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Evaluation of the SHIGA TOXIN QUIK CHEK after overnight enrichment as screening tool for Shiga toxin–producing *Escherichia coli* detection in human fecal samples

Sergio Sánchez *[,1], María Teresa Llorente, Raquel Ramiro, Laura Herrera-León, Silvia Herrera-León [1]

Reference and Research Laboratory of Food and Waterborne Bacterial Infections, National Center for Microbiology, Institute of Health Carlos III, Majadahonda, Madrid, Spain

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ABSTRACT

We evaluated the SHIGA TOXIN QUIK CHEK (STQC) on its suitability for Shiga toxin–producing *Escherichia coli* (STEC) testing on human fecal samples after overnight enrichment. Our in-house PCR-based protocol for STEC detection was used as the standard for comparison. STQC detected all described Shiga toxin subtypes with the only exception of Stx2f. In comparison to PCR, STQC performed with an overall sensitivity of 55.4%, specificity of 100.0%, positive predictive value of 100.0%, negative predictive value of 73.0%, infinite positive likelihood ratio, and negative likelihood ratio of 0.45. We conclude that STQC may not be considered a suitable screening tool for STEC detection in human fecal samples, although it could be useful for laboratories where PCR is not a routine tool for STEC screening yet, subject to the confirmation of negative samples by a reference laboratory with full diagnostic capabilities.

1. Introduction

Shiga toxin–producing *Escherichia coli* (STEC) are important foodborne zoonotic pathogens responsible for a broad spectrum of clinical symptoms in humans, ranging from mild diarrhea to hemorrhagic colitis and the life-threatening hemolytic uremic syndrome (HUS) (Nataro and Kaper, 1998). The main pathogenic mechanism of STEC is production of Shiga toxins (Stx), which induce cytotoxic effects on the microvascular endothelial cells. STEC produces 2 major antigenically distinct toxin types, Stx1 and Stx2, with different immunogenic and genetic properties, as well as different subtypes (Stx1a, Stx1c, Stx1d, Stx2a, Stx2b, Stx2c, Stx2d, Stx2e, Stx2f, and Stx2g) (Scheutz et al., 2012). Although serotype O157:H7 has been implicated in most outbreaks and in most cases of HUS, there is growing concern about the risk to human health associated with non-O157 STEC serotypes (Johnson et al., 2006), which may be as well responsible for important outbreaks (Bielaszewska et al., 2011), and therefore, detection of all STEC serotypes is a requirement of testing. However, routine testing of human fecal samples for STEC in clinical microbiology laboratories requires a practical, rapid, and sensitive method. In that respect, different enzyme immunoassays for Stx detection are commercially available, although most of them require an additional overnight enrichment step delaying the turnaround time, apart from failing to detect some or many Stx subtypes (Feng et al., 2011). In particular, SHIGA TOXIN QUIK CHEK (STQC) (Techlab®, Blacksburg, VA, USA) is a rapid membrane enzyme immunoassay which offers simultaneous and qualitative detection and differentiation of Stx1 and Stx2 and claims to be suitable for use with direct fecal samples. In addition, Boone et al. (Boone et al., 2016) have evaluated the performance of STQC in detection of Stx subtypes and concluded that this assay is able to detect all described Stx variants. However, the low sensitivity shown by STQC directly on stools in recent studies (De Rauw et al., 2016; Staples et al., 2017) does not recommend direct fecal testing but only testing after enrichment of the sample. In this study, we evaluated STQC on its ability to detect all known Stx subtypes and its suitability for testing on fecal samples after overnight enrichment, using our in-house PCR-based protocol for STEC detection as the standard, in order to assess its usefulness as screening tool for STEC detection in human fecal samples.

2. Material and methods

2.1. Detection of Shiga toxin subtypes

A selection of 25 *E. coli* reference strains and well-characterized clinical isolates representing all known Stx1 and Stx2 subtypes was used to evaluate the ability of STQC to detect the different Stx variants (Table 1). Reference strains were obtained from the Statens Serum Institut (Copenhagen, Denmark) through participation in the External Quality Assessment Program. With the exceptions of Stx1d, Stx2e, and Stx2g, at least 2 strains representing each unique subtype were tested. Bacteria were routinely maintained on in-house prepared 10% skim milk medium (Oxoid, Basingstoke, UK) and stored at −20 °C. Strains were

* Corresponding author. Tel.: +34-918223710; fax: +34-915097966.
E-mail address: sergio.sanchez@isciii.es (S. Sánchez).
[1] These authors contributed equally to this article.

