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# Detection of Enterotoxigenic *Clostridium perfringens* in Food and Fecal Samples with a Duplex PCR and the Slide Latex Agglutination Test

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**A duplex PCR procedure was evaluated for the detection of *Clostridium perfringens* in food and biological samples and for the identification of enterotoxigenic strains. This method uses two sets of primers which amplify in the same reaction two different DNA fragments simultaneously: the 283-bp *C. perfringens* phospholipase C gene fragment and the 426-bp enterotoxin gene fragment. Internal primers within the two primer sets confirmed the specificity of the method by DNA-DNA hybridization with the PCR products. No cross-reaction was observed with other *Clostridium* species or with other bacteria routinely found in food. The detection level was approximately $10^5$ *C. perfringens* cells per g of stool or food sample. When overnight enrichment culture was used, 10 *C. perfringens* cells per g was detected in 57 artificially contaminated food samples. The duplex PCR is a rapid, sensitive, and reliable method for the detection and identification of enterotoxigenic *C. perfringens* strains in food samples. A slide latex agglutination test was also evaluated as a rapid, simple technique for the detection of *C. perfringens* enterotoxin in stool samples.**

*Clostridium perfringens* type A is widely distributed in soil, sewage, and intestinal tracts of humans and animals. The enterotoxigenic strains are a common cause of food poisoning outbreaks worldwide (10, 14). The symptoms, predominantly diarrhea and abdominal pain, appear 6 to 24 h after ingestion of contaminated food. Vomiting and fever are unusual. Death occurs occasionally among debilitated patients, particularly the elderly (13). Enterotoxigenic *C. perfringens* has also been associated with sporadic cases of diarrhea and with some cases of sudden infant death syndrome (3, 15, 19, 20).

*C. perfringens* enterotoxin (CPE), which is produced during the sporulation phase, causes the symptoms of *C. perfringens* food poisoning (13, 17). The illness is caused by ingestion of food containing a large number ($>10^5$ bacteria per g) of vegetative enterotoxigenic *C. perfringens* cells (24). The ingested bacteria multiply and sporulate, releasing CPE into the intestine.

A *C. perfringens* count of $>10^6$ cells/g in fecal samples of patients is indicative of *C. perfringens* food poisoning (24). Direct detection of CPE in fecal samples is also a valuable diagnostic technique (1, 2, 9).

Epidemiological investigations involve enumerating *C. perfringens* in suspected food. Characterization of enterotoxigenic *C. perfringens* strains is not performed routinely, since *C. perfringens* sporulation, which is a prerequisite for CPE production, is limited in the usual culture media (17).

CPE and phospholipase C gene sequences have been determined (26, 28, 29). The phospholipase C gene is located on the chromosomal DNA in all *C. perfringens* toxin types (4), whereas distribution of the CPE gene is restricted. DNA-DNA hybridization experiments showed that only 6% of *C. perfringens* isolates from various origins carried the CPE gene (28). This percentage is higher (59%) among *C. perfringens* strains isolated from confirmed outbreaks of food poisoning (30).

We describe here a duplex PCR for the rapid detection and identification of enterotoxigenic *C. perfringens* strains in food and fecal samples. A slide agglutination test for the detection of CPE in stool samples was also evaluated.

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## MATERIALS AND METHODS

**Bacterial strains.** All of the bacterial strains used in this study are listed in Table 1. The *Clostridium* strains were grown in TYG medium under anaerobic conditions by using anaerobic jars containing 95% $H_2$ and 5% $CO_2$; this medium contained Trypticase (30 g/liter), yeast extract (20 g/liter), glucose (5 g/liter), and cysteine HCl (0.5 g/liter), and its pH was 7.2.

**Standard bacteriological methods.** For bacteriological analysis of food and feces samples, 10-g samples were weighed aseptically, placed into sterile stomacher bags, and homogenized for 2 min in 90 ml of peptone water. One-milliliter aliquots of 1:10 dilutions of the suspensions were mixed with 9 ml of SPS agar, which contained tryptone (15 g/liter), yeast extract (10 g/liter), ferric citrate (0.5 g/liter), sodium sulfite (0.5 g/liter), sodium thioglycolate (0.1 g/liter), Tween 80 (0.005 g/liter), polymyxin B sulfate (0.001 g/liter), disodium sulfadiazine (0.12 g/liter), and agar (14 g/liter) and had a pH of 7. After a 18 h of anaerobic incubation at 46°C, the sulfite-reducing *Clostridium* cells were counted. Colonies surrounded by the characteristic black precipitate were identified biochemically (by using lactose fermentation, nitrate reduction, gelatinase production, and motility tests).

