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Detection and Differentiation of Shiga Toxins 1 and 2 in Fecal Samples with a Rapid Immunoassay

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INTRODUCTION

Shiga toxin *Escherichia coli* (STEC) infection is a leading cause of foodborne illness related hospitalization in the United States. STEC infection is usually linked to the consumption of contaminated meat, produce, or dairy products, although person-to-person transmission is also possible. Early diagnosis of infected individuals is crucial, as the improper administration of antibiotics can exacerbate the disease. Current CDC guidelines recommend that fecal samples be tested only after overnight culture in an enrichment broth, as the level of toxin present in the feces may not be detectable, citing a publication by Cornick *et al*. to support this recommendation. However, in the Cornick study, due to HIPAA regulations, the researchers had to test fecal samples obtained several days (on average 5) after the original STEC diagnosis had been made. Because STEC levels in the feces decrease as the disease progresses, fecal samples should be obtained and tested as soon as possible after symptoms appear. Since Cornick *et al.* were unable to test the early fecal sample originally submitted for diagnosis, their results may not accurately reflect the efficacy of direct fecal testing for Shiga toxin. Here we present our results of direct testing of fecal samples for Shiga toxin without the need for a culture step using the *SHIGA TOXIN QUIK CHEK*.

METHODS

Fecal specimens (n=584) collected prospectively over an 8 month period were used for this study. Upon receipt, the Vero cell cytotoxicity neutralization assay was started and the specimens were tested with the *SHIGA TOXIN QUIK CHEK* per the package insert procedure for direct testing of fecal samples. Once a positive sample was identified by either method, GN broth, MacConkey broth, and SMAC plate cultures were started. Following an overnight (16-20 hour) 37°C incubation, broth cultures were tested for toxin by Vero cell assay, and broth and SMAC plate cultures tested with the *SHIGA TOXIN QUIK CHEK* following the package insert procedure for culture testing. O157 positive samples were identified using a combination of an O157 immunoassay and identification of clear colonies on SMAC plate cultures. Shiga toxin subtype was determined by PCR using a modification of the method described by Scheutz *et al*.

RESULTS AND DISCUSSION

The Vero cell cytotoxicity neutralization assay is considered the reference standard for detection of Shiga toxin in fecal samples because of its extreme (picogram level) analytical sensitivity. Although extremely sensitive, it is laborious and results are not available for 48-72 hours. In this study, the *SHIGA TOXIN QUIK CHEK* detected all fecal samples identified as positive by Vero cell testing. Of the 10 identified positive specimens, only 9 were detected by GN broth culture testing, 8 by MacConkey broth culture testing, and 8 by testing growth from a SMAC plate. As culturing was not performed on all samples, however, we do not know if fecal culture testing would have identified additional positive specimens missed by direct fecal testing. Samples that test positive by the direct fecal method but test negative when cultured could be explained by lack of viable STEC cells in the fecal specimen, low number of STEC cells that are outcompeted by other fecal organisms when cultured, or inhibitors present in the fecal sample such as antibiotics. Direct testing of fecal specimens with the *SHIGA TOXIN QUIK CHEK* allows for rapid, sensitive, and specific detection of Shiga toxin in fecal specimens with performance comparable to the Vero cell cytotoxicity assay, without the need for an overnight culture step.

Comparison of direct fecal and broth culture test results and further characterization of identified positive specimens

	Direct Fecal				Broth Culture[a]				SMAC Plate Culture			
	QUIK CHEK		Vero Assay		*QUIK CHEK*		Vero Assay		*QUIK CHEK*			
Sample	Stx1	Stx2	Stx1	Stx2	Stx1	Stx2	Stx1	Stx2	Stx1	Stx2	O157	Subtype
S1	+	-	+	-	-	-	tnp[b]	tnp[b]	-	-	-	Stx1a
S2	+	-	+	-	+	-	+	-	+	-	-	Stx1a
S3[c]	-	+	-	+	+	+	+	+	+	+	+/-	Stx1a/1c/2a
S4	+	+	+	+	+	+	+	+	-	-	+	Stx1a/2a
S5	+	-	+	-	+	-	+	-	+	-	-	Stx1a
S6	+	-	+	-	+	-	+	-	+	-	-	Stx1a
S7	+	-	+	-	+	-	+	-	+	-	-	Stx1a
S8	-	+	-	+	-	+	-	+	-	+	+	Stx2a
S9	+	-	+	-	+	-	+	-	+	-	-	Stx1a
S10	-	+	-	+	-	+	-	+	-	+	+	Stx1a/2a[d]

[a]Broth culture results shown are for GN, which agreed with the MacConkey results for all specimens except S4, which did not grow in MacConkey broth [b]tnp = test not performed; [c]Two STEC isolates were recovered: an O157/Stx1a/Stx2a strain and a non-O157/Stx1c strain; [d]Fecal sample was positive for both Stx1a and Stx2a, however, the isolate obtained from this specimen was Stx2a positive only

Negative

Positive Stx1/2

Positive Stx1

Positive Stx2

CONCLUSIONS

- The clinical performance of the *SHIGA TOXIN QUIK CHEK* is comparable to the Vero cell cytotoxicity assay
- Direct fecal testing (without a broth culture step) identified true positive specimens missed by traditional culture methods:
  - Ten positive samples were identified by direct fecal testing
  - Nine positive samples were identified by GN broth culture
  - Eight positive samples were identified by MacConkey broth culture
  - Eight positive samples were identified by SMAC plate culture
- Stx1a and Stx2a were the most prevalent Shiga toxin subtypes isolated from clinical specimens
- Shiga toxins produced by both O157 and non-O157 strains were detected
- Testing stool samples directly for Shiga toxin provides physicians with results a day sooner than methods requiring an overnight broth culture step

Shiga Toxin 1 (2)

n = 584	Vero +	Vero -
QUIK CHEK +	7 (4)[a]	0 (0)
QUIK CHEK -	0 (0)	577 (580)
Sensitivity:	100.0%	
Specificity:	100.0%	
PPV:	100.0%	
NPV:	100.0%	
Correlation:	100.0%	

[a]Ten positive samples were identified, one of which was positive for both Stx1 and Stx2, resulting in 11 positive results

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