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Table 1
E. coli reference strains and clinical isolates used to evaluate the ability of STQC to detect the different Stx variants.

Strain (source)	Serotype	*stx* subtype	STQC results		
			Control	Stx1	Stx2
960/14 (clinical isolate)	O91:H21	*stx1a, stx2d*	++	++	++
1505/14 (clinical isolate)	O73:H18	*stx2d*	++	−	+
2708/14 (clinical isolate)	O111:H8	*stx1a, stx2a*	++	++	++
908/14 (clinical isolate)	O157:H7	*stx1a, stx2c*	++	++	++
2238/14 (clinical isolate)	O157:H7	*stx1a, stx2a, stx2c*	++	++	++
2999/14 (clinical isolate)	O157:H7	*stx2a*	++	−	++
3210/14 (clinical isolate)	O157:H7	*stx2a, stx2c*	++	−	++
2368/11 (clinical isolate)	O104:H4	*stx2a*	++	−	++
1318/12 (clinical isolate)	O26:H11	*stx1a*	++	++	−
1497/12 (clinical isolate)	O145:H34	*stx2f*	++	−	−
2446/13 (clinical isolate)	O26:H11	*stx1a, stx2a*	++	++	++
3270/13 (clinical isolate)	O73:H18	*stx2d*	++	−	+
984/14 (clinical isolate)	O113:H4	*stx1c, stx2b*	++	++	++
1658/14 (clinical isolate)	O91:H14	*stx1a, stx2b*	++	++	++
3177/14 (clinical isolate)	O5:H-	*stx1a*	++	++	−
EDL933 (SSI)	O157:H7	*stx1a, stx2a*	++	++	++
F35790 (SSI)	O157:H7	*stx2c*	++	−	++
1112R15035 (SSI)	O166:H15	*stx2d*	++	−	+
C548-06 (SSI)	O145:H34	*stx2f*	++	−	−
DG131/3 (SSI)	O174:H8	*stx1c, stx2b*	++	++	−
MHI813 (SSI)	O8:Hrough	*stx1d*	++	++	−
EH250 (SSI)	O118:H12	*stx2b*	++	−	+
S1191 (SSI)[a]	O139:H1	*stx2e*	++	−	+
T4/97 (SSI)	O128ac:H2	*stx2f*	++	−	−
7v (SSI)	O2:H25	*stx2g*	++	−	+

STQC results are given semiquantitatively (−, no test lines visible, negative result; +, faint test line, less intense positive result; ++, dark test line, highly positive result). SSI, Statens Serum Institut, Copenhagen, Denmark.
[a] *stx2e*-positive strain nonexpressing a mucoid capsule.

thawed and plated on in-house prepared MacConkey (MAC) agar (Becton Dickinson, Franklin Lakes, NJ) and overnight incubated at 37 °C. An isolated colony from each culture was inoculated in 5 mL of in-house prepared buffered peptone water (BPW, Oxoid). Following overnight incubation at 37 °C, the BPW culture was tested with STQC as detailed in the 'Broth method' section of the manufacturer's instructions.

2.2. Screening of human fecal samples and characterization of STEC isolates

The fecal samples used in this study were fresh bulk stools originating from patients with gastrointestinal symptoms and/or HUS referred to the Reference and Research Laboratory of Food and Waterborne Bacterial Infections (Majadahonda, Spain) for routine STEC diagnosis from March 2015 to October 2016. Upon receipt, a stool impregnated cotton swab was inoculated in 5 mL of BPW and overnight incubated at 37 °C. No specific quantification was performed. After this nonselective enrichment step, the BPW culture was subcultured on MAC and overnight incubated at 37 °C. Our in-house PCR was performed starting from the bacterial growth on the MAC plate. Briefly, a loopful of bacterial growth taken from the first streaking area of the culture plate was suspended in 0.5 mL of sterile distilled water, boiled for 5 min to release the DNA, and centrifuged at 10,000 rpm for 5 min. The supernatant was used directly as a template in 2 conventional PCR assays using DreamTaq DNA Polymerase (Thermo Fisher Scientific, Waltham, MA), according to the manufacturer's instructions, and primers for the amplification of *stx1* (Pollard et al., 1990) and *stx2* genes (Olsen et al., 1995) (multiplex PCR assay), and *stx2f* gene (Scheutz et al., 2012) (single PCR assay). Thermal cycler conditions consisted of 25 cycles of denaturation at 94 °C for 30 s, annealing at 56 °C for 40 s, and extension at 72 °C for 1 min.