**Anti-CPE immunoglobulins.** CPE was purified from *C. perfringens* 8-6, and rabbit anti-CPE antibodies were prepared as described previously (21). Anti-CPE immunoglobulins were purified by an immunoaffinity procedure. CPE (3 to 5 mg) was coupled with 1 g of cyanogen bromide-activated Sepharose 4B (Pharmacia, Paris, France) according to the instructions of the manufacturer. Rabbit anti-CPE serum (5 ml) was passed over the immunoaffinity column. The column was washed with phosphate-buffered saline (PBS) until no further protein was detected in the eluate. The anti-CPE antibodies were then eluted with 1 M acetic acid. Fractions (200 μl) were collected in tubes containing 100 μl of 3 M Tris-HCl (pH 8). The fractions containing purified anti-CPE immunoglobulins were dialyzed against PBS.

**Preparation of latex beads.** Coating of latex beads (Bacto Latex 0.81; Difco, Detroit, Mich.) was performed as described previously (24). The latex suspension (2 to 5 ml) was diluted in 15 ml of glycine buffer (0.1 M glycine, 0.15 M NaCl; pH 8.2) containing 200 μg of purified anti-CPE immunoglobulin and homogenized for 1 min at room temperature. An equal volume of PBS–0.1% bovine serum albumin (BSA) was then added, and the mixture was vortexed and stored at 4°C. A negative latex control was prepared in the same way by using nonimmune immunoglobulin G (Sigma, Paris, France).

TABLE 1. Specificity of the duplex PCR: use of the duplex PCR to distinguish between enterotoxigenic *C. perfringens* strains and other *Clostridium* and enterobacterial strains

| Strain(s) |
|---|
| Strains yielding positive results with the PCR |
| *Clostridium perfringens* type A strains ATCC 13124, 8-6, 30, 1088.0, 4012, 4086, 4009, 4010, 4011, 1089.1, 1089.2, 1089.3, 1089.4, 1122, and 1513 |
| *Clostridium perfringens* type B strain CN39.22 |
| *Clostridium perfringens* type C strain CWC236 |
| *Clostridium perfringens* type D strains 2534, 250, A0, 48, 76, and 64/1 |
| *Clostridium perfringens* type E strain NCIB 10748 |
| Strains yielding negative results with the PCR for both phospholipase C and enterotoxin genes |
| *Clostridium botulinum* type A strain ATCC 25763 |
| *Clostridium botulinum* type B strain IPF11 |
| *Clostridium botulinum* type C strain IP468 |
| *Clostridium botulinum* type D strain IP1873 |
| *Clostridium botulinum* type E strain IP9009 |
| *Clostridium botulinum* type F strain NCIB10658 |
| *Clostridium botulinum* type G strain NCIB10714 |
| *Clostridium baratii* ATCC 27639 (*tox*) |
| *Clostridium baratii* ATCC 43756 (*toxF*) |
| *Clostridium butyricum* IPCB25-1 (*tox*) |
| *Clostridium butyricum* ATCC 43181 (*toxE*) |
| *Clostridium tetani* IPE19406 |
| *Clostridium sordelli* IP82 |
| *Clostridium innocuum* NCIB 10674 |
| *Clostridium sporogenes* ATCC 3584 |
| *Clostridium paraputrificum* ATCC 25780 |
| *Clostridium difficile* ATCC 9689 |
| *Clostridium difficile* IP203-1 |
| *Clostridium oedematiens* ATCC 17861 |
| *Clostridium spiroforme* IP247 |
| *Clostridium subterminale* ATCC 25774 |
| *Clostridium septicum* ATCC 12464 |
| *Clostridium limosum* IP384 |
| *Clostridium beijerinckii* VPI5481 |
| *Clostridium mangenotii* ATCC 25761 |
| *Clostridium chauvei* IP91 |
| *Clostridium bifermentans* ATCC 638 |
| *Bacillus thuringiensis* 14001 |
| *Bacillus subtilis* 168 |
| *Bacillus cereus* LCHA1203 |
| *Salmonella typhimurium* LCHA3612 |
| *Salmonella enteritidis* LCHA3625 |
| *Salmonella agona* LCHA3628 |
| *Listeria monocytogenes* 1/2a LCHA.A3 |
| *Listeria monocytogenes* 1/2b LCHA.I3 |
| *Listeria monocytogenes* 4b LCHA.O3 |
| *Listeria innocua* 6a LCHA.C3 |
| *Listeria welshimeri* 6b LCHA.E3 |
| *Listeria seeligeri* 1/2b LCHA.D3 |
| *Listeria ivanovii* 6b LCHA.X3 |
| *Escherichia coli* O157:H7 LCHA.P1 |
| *Escherichia coli* O6:H16 LCHA.877 |
| *Escherichia coli* O125:B15 LCHA.483 |
| *Proteus* sp. strain LCHA7208 |
| *Kurthia* sp. strain LCHAC4 |
| *Pseudomonas aeruginosa* LCHA32 |
| *Staphylococcus aureus* LCHAC3-0 |
| *Yersinia enterolitica* LCHAS31 |

