Vol. 41, No. 10

Comparison of a Shiga Toxin Enzyme-Linked Immunosorbent Assay and Two Types of PCR for Detection of Shiga Toxin-Producing *Escherichia coli* in Human Stool Specimens

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Shiga toxin (Stx)-producing *Escherichia coli* (STEC) is a major cause of sporadic cases of disease as well as serious outbreaks worldwide. The spectrum of illnesses includes mild nonbloody diarrhea, hemorrhagic colitis, and hemolytic-uremic syndrome. STEC produces one or more Stxs, which are subdivided into two major classes, Stx1 and Stx2. The ingestion of contaminated food or water, person-to-person spread, and contact with animals are the major transmission modes. The infective dose of STEC may be less than 100 organisms. Effective prevention of infection is dependent on rapid detection of the causative bacterial pathogen. In the present study, we examined 295 stool specimens for the presence of Stx-producing *E. coli* by three different methods: an Stx enzyme-linked immunosorbent assay, a conventional PCR assay, and a LightCycler PCR (LC-PCR) assay protocol recently developed by our laboratory at the Institute of Medical Microbiology at Hannover Medical School. Our intent was to compare these three methods and to examine the utility of the STEC LC-PCR protocol in a clinical laboratory. The addition of a control DNA to each sample to clearly discriminate inhibited specimens from negative ones enhanced the accuracy of the LC-PCR protocol. From our results, it can be concluded that LC-PCR is a very useful tool for the rapid and safe detection of STEC in clinical samples.

Shiga toxin (Stx)-producing *Escherichia coli* (STEC) is an emerging pathogen responsible for sporadic cases of infection as well as serious outbreaks worldwide. The potent cytotoxins produced by STEC are divided into two major classes, Stx1 and Stx2 (32; S. M. Scotland, H. R. Smith, and B. Rowe, Letter, Lancet **2:**885-886). STEC causes a spectrum of human diseases ranging from mild nonbloody diarrhea through hemorrhagic colitis to the extraintestinal manifestation hemolytic-uremic syndrome (HUS) (17, 18). HUS is characterized by hemolytic anemia, thrombocytopenia, and acute renal failure (29). It is the major reason for an acute loss of kidney function in childhood (26, 31). Stx2e is an Stx2 variant produced mainly by *E. coli* isolates associated with edema disease in pigs (21), although HUS caused by an STEC strain expressing Stx2e in humans has been reported (9). The infectious dose of STEC may be very low (22); as a result, the organisms can be transmitted efficiently not only through contaminated foods but also from person to person (27). Rapid detection of the causative pathogen therefore is an important contribution to effective prevention of infection.

The identification of STEC by culture methods is a challenging task, as pathogenic strains are similar to nonpathogenic strains of *E. coli* (24). Currently, the best accepted techniques for diagnosis involve the identification of Stxs, either through protein detection by a cytotoxicity assay or an enzyme-linked immunosorbent assay (ELISA) or at the genomic level by PCR (22, 24). Further, STEC-specific plating media are available. Sorbitol-MacConkey (SMAC) agar and other chromogenic culturing methods are widely used (3, 4, 20); special blood agar plates allow for detection of the enterohemolytic phenotype of STEC strains (5). Cytotoxicity assays are the most sensitive methods for detecting active Stxs (24), but they are cumbersome to perform and therefore are not often established for routine microbiological diagnosis. Stx-specific ELISAs are acceptable alternatives, with several tests being commercially available. However, compared to cytotoxicity assays, they are less sensitive (11) and are also known to generate some false-positive results (1, 6). The disadvantage of plating media is that STEC may be substantially underrepresented in stool specimens from patients or asymptomatic carriers; this possibility hampers the reliable detection of these pathogens by bacterial culturing (15). Furthermore, SMAC agar only works for STEC strains which are sorbitol negative, and an enterohemolytic phenotype can also be observed with other members of the family *Enterobacteriaceae*.