When culture tested positive, 10 individual *E. coli*–like colonies obtained from MAC plates were tested using the same PCR to obtain the STEC isolate, which was further confirmed biochemically as *E. coli* by the API 20E system (bioMérieux, Marcy l'Etoile, France). If no single colony was found to be positive among the first 10 colonies, at least 10 more were tested. If still none of the assayed colonies was positive in the PCR procedure, the sample was reported as PCR-positive without STEC isolation. For serotyping of STEC isolates, O antigen was identified by PCR (Iguchi et al., 2015), and H antigen was identified by PCR amplification of the *fliC* gene (Machado et al., 2000) and further sequencing of the PCR product. The identification of *stx1* and *stx2* subtypes was performed using a PCR-based method (Scheutz et al., 2012).

In order to limit costs of material and labor, we selected stools for STQC testing based on PCR results instead of performing blinded STQC testing for all specimens referred to our laboratory for routine STEC diagnosis. Immediately upon detection of an *stx*-positive culture, the positive stool and some *stx*-negative samples tested in the same PCR run were processed for STQC testing. Again, a stool impregnated cotton swab was inoculated in 5 mL of BPW and overnight incubated at 37 °C, and STQC analysis was performed as detailed in the 'Broth method' section of the manufacturer's instructions. In the meantime (no more than 48 h), stools were stored at 4 °C according to the manufacturer's instructions for collection, handling, and storage of fecal specimens. Although storage of fecal samples prior to STQC testing could be expected to influence the results, refrigeration at 4 °C has been recently associated with no significant alteration in fecal microbiota diversity or composition (Choo et al., 2015), and storage duration has not been found to affect the STEC isolation rate (van Duynhoven et al., 2008). A total of 163 fecal samples were screened with STQC, including all the *stx*-positive samples detected over the study period ($n = 74$) and a selection of *stx*-negative samples ($n = 89$).

When STQC failed to detect Stx in a *stx*-positive sample, the resulting STEC isolate (if obtained) was subsequently tested with STQC as described above in order to assess whether the discordant result between toxin detection and PCR could be attributed to the presence of an undetectable strain in the sample. Additionally, when STQC failed to detect Stx2e in an *stx2e*-positive isolate, it was plated on MAC to examine its colony appearance. If it was cultured as a mucoid colony type, we considered that it was expressing a mucoid capsule responsible for the negative result, as previously reported (Staples et al., 2017).

2.3. Statistical analysis

Sensitivity, specificity, positive (PPV) and negative (NPV) predictive values, and positive (LR+) and negative (LR−) likelihood ratios were calculated and presented along with its 95% confidence interval. The Fisher exact test was used to assess the significance of the differences in sensitivity between our results and those described in previous studies. All statistics were calculated using the website tool www.openepi.com, and statistical significance was defined by P values ≤0.05, with a confidence level of 95%.

3. Results

In the subtype study, the PCR and STQC results agreed for all Stx1 subtypes, but some differences were seen among the Stx2 subtypes. Variants Stx2a, Stx2c, Stx2d, Stx2e, and Stx2g were detected by both PCR and STQC, although a less intense STQC positive result (an obvious but faint test line) was observed when testing Stx2d, Stx2e, and Stx2g (Table 1). Detection of Stx2f with STQC was not possible regardless of the *stx2f*-positive strain tested. As for Stx2b, with 4 *stx2b*-positive strains tested, a less intense STQC positive result was observed in strain EH250, and detection was not possible in strain DG131/3 (Table 1). These less-intense reactions with STQC were considered positive results according to the manufacturer's instructions for interpretation of results. Strains 2238/14 and 3210/14 carried both *stx2a* and *stx2c* genes, and whether either one or both toxins were expressed was not determined.