[a] The following strains are enterotoxigenic: *C. perfringens* type A strains 8-6, 1088.0, 4012, and 4086; *C. perfringens* type D strains 76 and 64/1; and *C. perfringens* type E strain NCIB 10748.

TABLE 2. Sequences of the primers and probes used in the PCR

| Oligonucleotide | Sequence (5′-3′) | Position |
|---|---|---|
| PL3 | AAG TTA CCT TTG CTG CAT AAT CCC[a] | 1676–1699[b] |
| PL7 | ATA GAT ACT CCA TAT CAT CCT GCT | 1418–1440[b] |
| PLC | TCA AAA GAA TAT GCA AGA GGT | 1591–1611[b] |
| P145 | GAA AGA TCT GTA TCT ACA ACT GCT GGT CC | 472–500[c] |
| P146 | GCT GGC TAA GAT TCT ATA TTT TTG TCC AGT[a] | 868–897[c] |
| EntA | GAA CGC CAA TCA TAT AAA TTT CCA GCT GGG[a] | 783–812[c] |

[a] Complementary DNA strand.
[b] Position in the *C. perfringens* phospholipase C gene sequence (25).
[c] Position in the CPE gene sequence (28).

**SLAT for the detection of CPE.** A slide latex agglutination test (SLAT) was performed on a glass slide by using 25 μl of coated latex and 25-μl portions of 10-fold serial dilutions of samples in PBS–0.5% BSA. Each mixture was gently rotated, and the agglutination was recorded after 3 min. The presence of CPE in *C. perfringens* strains was monitored by the SLAT by using the supernatant fluids of cultures grown in Duncan-Strong sporulation medium (30).

Fecal specimens were diluted 1:10 in PBS–0.5% BSA, homogenized by vortexing, and centrifuged at 15,000 × *g* for 3 min. The clarified supernatant fluid was analyzed by the SLAT.

**Primers used in the duplex PCR.** The six oligonucleotides used for PCR amplification and hybridization in this study are listed in Table 2. PL3 and PL7 have been described previously (26), PlC was derived from the *C. perfringens* alpha-toxin gene (26), and P145, P146, and probe EntA were derived from the enterotoxin gene (29).

Primers were selected by using PC Gene (IntelliGenetics, Geel, Belgium) according to the following criteria: no cross-hybridization with the other known enterotoxin or phospholipase C genes, high G+C content at the 3′ end, and limited dimer formation and self-complementarity. All of the oligonucleotides were chemically synthesized with a nucleic acid synthesizer (model 380B; Applied Biosystems, Inc., Foster City, Calif.).

**Duplex PCR with broth culture.** One milliliter of an enrichment culture was centrifuged at 12,000 × *g* for 3 min, and the pellet was washed in 2 ml of distilled water, centrifuged, and incubated at 56°C for 30 min with 200 μl of InstaGene matrix (Bio-Rad, Paris, France). The mixture was then vortexed for 10 s, incubated at 100°C for 8 min, vigorously vortexed, and centrifuged at 12,000 × *g* for 2 min. The PCR was performed with a model PTC-100 programmable thermal controller (MJ Research, Inc., Watertown, Mass.) by using 5 μl of supernatant fluid. Each reaction tube contained 50 μl of 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.1 mg of gelatin per ml, each deoxynucleotide (Boehringer, Mannheim, Germany) at a concentration of 200 μM, each primer at a concentration of 0.5 μM, and a DNA sample. Evaporation within the tube was prevented by the addition of 100 μl of mineral oil (Sigma, St. Louis, Mo.). The reaction mixture was incubated at 94°C for 5 min to denature the DNA, and 2.5 U of *Taq* polymerase (Boehringer) was then added to each tube. The PCR involved 30 cycles consisting of the following optimized thermal profile: 30 s of denaturation at 94°C, 30 s of primer annealing at 55°C, and 30 s of primer extension at 72°C. After the 30th cycle, primer extension was continued for an additional 10 min at 72°C to ensure that the final extension step was complete. Negative control experiments were performed with all of the reagents except the template DNA. Sample preparation, PCR amplification, and electrophoresis were performed in three different rooms to avoid contamination.