The detection of *stx* genes by PCR offers certain advantages over the above-described diagnostic tools. It is exquisitely sensitive and highly specific. Therefore, even fewer than 10 Stx-producing *E. coli* organisms per ml of culture against a background of more than 10^9 other organisms per ml can be reliably detected in stool specimens (23). The serotype of the

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TABLE 1. Nucleotide sequences of primers and probes used in this study[a]

Primer or probe and target	Name	Sequence (5′ → 3′)	Function	Positions	T_m (°C)	Reference
Primers						
stx_1	KS7	CCC GGA TCC ATG AAA AAA ACA TTA TTA ATA GC	Sense	1120–1142	54.0	30
	KS8	CCC GAA TCC AGC TAT TCT GAG TCA ACG	Antisense	1401–1384	52.0	30
	StxA1 598	AGT CGT ACG GGG ATG CAG ATA AAT	Sense	598–621	56.9	2
	StxA1 1015	CCG GAC ACA TAG AAG GAA ACT CAT	Antisense	1015–992	55.3	2
stx_2	LP43	ATC CTA TTC CCG GGA GTT TAC G	Sense	295–316	57.0	8
	LP44	GCG TCA TCG TAT ACA CAG GAG C	Antisense	881–860	57.0	8
	StxA2 679	TTC CGG AAT GCA AAT CAG TC	Sense	679–698	52.5	2
	StxA2 942	CGA TAC TCC GGA AGC ACA TTG	Antisense	942–922	54.6	2
Probes						
stx_1	StxA1 FL 724	CTG TCA CAG TAA CAA ACC GTA ACA TCG CTC-X	FL probe	724–695	65.5	2
	StxA1 LC 693 R7	LC-TGC CAC AGA CTG CGT CAG TGA GGT-ph	Red 705 probe	693–670	67.5	2
stx_2	StxA2 FL 769	CAG AGC AGT TCT GCG TTT TGT CAC TGT CA-X	FL probe	769–797	65.0	2
	StxA2 LC 799 R6	LC-AGC AGA AGC CTT ACG CTT CAG GC-ph	Red 640 probe	799–821	63.3	2

[a] Sequence M19473 (14) was used as a reference for nucleotide positions of stx_1-specific primers and probes, and sequence X07865 (13) was used as a reference for all stx_2-specific oligonucleotides. T_m, melting temperature. X, 3′ fluoresceine; LC, 5′ LightCycler dye; ph, 3′ phosphate. FL, fluoresceine Red 640, LightCycler dye Red 640; Red 705, LightCycler dye Red 705.

respective strain does not influence the PCR diagnosis of STEC, as detection is solely based on the presence or absence of *stx* genes. With new developments in PCR technology, such as using fluorescence for the identification and verification of PCR products (12, 33), the molecular identification of emerging enteropathogens becomes an economical alternative to the previously used methods.

In this study, we simultaneously examined 295 stool specimens from 271 diarrheic patients and patients with suspected STEC infections for the presence of STEC by three different methods: an Stx-specific ELISA (11), a conventional PCR (8, 30), and a recently developed LightCycler PCR (LC-PCR) (2). Our intent was to compare these three methods and to examine the utility of the STEC LC-PCR in a clinical laboratory. Further, we used an internal control DNA to identify inhibited samples.

MATERIALS AND METHODS

Collection of samples and preparation of template DNA for PCR. A total of 295 stool specimens were collected from 271 patients with diarrhea over the periods from 1 September 2000 to 31 January 2001, 1 April 2001 to 31 January 2002, and 28 April 2003 to 8 May 2003. A small swab or 250 μl of liquid stool was incubated in EHEC toxin medium (Heipha, Heidelberg, Germany) in a horizontal shaker overnight at 37°C. Two loops (30 μl) of this broth culture were then plated directly on both blood agar and SMAC agar plates (Oxoid, Wesel, Germany) and again incubated overnight at 37°C. All colonies were suspended in 1 ml of 0.9% NaCl. The suspension was diluted 1:20 in PCR-grade H$_2$O (Applichem, Darmstadt, Germany); 10 μl was taken from this mixture for PCR with either a conventional block cycler or the LightCycler instrument.

Stx-specific ELISA. An Stx-specific ELISA was performed according to the manufacturer's instructions. Briefly, the RIDASCREEN Verotoxin ELISA (R-Biopharm, Darmstadt, Germany) detects the presence of Stx1 and Stx2 by using immobilized mouse monoclonal antibodies against these two toxin molecules. For ELISA testing, 100 μl of a liquid culture from EHEC toxin medium was used. Absorbances were measured at 450 nm by using a spectrophotometer. The threshold was calculated according to the manufacturer's instructions, and positive and negative controls were run with each test.