Regarding the screening of human fecal samples, results are summarized in Table 2. Overall, 33 out of the 74 *stx*-positive samples tested negative with STQC, resulting in a sensitivity of 55.4% [95% CI: 44.1–

Table 2
Summary of STQC testing results in comparison to the in-house PCR-based protocol for STEC detection.

	PCR	
n = 163	*stx*+ (*n* = 74)	*stx*− (*n* = 89)
No. of STQC+ samples	41	0
No. of STQC− samples	33	89
Sensitivity[a]	55.4% [95% CI: 44.1–66.2]	
Specificity	100.0% [95% CI: 95.9–100.0]	
PPV	100.0% [95% CI: 91.4–100.0]	
NPV	73.0% [95% CI: 64.5–80.0]	
LR+	∞	
LR−	0.45 [95% CI: 0.42–0.47]	

PPV, positive predictive value; NPV, negative predictive value; LR+, positive likelihood ratio; LR−, negative likelihood ratio.
[a] Sensitivity rose to 64.1% [95% CI: 51.8–75.7] when excluding samples carrying undetectable strains (*n* = 10).

66.2]. The specificity and PPV were 100.0% [95% CI: 95.9–100.0] and 100.0% [95% CI: 91.4–100.0], respectively, as all 89 *stx*-negative samples tested negative with STQC and therefore no false-positive results were obtained, and NPV was 73.0% [95% CI: 64.5–80.0], indicating a noteworthy proportion of false-negative results. The assay performed with an infinite LR+ and an LR– of 0.45 [95% CI: 0.42–0.47].

STEC was isolated from 68 out of the 74 *stx*-positive samples, 40 out of the 41 STQC-positive samples, and 28 out of the 33 STQC-negative ones, although the 6 samples without isolation remained PCR-positive after culture. Isolates belonged to 22 different serotypes and carried 8 different *stx* subtypes (*stx1a*, *stx1c*, *stx2a*, *stx2b*, *stx2c*, *stx2d*, *stx2e*, and *stx2f*) (Table 3). Nine out of the 28 isolates that were subsequently tested with STQC were found negative (Table 3). Concretely, 6 of them were *stx2f*-positive strains and therefore undetectable with STQC, according to our subtype study, two of them were *stx2e*-positive strains expressing a mucoid capsule, and another 1 probably carried a nonfunctional *stx2* gene, as suggested by the presence of an insertion sequence element in the *stx2* gene revealed when sequencing its unusually large PCR product (data not shown). The remaining 19 isolates were found positive when subsequently tested with STQC (Table 3), and therefore, the discordant result between STQC and PCR obtained in these samples could not be attributed to the presence of an undetectable strain in the sample. When excluding samples carrying such undetectable strains, i.e., *stx2f*-positive samples (*n* = 7), samples carrying *stx2e*-positive strains expressing a mucoid capsule (*n* = 2), and samples carrying strains with nonfunctional *stx* genes (*n* = 1), the sensitivity of the assay rose to 64.1% [95% CI: 51.8–75.7].

4. Discussion

In our study, STQC detected all described Stx subtypes, with the only exception of Stx2f, as reported in recent studies by De Rauw et al. (2016) and Staples et al. (2017). In contrast, Boone et al. (2016) were able to detect Stx2f with STQC and additionally verified this toxin production by Vero cell assay. This discrepancy could be due to the fact that Stx production may vary depending upon factors like enrichment broth type, inducing agent, and culture conditions (Beutin et al., 2007). Unfortunately, our study and the previous ones used a PCR assay as the reference method instead of a Vero cell cytotoxicity assay, and therefore, Stx production among the *stx2f*-positive strains was not confirmed, which is a significant limitation to our study. Remarkably, Boone et al. (2016) detected Stx2f from Gram-negative (GN) broth cultures in contrast to our study and previous ones (Chui et al., 2015; De Rauw et al., 2016) using BPW, modified tryptone soya broth (mTSB), and MAC broth cultures, respectively. Along these lines, although STQC was able to detect Stx2b in our study, detection was not possible in the *stx2b*-positive strain DG131/3 from a BPW culture. Boone et al. (2016) also failed to detect Stx2b in strain DG131/3 by both STQC and the Vero cell assay from a GN broth culture even after using mitomycin C or ciprofloxacin as induction agents, but they were able to detect it from a MAC broth culture, as reported by De Rauw et al. (2016) from an mTSB culture. The ability of STQC to detect Stx2a, Stx2c, and Stx2d is particularly remarkable, as they are generally considered the most clinically relevant Stx subtypes, implicated in most outbreaks and in most cases of HUS (EFSA Panel of Biological Hazards (BIOHAZ), 2013). However, its inability to detect Stx2f could be concerning, as recent studies have shown that the occurrence of this subtype in human infections is more frequent than previously thought (Friesema et al., 2014), and even a few cases of HUS have been associated with *st2f*-positive STEC (Grande et al., 2016). Regarding the failure of STQC to detect Stx2e from isolates expressing a mucoid capsule, it could be considered of less importance from a clinical testing standpoint, as Stx2e producing STEC strains are probably not human pathogens (Scheutz et al., 2012).