**Duplex PCR with stool samples.** Stool samples (0.1 g) were weighed aseptically, placed into sterile tubes, and homogenized with 0.9 ml of water and then were treated with InstaGene (Bio-Rad) and PCR amplified as described above.

**Duplex PCR with food samples.** Food samples (10 g) were suspended in 90 ml of peptone water and homogenized in stomacher bags for 2 min. In artificial food contamination experiments, 1 ml containing $10^2$ *C. perfringens* 8-6 cells was added to 100 ml of a food suspension; this represented a contamination level of 10 *C. perfringens* cells per g of food. The food suspension was incubated under anaerobic conditions at 37°C for 18 h. One milliliter of the enrichment culture was then treated with InstaGene (Bio-Rad), and the DNA sequences were amplified by the PCR as described above.

**Analysis of PCR products.** Agarose gel electrophoresis, transfer to nylon membranes, and hybridization with digoxigenin-labeled probes were all performed as described previously (8).

## RESULTS

***C. perfringens* duplex PCR specificity and characterization of enterotoxigenic strains.** The specificity of the duplex PCR with the two sets of primers derived from the phospholipase C and CPE genes was assessed with different *Clostridium* species and

other bacterial strains frequently associated with food (Table 1).

The 283-bp amplified fragment from the 24 *C. perfringens* strains tested was observed by agarose gel electrophoresis (Table 1). This PCR product had the expected size of the phospholipase C gene DNA fragment amplified with PL3 and PL7, and it hybridized with internal primer PlC. The other bacterial strains tested, including the *Clostridium bifermentans* strain, which produces a phospholipase related to that of *C. perfringens* (27) (Table 1), did not yield amplified fragments with PL3 and PL7 or with P145 and P146.

Seven of the 24 *C. perfringens* strains tested yielded an additional 426-bp PCR product, a product having the predicted size for the CPE gene DNA fragment amplified with P145 and P146. This 426-bp PCR product hybridized with the EntA primer located on the CPE DNA sequence between P145 and P146 (29), but not with PlC.

The duplex PCR procedure data indicated that *C. perfringens* 8-6 was enterotoxigenic (Table 1). These data are consistent with the fact that this strain produced CPE, as determined by Vero cell cytoxicity, mouse lethality, and immunoprecipitation (22). The other *C. perfringens* strains were not tested for CPE production, since they did not sporulate under the experimental conditions used.

**Sensitivity of the duplex PCR with a broth culture.** The duplex PCR was performed directly with an enterotoxigenic *C. perfringens* 8-6 broth culture to determine the sensitivity of the technique. Bacteria in the culture sample were counted microscopically in a Petrov chamber. As few as 50 bacteria in the reaction mixture yielded a positive result as determined by agarose gel electrophoresis. This sensitivity was increased 10-fold by Southern blotting with digoxigenin-labeled internal probes EntA and Plc.

**Application of the duplex PCR to stool samples.** Twenty-three stool samples were collected after a suspected *C. perfringens* food poisoning outbreak in a school restaurant and were analyzed by both the duplex PCR method and the standard method. Eighteen of these samples contained enterotoxigenic *C. perfringens* as determined by the duplex PCR technique (Table 3) and yielded two amplified fragments of the sizes predicted for phospholipase C and CPE gene DNA fragments amplified with PL3-PL7 and P145-P146 and hybridized with internal primers PlC and EntA, respectively. The number of sulfite-reducing bacteria as determined by the standard method ranged from $<10^4$ to $10^7$ bacteria per g. Five feces samples did not contain enterotoxigenic *C. perfringens* as determined by the duplex PCR technique and Southern blotting. The corresponding sulfite-reducing bacterial counts were less than $10^4$ bacteria per g for three samples and between $10^5$ and $2 \times 10^5$ bacteria per g for the two other samples (Table 3).

