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Evaluation of the Great Basin Shiga Toxin Direct Molecular Assay for the Detection of Shiga-like Toxin Producing *Escherichia coli* (STEC) in Diarrheal Stool Specimens

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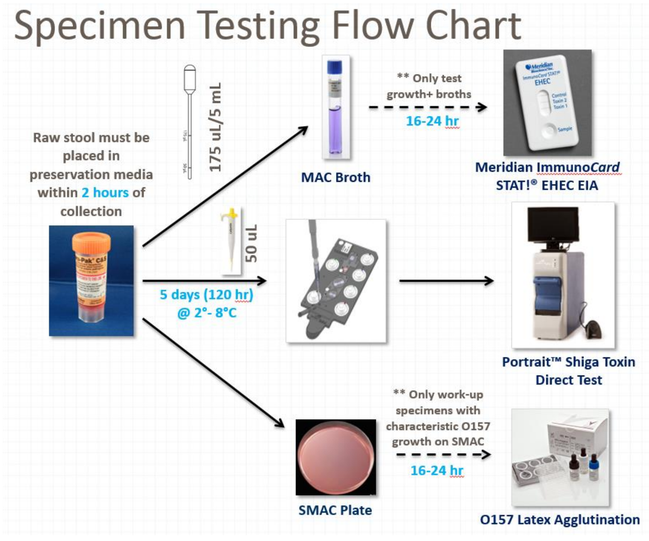
Introduction

E. coli carrying the Shiga-like toxin genes *stx1* and *stx2* (STEC) cause acute gastroenteritis (AGE) and has the potential to cause HUS. Serotype O157:H7 is most commonly associated with STEC, however, over 100 other *E. coli* serotypes may carry the Shiga Toxin genes. Detection of STEC using EIAs or selective growth medium requires a minimum of 24 h and is 29-80% sensitive compared to NAAT. The Shiga Toxin Direct Test (ST Direct) is a 2 h sample to result test that uses nucleic acid amplification to identify *stx1*, *stx2*, and *E. coli* serotype O:157 in preserved stool specimens.

Methods and Instrumentation

Stool specimens (n=1,082) were prospectively collected at 5 clinical centers and tested in parallel using ST Direct and culture. Each specimen was inoculated to SMAC agar and MacConkey broth and incubated 18-24 h. Colorless colonies on SMAC agar were tested with latex agglutination to confirm serotype O:157. Positive broths were tested with a STEC EIA. A panel of retrospectively collected specimens containing 55 STEC positive (23 O:157 positive) and 33 STEC negative stools was used to augment results from the prospective study set.



The Portrait Shiga Toxin Direct Test (Great Basin, Salt Lake City, UT) is a sample to result, multiplexed nucleic acid amplification assay that uses chip-based detection for shiga toxin encoding *stx1, stx2*, and O157 antigen encoding *rfbE* DNA targets present in STEC. If either stx1 or stx2 is detected, a result of "STEC POSITIVE" is reported along with the O157 result. If stx is not detected, the O157 result is not reported. Each test card contains all reagents for nucleic acid extraction, targets amplification, and detection as well as an internal process control to monitor for inhibition. The test is complete in <2 h.

Tables 1 and 2. Fresh prospective specimens. Shiga Toxin Direct Test compared to *stx1/2* EIA, culture, and latex typing (gold standard).

Shiga Toxin						
Site	TP	FP	TN	FN	Sensitivity (95% C.I.)	Specificity (95% C.I.)
A	1	2	296	0	100% (3-100)	99.3% (98-100)
B	1	3	300	0	100% (3-100)	99.8% (97-100)
C	0	1	173	0	ND	99.4% (97-100)
D	0	0	35	0	ND	100% (90-100)
E	2	2	266	0	100% (16-100)	99.3% (97-100)
Total	**4**	**8[a]**	**1,070**	**0**	**100% (40-100)**	**99.8% (99-100)**

O157						
Site	TP	FP	TN	FN	Sensitivity (95% C.I.)	Specificity (95% C.I.)
A	0	0	299	0	ND	100% (99-100)
B	0	1	303	0	ND	99.7% (98-100)
C	0	1	173	0	ND	99.4% (97-100)
D	0	0	35	0	ND	100% (90-100)
E	0	0	270	0	ND	100% (99-100)
Total	**0**	**2[b]**	**1,080**	**0**	**ND**	**99.8% (99-100)**

[a]Preserved stool specimens and enrichment broths subjected to bi-directional sequence analysis (LoD ~ 10^4 CFU/mL). Sequence for *stx1*, *stx2*, or both was confirmed in all 8 samples
[b]Both specimens were reported as positive for *E. coli* O157 using an alternative molecular IVD assay.

Table 3. Frozen retrospective specimens[a]. Shiga Toxin Direct Test.

Target	TP	FP	TN	FN	PPA (95% C.I.)	NPA (95% C.I.)
stx1/2	51	0	33	4[b]	92.7% (82-98)	100% (89-100)
O157	22	0	24	1	95.7% (78-100)	100% (86-100)

[a]The retrospective panel consists of 55 STEC positive (23 O:157 positive) and 33 STEC negative stools. Historical positive results for both Shiga toxin and O:157 were confirmed by an alternative molecular IVD assay for each specimen used in the retrospective panel
[b]All 4 FN were positive by alternative molecular IVD but negative by bi-directional sequence analysis

Table 4 . Comparison of Shiga Toxin Direct Test to molecular IVD assay

stx1/2		IVD Assay		
		Pos	Neg	Total
ST Direct	Pos	12	0	12
	Neg	0	0	0
	Total	12	0	12

O157		IVD Assay		
		Pos	Neg	Total
ST Direct	Pos	2	0	2
	Neg	0	10	10
	Total	2	10	12

All specimens testing positive by the Shiga Toxin Direct Test were also tested by an alternative molecular IVD assay. This demonstrated 100% PPA Shiga Toxin Direct.

Table 5. Shiga Toxin Direct Test invalid rate

Site	INV/Total Run	Initial INV Rate	INV after Single Repeat	Final INV Rate
A	7/306	2.3%	1/306	0.3%
B	5/315	1.6%	1/315	0.3%
C	9/184	4.9%	0/184	0.0%
D	0/35	0.0%	N/A	0.0%
E	2/303	0.3%	0/303	0.0%
Total	**23/1,143**	**2.0%**	**2/1,143**	**0.2%**

Conclusion

- In this study the ST Direct assay is 100% sensitive for detection of STEC, including serotype O:157, compared to culture and EIA test.
- ST Direct identified an additional 8 specimens, including 2 serotype O:157, that were missed by culture.
- Compared to ST Direct, EIA is only 33% sensitive for detection of STEC and culture is insensitive for the detection of O:157
- ST Direct provides a rapid and sensitive alternative to culture and EIA for the detection of STEC in preserved stool specimens