Cytotoxicity testing. Vero cells were grown as monolayers in 100 μl of Dulbecco's minimal essential medium (Biochrom, Berlin, Germany) supplemented with L-glutamine, antibiotics (penicillin and streptomycin), and 5% fetal bovine

serum on 96-well plates (Greiner Bio-One, Frickenhausen, Germany). For the Stx-specific ELISA, supernatants from EHEC toxin medium were filter sterilized (Minisart; Sartorius, Göttingen, Germany) and diluted 1:5 and 1:25 in tissue culture medium. From each dilution step, 100 μl was added to the Vero cell culture, leading to final dilutions of 1:10 and 1:50, respectively. Assays were run in duplicate with purified Stx2 as a positive control. Cells were incubated for 24 to 48 h at 37°C in a 5% CO$_2$ atmosphere. Cytopathic effects, characterized by lysis, vacuolization, and rounding of the cells, were measured photometrically after staining of the adherent monolayer. For staining, cells were fixed with 2% (vol/vol) formalin in phosphate-buffered saline for 15 min and then stained with a crystal violet solution (5% [vol/vol] ethanol, 2% [vol/vol] formalin, 0.13% [wt/vol] crystal violet; all chemicals were from Applichem) for 15 min at room temperature. The dye was redissolved with 100 μl of 70% ethanol, and then spectrophotometric absorbances were measured at 540 nm and compared to those for the positive and negative controls.

Conventional PCR. Conventional PCR was performed with 50-μl assay mixtures and $stxB_1$- and $stxA_2$-specific primers KS7-KS8 and LP43-LP44 (all synthesized by MWG-Biotech, Ebersberg, Germany) as described by Schmidt et al. (30) and Cebula et al. (8). A culture isolate of STEC strain EDL933 (25) served as a positive control. DNA amplicons were subjected to submarine gel electrophoresis with 2% agarose gels, visualized by ethidium bromide staining, and compared to the respective PCR products from the stx_1- and stx_2-positive control strain.

LC-PCR. Detection of *stx* genes with the LightCycler instrument was performed with a single capillary tube by using melting point analysis for the detection of the stx_1, stx_2, and stx_{2e} genes as described recently (2). Primers and probes were synthesized by TIB MOLBIOL (Berlin, Germany). All DNA oligonucleotides used for conventional PCR as well as LC-PCR are listed in Table 1. As in conventional PCR, genomic DNA from STEC strain EDL933 was used as a positive control. To identify inhibited samples, we added to each capillary tube a plasmid control DNA which was amplified and detected together with the target sequences by means of the $stxA_2$-specific primers and fluorescent hybridization probes. The construction and validation of this inhibition plasmid control DNA are described elsewhere (T. Bellin, A. Meuser, A. Karow, M. Hartmann, A. Matussek, S. Kafert, and F. Gunzer, submitted for publication). Briefly, a 917-bp fragment of the $stxA_2$ gene was amplified by PCR with primers GK1a (GCT CTA GAA TGA AGT GTA TAT TAT TT) and AG1 (ACG CTG CAG CTG TAT TAC), bearing recognition sites for restriction endonucleases *Xba*I and *Pst*I. The DNA amplicon was cloned into pUC19 linearized with *Xba*I and *Pst*I. With a QuikChange site-directed mutagenesis kit from Stratagene (Amsterdam, The Netherlands), three point mutations were introduced into the probe binding region of the cloned $stxA_2$ sequence by PCR-guided mutagenesis, thereby lowering the melting point of the control amplicon to approximately 58°C. The LightCycler signal generated by the plasmid was readily detected in samples that did not contain inhibitors.

Isolation of STEC from stool specimens. First, we tried to isolate STEC strains from positive stool specimens on EHEC agar (Heipha), a solid plating medium consisting of enterohemolysin blood agar on one half and chromogenic BCM agar on the other half of a petri dish. If these efforts were unsuccessful, colony hybridization was performed. From the plate suspension used for PCR analysis, serial 10-fold dilutions were made by using phosphate-buffered saline, and 100 μl of the 10^{-5}, 10^{-6}, and 10^{-7} dilution steps was spread on SMAC agar plates. The contents of plates with 200 to 300 well-separated bacterial colonies were replicated on polyamide round filters (Sartolon; Sartorius, Göttingen, Germany). Membrane-bound bacterial colonies were lysed, and genomic DNA was released through incubation of the filters in DNA I solution (0.5 M NaOH, 1.5 M NaCl) for 15 min, followed by a 5-min neutralization step with DNA II solution (1.0 M Tris-HCl [pH 7.5], 1.5 M NaCl) and a 20-min incubation step with 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate [pH 7.4]). The filters were then washed in 2× SSC– 0.1% sodium dodecyl sulfate for 2 h at 65°C in a hybridization oven. The wash buffer was changed after 1 h, and the filters were scrubbed thoroughly with a cotton towel to remove any residues from bacterial colonies, which might give false-positive signals. The hybridization membranes were then incubated at 42°C with digoxigenin-labeled PCR products amplified with Light-Cycler primers StxA1 598 and StxA1 1015 or StxA2 679 and StxA2 942 (2) by using Dig Easy Hyb solutions (Roche Diagnostics, Mannheim, Germany) and a digoxigenin DNA labeling and detection kit (Roche) according to the instructions provided by the manufacturer. Probe-positive colonies were visualized by chemoluminescence on X-ray film with CSPD (Applied Biosystems, Langen, Germany) as a substrate. With the aid of a light box, the corresponding colonies could then be isolated from the original plates.