Despite the good expectations derived from the subtype study, in contrast to the vast majority of rapid Stx detection assays on the market (Feng et al., 2011), the overall sensitivity of STQC after overnight enrichment observed in our study was 55.4%. This sensitivity is slightly lower than that reported after overnight enrichment by De Rauw et al. (2016) but significantly lower than that previously reported by Chui et al. (2015) (55.4% vs. 85.0%; $P = 0.02561$). These differences in sensitivity might not be explained by differences in clinical presentation of the STEC-positive patients, as only 3 out of the 33 samples showing discordant results between STQC and PCR in our study originated from patients suffering from HUS (Table 3), which is typically diagnosed at least 1 week after onset of diarrhea, when free fecal Stx is often low (Cornick et al., 2002). As pointed out above, another possible explanation could be found in the broth we used for the overnight enrichment, i.e., BPW vs. MAC broth, although Boone et al. (2016) failed to detect several STEC-positive fecal samples with STQC after an overnight enrichment in MAC broth. In any case, no such effect of the enrichment broth type over the STQC result could be observed in our study, as the 19 STEC isolates from samples showing discordant results that were found positive when subsequently tested with STQC had been tested from BPW cultures, just like the overnight enrichments of the corresponding STQC-negative samples. Nevertheless, these discrepancies in sensitivity could be due to the higher number of positive samples included in this study and mostly to the broader range of Stx subtypes covered by them (8 out of the 10 described subtypes, including Stx2f and Stx2e), in contrast to all previous studies (Boone et al., 2016; Chui et al., 2015; De Rauw et al., 2016; Staples et al., 2017), which did not include fecal samples carrying any of the aforementioned undetectable STEC strains. Actually, when excluding these kinds of samples in our study (*n* = 10), the difference in sensitivity between our results and those described by Chui et al. (Chui et al., 2015) was not significant anymore (64.1% vs. 85.0%; $P = 0.1279$).

According to our results, when testing on fecal samples after overnight enrichment, STQC was reliable in the analysis of true STEC-negative specimens (specificity 100.0%), with a positive STQC result representing a true STEC-positive specimen in every case tested (PPV 100.0%) and having a large effect on increasing the probability of disease presence (LR+ ∞) (McGee, 2002). However, the assay performed with low sensitivity (54.1%), with a negative STQC result representing a false STEC-negative specimen in many cases (NPV 73.0%) and having a moderate effect on decreasing the probability of disease presence (LR − 0.45) (McGee, 2002). Regarding the low sensitivity displayed by STQC in our study, of the 33 samples showing discordant results between STQC and PCR (false negatives), only 10 could be attributed to the presence of an undetectable STEC strain. Altogether, 19 out of the remaining 23 samples with discordant results failed to be detected with STQC despite carrying detectable STEC strains. As described above, our PCR-based protocol for STEC detection involved the enrichment and culture of the fecal samples, and therefore, these discordant results might not be explained by the presence and shedding of free Stx-converting bacteriophages in the stools. The most probable explanation for the failure to detect Stx in these samples is the absence of *stx*

Table 3
Summary of the 74 *stx*-positive samples used in this study and characterization of the STEC isolates obtained from them. Only those originating from STQC-negative samples were subsequently tested with STQC.

