compared to other methods, TAT and sensitivity would be improved. Also, specimen handling and labor may be minimized using this approach. Culture was limited by sensitivity, and also, its inability to detect non-0157 strains.

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Background

- Some strains of *E.coli* produce toxins called Shiga toxin 1 and Shiga toxin 2 (STX-1 and STX-2).
- Shiga toxin-producing *E. coli* (STEC) can cause severe disease. Hemolytic uremic syndrome (HUS) occurs mostly in young children.
- Outbreaks are frequent in the United States. The most common strain is *E.coli* 0157:H7 but many other disease-causing serogroups are documented.
- The Centers for Disease Control and Prevention recommends that *E. coli* 0157 culture and Shiga toxin testing both be performed on stool specimens.
- EIA and PCR perform best after incubation of stool in enteric broth, which delays results. The Shiga Toxin Quik Chek test is a rapid, 30-minute, cassette-based test that can be performed both directly from stool and enteric broth.

Objectives

- To evaluate the performance of the Shiga Toxin Quik Chek test (Techlab, Blacksburg, VA), the Premier EHEC enzyme immunoassay (Meridian Biosciences, Cincinnati, OH), culture on CHROMagar 0157 (Hardy Diagnostics, Santa Maria, CA) in children.
- To compare yield of the Shiga Toxin Quik Chek assay when performed directly on stool samples upon receipt versus following incubation of stool in Remel GN broth

Figure 1. Shiga Toxin Quik Chek Procedure

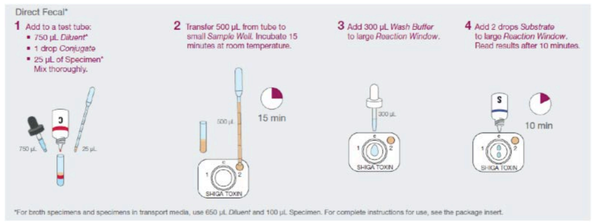

Figure 2. Shiga Toxin Quik Chek Interpretation

Methods

Specimens

- All stool samples submitted for culture to the Clinical Microbiology Laboratory at The Children's Hospital of Philadelphia were considered for inclusion (fresh and in Cary Blair medium).
- Only first stool specimens submitted to the laboratory were included. Duplicate specimens were excluded.

Specimen processing

Routine procedures

- Stool specimens were inoculated on to 5% sheep's blood, MacConkey, XLD, CIN and *Campylobacter* blood media; and GN broth (Remel, Lenexa, KS) and incubated for 18-24 hours in O_2 at 35-37°C.
- Stool specimens were also inoculated on to 0157 CHROMagar (BD Diagnostics, Sparks, MD) and examined after incubation for 18-24 hours in O_2 at 35-37°C in a darkened incubator.
- Cultures were processed according to routine procedures on the stool bench.
- The Premier EHEC enzyme immunoassay (Meridian Bioscience, Cincinnati, OH) was performed according to manufacturer's instructions on inoculated GN broth after incubation for 16-24 hours.

Shiga Toxin Quik Chek

- See Figure 1.
- STQC was performed according to manufacturer's instructions on all stool specimens at two points: directly on stool when received and on inoculated GN broth following incubation for 16-24 hours.
- Diluent was added to a test tube. A drop of conjugate was added followed by a small amount of well-mixed stool or GN broth following incubation.
- The diluted specimen was then added to the specimen well of the membrane device and incubated at room temperature for 15 minutes.
- Wash Buffer was added to the Reaction Window followed by another 10-minute room temperature incubation.
- The test was interpreted as per Figure 2.

Discordant specimens and statistical analysis

- Specimens with any positive test (0157 CHROMagar, EIA, STQC) were sent to the Pennsylvania Public Health Laboratory for Stx1 and Stx2 PCR testing.
- Specimens that tested positive by 2 of the 3 testing methods (ie. EIA, STQC, and culture) were considered "true positive". Results that were positive only by one method were adjudicated by reference *stx1/stx2* PCR.

Results

- 891 specimens were tested (see Table 1).
- 13 specimens tested positive by at least one method.
- 10 met "true positive" status (prevalence of 1.1%).
- Three specimens were positive by EIA only. All 3 were negative by *stx 1/stx 2* PCR.
- One specimen was positive by STQC (direct testing on stool) only. It was also negative by PCR.
- Sensitivity of STQC was highest following specimen incubation on broth and was superior to enzyme immunoassay and culture.

Table 1. Performance of STQC, EIA, and Culture

Test	SENSITIVITY (%)	SPECIFICITY (%)	POS. PRED. VALUE (%)	NEG. PRED. VALUE (%)
STQC (direct)	80.0	99.9	88.9	99.8
STQC (broth)	100	100	100	100
EIA	70.0	99.8	77.8	99.7
Culture	70.0	100	100	99.7

Conclusions

- The Shiga Toxin Quik Chek test was rapid, user-friendly, and appropriate for use in a clinical microbiology laboratory.
- The sensitivity of Shiga Toxin Quik Chek was superior from broth compared to direct testing on stool.
- In an outbreak setting, stool could be tested directly and specimens with negative tests could be reflexed to re-testing after broth incubation.
- Use of STQC after broth incubation would improve sensitivity over culture and EIA, and reduce labor.
- Culture was limited by poor sensitivity (70%), and its inability to detect non-0157 strains.

Acknowledgments

- We thank Lan Nguyen and Lauren Gallagher for their contributions to specimen testing.
- Shiga Toxin Quik Chek kits were provided by Alere Inc.