Phenotypic methods. STEC isolates were serotyped according to the method of Bockemühl et al. (7) by using antisera for *E. coli* somatic (O) antigens O1 to O170 and flagellar (H) antigens H1 to H56.

Statistical analysis. Differences between groups were assessed by use of an alternative chi-square test, called McNemar's chi-square test (19), which is based on the numbers of discordant pairs r and s, as follows:

$$\chi^2_{\text{paired}} = \frac{(|r - s| - 1)^2}{r + s}, \ df = 1\chi$$

McNemar's chi-square test values of >3.841 were considered to be statistically significant. The use of McNemar's chi-square test is valid provided that the total number of discordant pairs is at least 10. In our studies, there were sometimes fewer than 10 discordant pairs; therefore, beyond that number, we calculated exact binomial probabilities. A P value of <0.05 was considered statistically significant. Both tests showed the same results for all comparisons; therefore, we consider McNemar's chi-square test to be robust.

RESULTS

Comparison of ELISA, conventional PCR, and LC-PCR. The STEC LC-PCR detected one or more *stx* genes in 51 (17.2%) of the 295 stool specimens. The PCR profile and ELISA signal of the samples as well as the serotype and *stx* genotype of the isolated bacterial strains are listed in Table 2. Compared to conventional PCR, LC-PCR detected *stx* genes in five additional samples (Table 3). Four samples were positive for the *stx*$_1$ gene, and one sample produced signals for the *stx*$_1$ and *stx*$_{2e}$ genes (Table 2). None of them was found positive by the ELISA. Although the advantage of LC-PCR over conventional PCR in detecting STEC was not statistically significant ($P = 0.063$; $\chi^2 = 3.2$), the trend was that LC-PCR could identify such organisms in clinical samples more often. The *stx* genes discovered were identical in 44 of the 46 samples found positive by both PCR methods. Conventional PCR failed to detect *stx*$_1$ two times in STEC strains that were found positive for both *stx*$_1$ and *stx*$_2$ by LC-PCR (Table 2, sample no. 38 and 40). Comparing both PCR methods to the Stx-specific ELISA revealed that significantly more STEC-positive stool specimens were identified by the molecular techniques (Table 3). Twenty additional samples harboring STEC were detected by LC-PCR ($P < 0.001$; $\chi^2 = 18.05$), and the immunoassay still failed 15

TABLE 2. Results from conventional PCR, Stx-specific ELISA, and LC-PCR as well as serotypes of STEC strains isolated from 51 stool samples testing positive for *stx* genes with LC-PCR[a]