Sample	Serotype	*stx* subtype	Disease	STQC results (feces)			STQC results (isolate)		
				Control	Stx1	Stx2	Control	Stx1	Stx2
439/15	O157:H7	*stx1a, stx2c*	BD	++	++	++	ND	ND	ND
835/15	O26:H11	*stx2a*	HUS	++	−	+	ND	ND	ND
908/15	O157:H7	*stx1a, stx2c*	BD	++	++	++	ND	ND	ND
1395/15	O157:H7	*stx2a, stx2c*	HUS	++	−	++	ND	ND	ND
1398/15	O157:H7	*stx2a, stx2c*	ASY	++	−	++	ND	ND	ND
1574/15	N/A	N/A (*stx1*)	D	++	−	−	N/A	N/A	N/A
2034/15	O128:H2	*stx2b*	BD	++	−	−	++	−	++
2073/15	O157:H7	*stx1a, stx2c*	BD	++	++	++	ND	ND	ND
2074/15	O26:H11	*stx2a*	D	++	−	+	ND	ND	ND
2076/15	O26:H11	*stx1a, stx2a*	D	++	++	++	ND	ND	ND
2197/15	O26:H11	*stx1a*	BD	++	++	−	ND	ND	ND
2315/15	O157:H7	*stx1a, stx2c*	D	++	++	+	ND	ND	ND
2506/15	O128:H2	*stx2b*	D	++	−	−	++	−	++
2533/15	O157:H7	*stx2c*	D	++	−	+	ND	ND	ND
2656/15	O5:H-	*stx1a*	D	++	++	−	ND	ND	ND
2661/15	O103:H2	*stx1a*	D	++	++	−	ND	ND	ND
2709/15	ONT:H21	*stx2d*	D	++	−	−	++	−	++
2894/15	O26:H11	*stx1a*	D	++	++	−	ND	ND	ND
2929/15	O63:H6	*stx2f*	D	++	−	−	++	−	−
2970/15	N/A	N/A (*stx1, stx2*)	BD	++	++	+	N/A	N/A	N/A
3373/15	O157:H7	*stx1a, stx2c*	BD	++	++	+	ND	ND	ND
3641/15	O13:H2	*stx2f*	D	++	−	−	++	−	−
3650/15	O153/O178:H10	*stx2b*	D	++	−	+	ND	ND	ND
3672/15	O157:H7	*stx2a, stx2c*	BD	++	−	++	ND	ND	ND
3673/15	O181:H49	*stx1a, stx2a, stx2d*	HUS	++	−	−	++	++	++
3680/15	O26:H11	*stx1a*	BD	++	+	−	ND	ND	ND
3688/15	O145:H28	*stx2a*	HUS	++	−	−	++	−	++
3722/15	O26:H11	*stx1a*	D	++	++	−	ND	ND	ND
3770/15	O108:H25	*stx1a*	ASY	++	−	−	++	++	−
3794/15	O157:H7	*stx2a, stx2c*	BD	++	−	++	ND	ND	ND
3861/15	O108:H25	*stx1a*	ASY	++	−	−	++	++	−
4068/15[a]	O157:H7	*stx2c*	D	++	−	−	++	−	−
4065/15	O166:H28	*stx1c, stx2b*	D	++	−	−	++	++	−
4107/15	O76:H19	*stx1c*	D	++	−	−	++	++	−
4223/15	O157:H7	*stx2a, stx2c*	UNK	++	−	−	++	−	++
4224/15[b]	O9:H30	*stx2e*	D	++	−	−	++	−	−
4303/15	O166:H28	*stx1c, stx2b*	D	++	−	−	++	++	−
4514/15	O26:H11	*stx1a*	D	++	++	−	ND	ND	ND
4521/15	O63:H6	*stx2f*	D	++	−	−	++	−	−
4756/15	O63:H6	*stx2f*	D	++	−	−	++	−	−
4972/15	O125ac:H6	*stx2f*	D	++	−	−	++	−	−
5137/15	O157:H7	*stx1a, stx2c*	D	++	−	−	++	++	++
5138/15	O157:H7	*stx1a, stx2c*	D	++	−	−	++	++	++
5139/15	O157:H7	*stx1a, stx2c*	D	++	−	−	++	++	++
5148/15	O157:H7	*stx1a, stx2c*	BD	++	++	++	ND	ND	ND
5167/15[b]	O100:H20	*stx2e*	D	++	−	−	++	−	−
5192/15	O157:H7	*stx1a, stx2c*	D	++	++	++	ND	ND	ND
5233/15	N/A	N/A (*stx1, stx2*)	D	++	−	−	N/A	N/A	N/A
5241/15	O26:H11	*stx1a*	D	++	++	−	ND	ND	ND
5242/15	N/A	N/A (*stx2*)	D	++	−	−	N/A	N/A	N/A
5528/15	N/A	*stx2f*[d]	D	++	−	−	N/A	N/A	N/A
5608/15	O145:H34	*stx2f*	D	++	−	−	++	−	−
73/16	O187:H52	*stx1c*	D	++	−	−	++	++	−
176/16	O157:H7	*stx1a, stx2c*	BD	++	++	++	ND	ND	ND
497/16	O117:H7	*stx1a*	D	++	−	−	++	++	−
574/16[c]	O8:H19	*stx2e*	BD	++	−	++	ND	ND	ND
758/16	O157:H7	*stx1a, stx2c*	D	++	−	−	++	++	++
759/16	O157:H7	*stx1a, stx2c*	HUS	++	−	−	++	++	++
760/16	O157:H7	*stx1a, stx2c*	D	++	−	++	ND	ND	ND
1011/16	O128:H2	*stx2b*	D	++	−	++	ND	ND	ND
1056/16	O146:H28	*stx2b*	BD	++	−	++	ND	ND	ND
2022/16	O157:H7	*stx2c*	BD	++	−	−	++	−	++
2103/16	O157:H7	*stx1a, stx2c*	D	++	++	++	ND	ND	ND
2533/16	O157:H7	*stx2a, stx2c*	BD	++	−	++	ND	ND	ND
2646/16	N/A	N/A (*stx2*)	D	++	−	−	N/A	N/A	N/A
2818/16	O157:H7	*stx1a, stx2c*	BD	++	++	+	ND	ND	ND
2983/16	O26:H11	*stx1a*	D	++	++	−	ND	ND	ND
3332/16	O157:H7	*stx2a*	BD	++	−	++	ND	ND	ND
3432/16	O157:H7	*stx1a, stx2c*	BD	++	++	+	ND	ND	ND
3744/16	O128:H2	*stx2b*	D	++	−	++	ND	ND	ND
3754/16	O113:H4	*stx1c*	BD	++	++	−	ND	ND	ND
3802/16	O157:H7	*stx2a, stx2c*	HUS	++	−	++	ND	ND	ND