**CPE SLAT with stool samples.** CPE was detected in 18 of 23 stool samples with the SLAT (Table 3). Enterotoxigenic *C. perfringens* was identified in 17 of these 18 SLAT-positive stool samples by the duplex PCR. One stool sample (Table 3, sample 4) contained enterotoxigenic *C. perfringens* as determined by the duplex PCR but not by the SLAT. The low number of sulfite-reducing bacteria in this sample ($<10^4$ bacteria per g) suggested that CPE was absent. The other SLAT-positive samples contained at least $10^4$ sulfite-reducing bacteria per g (Table 3).

CPE was detected in one stool sample by the SLAT (Table 3, sample 11), but this sample was negative as determined by the duplex PCR. There were $10^5$ sulfite-reducing bacteria per g in this sample. Further analysis of 20 *C. perfringens* clones from this sample grown on sheep blood agar identified them as enterotoxigenic *C. perfringens* clones as determined by the PCR (data not shown).

TABLE 3. Stool sample analysis performed with the PCR and SLAT

| Sample | No. of sulfite-reducing bacteria per ml | Results with the following detection methods: | |
|---|---|---|---|
| | | Duplex PCR | SLAT |
| 1 | $<10^4$ | −[a] | − |
| 2 | $<10^4$ | − | − |
| 3 | $<10^4$ | − | − |
| 4 | $<10^4$ | + | − |
| 5 | $10^4$ | + | + |
| 6 | $10^4$ | + | + |
| 7 | $10^4$ | + | + |
| 8 | $2 \times 10^4$ | + | + |
| 9 | $5 \times 10^4$ | + | + |
| 10 | $5 \times 10^4$ | + | + |
| 11 | $10^5$ | − | + |
| 12 | $10^5$ | + | + |
| 13 | $10^5$ | + | + |
| 14 | $2 \times 10^5$ | − | − |
| 15 | $2 \times 10^5$ | + | + |
| 16 | $8 \times 10^5$ | + | + |
| 17 | $10^6$ | + | + |
| 18 | $2 \times 10^6$ | + | + |
| 19 | $2 \times 10^6$ | + | + |
| 20 | $10^7$ | + | + |
| 21 | $10^7$ | + | + |
| 22 | $10^7$ | + | + |
| 23 | $10^7$ | + | + |

[a] −, *C. perfringens* not present in sample; +, *C. perfringens* present in sample.

**Application of the duplex PCR to naturally and artificially contaminated food samples.** The food in the school restaurant responsible for the outbreak of food poisoning contained enterotoxigenic *C. perfringens* as determined by the duplex PCR performed without culture enrichment. The corresponding sulfite-reducing bacterial count was $10^5$ bacteria per g, and CPE was not detected by the SLAT.

The sensitivity of the duplex PCR method was investigated by using naturally and artificially contaminated food samples. The limit of detection was $10^5$ *C. perfringens* cells per g (data not shown). The sensitivity of the technique was improved by overnight culture enrichment. Of 59 naturally contaminated food samples, 2 contained $5 \times 10^5$ and $10^3$ *C. perfringens* cells per g as determined by the standard method and gave positive results as determined by the duplex PCR (Table 4).

The 57 food samples which did not contain sulfite-reducing bacteria were artificially contaminated (10 *C. perfringens* cells per g). All gave a positive result as determined by the duplex PCR after culture enrichment (Table 4).

## DISCUSSION

We developed a duplex PCR for detecting *C. perfringens* and identifying which strains are enterotoxigenic. One pair of primers (PL3 and PL7) was derived from the phospholipase C gene present in all strains of *C. perfringens* (7). The other pair (P145 and P146) was derived from the enterotoxin gene found predominantly in *C. perfringens* strains associated with outbreaks of food poisoning (29, 30). The PCR products were analyzed by DNA-DNA hybridization by using specific probes for the phospholipase C gene (PLC) and CPE (EntA).