No.	Name	Conventional PCR		ELISA	LC-PCR			Serotype
		stx$_1$	stx$_2$		stx$_1$	stx$_2$	stx$_{2e}$	
1	D12 2001	+	−	+	+	−	−	O55:H7
2	D20 2001	+	−	−	+	−	−	O91:H14
3	D20 2002	+	−	+	+	−	−	O91:H⁻
4	D20 2001 II	+	−	−	+	−	−	O91:H14
5	D24 2001	−	+	−	−	+	−	ND
6	D25 2002	−	+	+	−	+	−	ND
7	D25 2004	−	−	−	−	+	−	ND
8	D27 2004	+	−	+	+	−	−	O145:H⁻
9	E21 2002	−	+	−	−	−	+	ND
10	E21 2003	−	+	−	−	−	+	ND
11	E22 2003	+	−	+	+	−	−	O142:H33
12	E22 2004	+	−	+	+	−	−	O91:H⁻
13	E22 2003 II	+	−	+	+	−	−	O142:H33
14	E25 5020	+	−	+	+	−	−	O91:H⁻
15	E25 5021	−	−	−	+	−	+	ND
16	E28 5009	−	−	−	+	−	−	ND
17	E29 2004	+	−	+	+	−	−	O142:H33
18	eh 156	+	+	+	+	−	+	O128:H2
19	eh 179	+	+	+	+	−	+	O113:H17
20	eh 181	−	+	−	−	+	−	O2:H⁻
21	eh 241	+	−	−	+	−	+	O128:H2
22	eh 507	+	−	−	+	−	−	O103:H2
23	eh 1478	+	+	+	+	+	−	O157:H⁻
24	eh 1493	−	+	+	−	+	−	O157:H7
25	eh 1625	−	+	+	−	+	−	O157:H7
26	eh 1829	+	−	+	+	−	−	O91:H14
27	eh 1935	+	−	+	+	−	−	O145:HNT
28	eh 134 II	+	−	−	+	−	−	O26:H1
29	eh 1413 I	+	−	+	+	−	−	O111:H⁻
30	eh 1493 II	−	+	+	−	+	−	O111:H⁻
31	eh 1776 I	−	+	+	−	+	−	O157:H7
32	eh 1870 I	+	−	−	+	−	−	ND
33	eh 1933 II	+	−	+	+	−	−	O145:HNT
34	eh 1962 II	+	−	+	+	−	−	O91:H14
35	eh 2	+	−	+	+	−	−	O103:H2
36	eh 2086 II	+	−	+	+	−	−	O26:H11
37	F01 2002	+	−	+	+	−	−	O55:H7
38	1K28 2001	−	+	−	+	+	−	O157:H7
39	1H20 2002	−	+	+	−	+	−	ONT:H⁻
40	1I27 2001	−	+	+	+	+	−	O145:H⁻
41	1J04 2001	+	−	−	+	−	−	ONT:H⁻
42	1J18 5018	+	−	−	+	−	−	O91:H⁻
43	1J20 2003	−	−	−	+	−	−	ND
44	1K06 2001	−	−	−	+	−	−	ND
45	2A21 2004	+	−	+	+	−	−	O91:H⁻
46	1K20 2005	+	+	+	+	+	−	O157:H⁻
47	204943 2002	−	+	+	−	+	−	O157:H7
48	199414 2002	−	+	+	−	+	−	ND
49	287887 2003	+	−	+	+	−	−	O91:H⁻
50	288597 2003	−	+	+	−	+	−	O157:H⁻
51	288991 2003	−	+	+	−	+	−	O157:H⁻

[a] +, positive; −, negative; ND, not detected. PCR-only-positive results were obtained for samples 5, 7, 9, 10, 15, 16, 32, 43, and 44.

times when it was compared to conventional PCR ($P < 0.001$; $\chi^2 = 13.07$). Further, the Stx-specific ELISA produced eight false-positive results. In total, we were able to isolate STEC strains of various serotypes from 40 (78.4%) of the 51 PCR-positive stool specimens.

Serotypes of STEC strains isolated from stool specimens. Overall, isolated strains belonged to 11 different serogroups

TABLE 3. Checkerboard for comparison of positive and negative
results of Stx-specific ELISA, LC-PCR, and conventional
PCR for all 295 stool specimens

Result	No. of specimens with the following result:			
	LC-PCR +	LC-PCR −	PCR +	PCR −
ELISA +	31	8	31	8
ELISA −	20	236	15	241
PCR +	46	0		
PCR −	5	244		

(Table 2). They contained the following O antigens (number of strains): O2 (1), O26 (2), O55 (2), O91 (10), O103 (2), O111 (2), O113 (1), O128 (2), O142 (3), O145 (4), and O157 (9). Two strains were not typeable. Five of the nine O157 STEC strains possessed H7 flagella; the four remaining ones were nonmotile (H⁻).

stx genes of STEC strains isolated from stool specimens. Twenty-nine stool specimens were positive for the stx_1 gene, and 14 had the stx_2 and stx_{2e} genes. Eight stool specimens contained STEC with two toxin genes, appearing either as stx_1 and stx_2 ($n = 4$) or as stx_1 and stx_{2e} ($n = 4$) genotypes. With respect to the isolated strains, 24 of them harbored stx_1 genes, 9 were positive for stx_2 genes, and 7 contained both stx_1 and stx_2 genes ($n = 4$) or both stx_1 and stx_{2e} genes ($n = 3$). No bacteria could be isolated from five stx_2-positive stool specimens, three stx_1-positive stool specimens, two stx_{2e}-positive specimens, and one stool specimen harboring both stx_1 and stx_{2e} genes. Among these were five specimens found positive for stx genes solely by LC-PCR. ELISA was negative for 12 stx_1-positive samples, 2 stx_2-positive samples, 2 stx_{2e}-positive samples, 1 stx_1- and stx_2-positive sample, and 3 stx_1- and stx_{2e}-positive samples. Isolation of bacteria from nine of these ELISA-negative stool specimens was not successful.