(continued on next page)

Table 3 *(continued)*

Sample	Serotype	*stx* subtype	Disease	STQC results (feces)			STQC results (isolate)		
				Control	Stx1	Stx2	Control	Stx1	Stx2
4061/16	O157:H7	*stx1a, stx2c*	UNK	++	++	++	ND	ND	ND
5177/16	O26:H11	*stx1a, stx2a*	HUS	++	++	++	ND	ND	ND

STQC results are given semiquantitatively (−, no test lines visible, negative result; +, faint test line, less-intense positive result; ++, dark test line, highly positive result). D = diarrhea; BD = bloody diarrhea; HUS = hemolytic uremic syndrome; ASY = asymptomatic; UNK = unknown; ND = not determined; N/A = no isolate obtained for serotyping, *stx* subtyping, and subsequent STQC testing.

[a] Isolate probably carrying a nonfunctional *stx* gene.

[b] *stx2e*-positive isolate expressing a mucoid capsule.

[c] *stx2e*-positive isolate nonexpressing a mucoid capsule.

[d] Although no isolate was obtained for *stx* subtyping, the sample tested *stx2f*-positive in the PCR protocol for *stx* detection.

expression or the low amount of Stx produced under the growth conditions used in our study, below the detection limit of the STQC. Such differences in Stx production might also explain the less intense STQC positive results observed when testing certain strains and samples. Unfortunately, as pointed out above, Stx production was not confirmed by Vero cell assay in our study.

According to the low sensitivity, NPV, and LR− shown even after overnight enrichment, we conclude that STQC may not be considered a suitable screening tool for STEC detection in human fecal samples. Nevertheless, its high specificity, PPV, and LR+ suggest that STQC could be useful for laboratories where PCR is not a routine tool for STEC screening yet, always subject to the confirmation of negative samples with a clinical history indicative of STEC by a reference laboratory with full diagnostic capabilities.

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Conflict of interests

None.

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