SHIGA TOXIN CHEK and SHIGA TOXIN QUIK CHEK
Detect *Escherichia coli* Subtypes Associated with Human Disease

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PURPOSE

This study was conducted to evaluate the new *SHIGA TOXIN CHEK* and *SHIGA TOXIN QUIK CHEK* tests for their ability to detect Stx1 and Stx2 subtypes associated with human disease.

BACKGROUND

Shiga toxin-producing *Escherichia coli* (STEC) were first isolated from undercooked beef in 1982. STEC infections may lead to diarrhea and hemolytic uremic syndrome (HUS) through the production of Shiga toxin 1 (Stx1) and/or Shiga toxin 2 (Stx2). Subtypes of Stx1 and Stx2 toxins have been identified that are associated with human disease. Stx1a, Stx1c, Stx2a, Stx2c, and Stx2d have been detected in patients with clinical symptoms that include diarrhea, hemorrhagic colitis (HC) and hemolytic uremic syndrome (HUS). Stx1c is associated with less severe disease, unlike Stx1a that has been isolated from patients with HUS. Stx2c has been associated with O157:H7 serotype and Stx2d has been associated with non-O157:H7 in patients with clinical symptoms.

MATERIALS AND METHODS

Vero cell cytotoxicity assay: The cytotoxicity assay was performed by adding diluted and filtered 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.

Sorbitol MacConkey Agar (SMAC) plates: Plates were streaked from a chopped meat broth to obtain isolated colonies. The plates were stored at 37 ± 2°C for 16-24 hours.

Broth Cultures: 5 mL of Gram Negative broth were inoculated with an isolated colony from SMAC plates. Inoculated tubes were incubated overnight at 37± 2°C for 16 -24 hours before testing. Broth cultures with no growth were not tested.

Reference Subtype Standards: Scheutz F, Teel LD, Beutin L, Piérard D, Buvens G, Karch H, Mellmann A, Caprioli A, Tozzoli R, Morabito S, Strockbine NA, Melton-Celsa AR, Sanchez M, Persson S, O'Brien AD. J Clin Microbiol. 2012 Sep;50(9):2951-63.

MSU Strains: Purchased from the STEC Center that is based at Michigan State University (MSU) in the Department of Microbiology and Molecular Genetics.

Extraction of DNA: DNA extraction was performed on the BioMerieux Nuclisens® EASYMAG®.

Real-Time PCR (qPCR): Real-Time PCR was conducted on the CFX96™ Real-Time System C100 Thermal Cycler.

DISCUSSION

•Stx1a, Stx1c, Stx2a, Stx2c, and Stx2d have been shown to cause disease in humans and were detected by the *SHIGA TOXIN CHEK* and *SHIGA TOXIN QUIK CHEK* assays.

•Isolates were confirmed by Vero cells and correlated with the *SHIGA TOXIN CHEK* and *SHIGA TOXIN QUIK CHEK*.

Isolates Tested N = 55	*SHIGA TOXIN CHEK* (ELISA)	*SHIGA TOXIN QUIK CHEK* (Rapid)	Real-Time PCR	Vero Cell Cytotoxicity
N = 30 Stx1 Isolates	All Isolates were Positive	All Isolates were Stx1 Positive	29 Isolates were Stx1a 1 Isolate was Stx1c	All Isolates were Stx1 Positive
N = 10 Stx1/ Stx2 Isolates	All Isolates were Positive	All Isolates were Stx1/Stx2 Positive	All Isolates were Stx1a/ Stx2a Positive	All Isolates were Stx1/ Stx2 Positive
N = 15 Stx2 Isolates	All Isolates were Positive	All Isolates were Stx2 Positive	10 Isolates were Stx2a 1 Isolate was Stx2b 2 Isolate were Stx2c 2 Isolate were Stx2d	All Isolates were Stx2 Positive

Subtype	Isolates Tested	*SHIGA TOXIN CHEK* (ELISA)	*SHIGA TOXIN QUIK CHEK* (Rapid)	Vero Cell Cytotoxicity Testing
Stx1a	29	✓	✓	Stx1 Positive
Stx1c	1	✓	✓	Stx1 Positive
Stx1a/Stx2a	10	✓	✓	Stx1/Stx2 Positive
Stx2a	10	✓	✓	Stx2 Positive
Stx2b	1	✓	✓	Stx2 Positive
Stx2c	2	✓	✓	Stx2 Positive
Stx2d	2	✓	✓	Stx2 Positive

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Gene	Primer	Sequence (5' to 3')
Stx1a	Forward	CGCGAGTTGCCAGAATGGCATCTG
	Reverse	CATTTTACCCCCTCAACTGC
Stx1c*	Forward	CCTTTCCTGGTACAACTGCGGTT
	Reverse	CAAGTGTTGTACGAAATCCCCTCTGA
Stx1d*	Forward	CAGTTAATGCGATTGCTAAGGAGTTT
	Reverse	CTCTTCCTCTGGTTCTAACCCCATGATA

*Scheutz, F. et al. Multicenter Evaluation of a Sequence-Based Protocol for Subtyping Shiga Toxins and Standardizing Stx Nomenclature. (2012). *Journal of Clinical Microbiology*, *50*(9), 2951-2963. doi: 10.1128/JCM.00860-12

Gene	Primer Name	Sequence (5' to 3')
Stx2a*	Forward	GCGATACTGRGBACTGTGGCC
	Reverse	GCCACCTTCACTGTGAATGTG
Stx2b*	Forward	AAATATGAAGAAGATATTTGTAGCGGC
	Reverse	CAGCAAATCCTGAACCTGACG
Stx2c*	Forward	GAAAGTCACAGTTTTTATATACAACGGGTA
	Reverse	CCGGCCACYTTTACTGTGAATGTA
Stx2d*	Forward	AAARTCACAGTCTTTATATACAACGGGTG
	Reverse	TTYCCGGCCACTTTTACTGTG
Stx2e	Forward	CGGAGTATCGGGGAGAGGC
	Reverse	TCATTCACCAGTTGTATATAAAGG
Stx2f	Forward	TGACGGCTCAGGATGTTGAC
	Reverse	GCAACACTTCCGAGAATCGC
Stx2g*	Forward	CACCGGGTAGTTATATTTCTGTGGATATC
	Reverse	GATGGCAATTCAGAATAACCGCT

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CONCLUSIONS

❖The *SHIGA TOXIN CHEK* and *SHIGA TOXIN QUIK CHEK* can detect subtypes of Stx1 and Stx2 associated with human disease.

❖The *SHIGA TOXIN CHEK* and *SHIGA TOXIN QUIK CHEK* are comparable to Vero cell cytotoxicity assay.

❖The *SHIGA TOXIN CHEK* and *SHIGA TOXIN QUIK CHEK* allow for rapid identification of STEC patients.