Cytotoxic activity of samples found positive only by PCR. None of the nine stool specimens found positive for stx genes by one or both PCR methods (Table 2) exhibited cytotoxic activity toward Vero cells. Although we screened 2,000 to 3,000 individual colonies from each of the respective bacterial suspensions for the presence of stx genes by using colony hybridization, no STEC could be isolated.

DISCUSSION

Due to the fact that the identification of STEC by culture methods is difficult, more efficient techniques for the diagnosis of these emerging pathogens must be developed. In this study, we have shown that the detection of STEC by molecular methods is significantly more effective than detection by a licensed commercially available immunoassay. The ELISA that we used failed to detect 15 and 20 STEC-positive stool specimens identified by conventional PCR and LC-PCR, respectively. Furthermore, it delivered eight false-positive results. These findings clearly demonstrate that the Stx-specific ELISA used here has limitations for the detection of STEC in stool specimens. An additional restraint on the use of Stx detection assays is that not all *E. coli* strains which harbor stx genes produce the encoded protein under laboratory conditions (16).

Conventional PCR failed to identify five STEC-positive stool specimens detected by LC-PCR. Although this difference does not reach statistical significance, we assume that our choice of primers and the optimized PCR protocol account for the improved STEC detection. LC-PCR also has the advantage of rapid cycling combined with fluorescence-based identification and verification of PCR products. Further, the stx_1 and stx_2 genes are detected in a single reaction capillary tube, reducing the effort and time needed for diagnosis. The fact that, despite our endeavors, no STEC could be isolated from 9 of the 20 ELISA-negative stool specimens is most likely due to very low numbers of organisms in these specimens. The lack of cytotoxic activity toward Vero cells can be explained in this way also. These observations indicate the superior sensitivity of molecular techniques for the detection of STEC. Many of the traditional culture methods used for identifying STEC do not reveal the presence of non-O157 *E. coli* (3; H. Karch, Editorial, Eur. J. Clin. Microbiol. Infect. Dis. **15:**276-280), and the detection of such organisms is beyond the scope of most routine laboratories. The use of molecular techniques is the best way to address this problem, and our study demonstrates the usefulness of LC-PCR for this purpose.

A complete analysis of 32 probes with a LightCycler instrument, including time for preparation of samples, takes about 180 min. Conventional PCR takes almost 2.5 times as long (440 min) and does not include the verification of positive PCR products by hybridization. The LC-PCR assay delivers very specific results quickly; therefore, this application is especially attractive for work involving a large number of samples, such as stool diagnostic procedures in clinical microbiology or food safety surveillance. Furthermore, melting point analysis allows discrimination between stx_2 and stx_{2e}, the gene encoding the pig edema disease toxin (21), a feature potentially interesting for epidemiological studies and risk assessment of stx-positive samples from asymptomatic patients. Friedrich et al. showed recently that infection with STEC possessing stx_{2e} genes bears only a minimal risk of severe complications, such as hemorrhagic colitis and HUS (10).

When PCR is used to analyze clinical samples, inhibitory specimens must be safely identified to avoid false-negative results (28). Therefore, an internal standard was added to each test sample. A positive signal from this target demonstrates successful amplification and thereby validates a negative result for the primary target. Our approach is generally useful for all real-time PCR applications where melting curve analysis can be performed and certainly increases the security of any PCR-based diagnostic method. Bacterial strains were isolated from 40 out of 51 LC-PCR-positive samples and serotyped. The fact that we found an STEC content of 17.2% in the stool specimens investigated can be explained by the choice of specimens, which were taken from patients with diarrhea as well as from individuals with suspected STEC infections.

In conclusion, we have shown that LC-PCR is a very useful tool for identifying STEC in clinical samples. Compared to the ELISA used in this study, LC-PCR was significantly more effective in detecting pathogens. In addition, the costly and time-consuming hybridization of DNA amplicons for verification after conventional PCR becomes redundant. However, in addition to the rapid detection of STEC, culturing the organisms is desirable to assess their antibiotic resistance profiles and, from a public health perspective, to perform subtyping

beyond *stx* gene detection for surveillance and outbreak investigations. Better methods are therefore still needed to isolate STEC from PCR-positive stool specimens.

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