The specificity of the duplex PCR was confirmed by studying

TABLE 4. Analysis of food samples for *C. perfringens* by the standard method and the duplex PCR method

| Food | No. of samples | No. of positive samples | | | |
|---|---|---|---|---|---|
| | | Naturally contaminated | | Artificially contaminated[a] | |
| | | Standard method | Duplex PCR | Standard method | Duplex PCR |
| Cooked food | 31 | 1 ($5 \times 10^5$)[b] | 1 | 30 | 30 |
| Pork butchery | 13 | 1 ($10^3$) | 1 | 12 | 12 |
| Raw meat | 11 | 0 | 0 | 11 | 11 |
| Milk product | 2 | 0 | 0 | 2 | 2 |
| Salad | 2 | 0 | 0 | 2 | 0 |

[a] Artificially contaminated food samples were inoculated with 10 *C. perfringens* cells per g, grown for 18 h in TYG medium, and analyzed by the duplex PCR as described in Materials and Methods. The standard method involved counting the number of *C. perfringens* cells per g of food sample.

[b] The values in parentheses are numbers of *C. perfringens* cells per gram.

24 *C. perfringens* strains, 27 different *Clostridium* species, and 20 bacterial strains commonly found in food samples.

The identification of enterotoxigenic *C. perfringens* isolates by immunological methods requires in vitro sporulation to obtain detectable levels of CPE, and since *C. perfringens* sporulates poorly in culture medium, this technique is unsatisfactory (6, 21). The duplex PCR and DNA-DNA hybridization methods are rapid, simple techniques for the identification of enterotoxigenic *C. perfringens* isolates (12, 23, 27, 30). The PCR and hybridization analysis have also been reported to be useful methods for *C. perfringens* typing (5, 18).

Our results show that the PCR is a useful technique for the analysis of biological and food samples. Enterotoxigenic *C. perfringens* was detected directly in 18 of 23 stool samples from patients suffering from food poisoning. These results were consistent with results obtained by using the standard method of counting sulfite-reducing bacteria with detection of CPE by the SLAT, except in two cases. One stool sample that contained $10^5$ sulfite-reducing bacteria per g and was CPE positive as determined by the SLAT gave a negative result with the duplex PCR. However, *C. perfringens* clones isolated from this stool sample were subsequently identified as enterotoxigenic *C. perfringens* by using the duplex PCR. The negative result may have been due to PCR inhibitors in the sample, and a more appropriate method of DNA extraction could have been used. The other stool sample contained $10^4$ sulfite-reducing bacteria per g and no detectable CPE as determined by the SLAT but was positive as determined by the duplex PCR technique.

The duplex PCR is a sensitive diagnostic technique; enterotoxigenic *C. perfringens* is detectable in stool samples at a concentration of $10^4$ to $10^5$ bacteria per g. Since stools of patients suffering from *C. perfringens* food poisoning harbor $10^6$ or more bacteria per g (2, 24), the duplex PCR is an appropriate diagnostic tool. The SLAT is also a sensitive, reliable, and rapid diagnostic technique for CPE detection (detection limit, 0.1 ng of CPE per ml [data not shown]). The use of the SLAT and latex agglutination on microplates for specific CPE detection has been described previously (1, 16).

Monitoring levels of *C. perfringens* food contamination is important in the food industry for preventing food poisoning. The standard method used routinely detects sulfite-reducing bacteria, including *C. perfringens* strains and other *Clostridium* strains. The duplex PCR method reported here is specific to *C. perfringens* and discriminates the enterotoxigenic strains of this species. The sensitivity of this method (10 *C. perfringens* cells per g) with culture enrichment of food samples is compatible with the detection levels required for food testing. Without culture enrichment, $10^5$ *C. perfringens* cells per g is detectable by the duplex PCR. Since food samples responsible for *C. perfringens* intoxication usually contain more than $10^5$ bacteria per g (24), the duplex PCR may be used directly in the first instance when *C. perfringens* food poisoning is suspected. The PCR method as described here can be used for rapid screening for *C. perfringens* in routine testing of food but does not provide quantitative results. A quantitative detection technique based on the most-probable-number method, consisting of inoculating serial dilutions of food samples into enrichment medium and performing PCR with each dilution culture, has been proposed for enumeration of *Clostridium botulinum* (11) and could be used for *C. perfringens* enumeration.

In conclusion, the duplex PCR method is a rapid, sensitive detection method for enterotoxigenic *C. perfringens* present in stool samples and in contaminated food. For routine food tests, culture enrichment is necessary to obtain a detection level of 10 *C. perfringens* cells per g. Detection of CPE in stools by the SLAT allows early diagnosis of *C. perfringens* intoxication and confirms the production of toxin by enterotoxigenic *C. perfringens* as detected by the duplex PCR.

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