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Evaluation of a Real-Time PCR Kit for Detecting *Escherichia coli* O157 in Bovine Fecal Samples

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A commercially available real-time, rapid PCR test was evaluated for its ability to detect *Escherichia coli* O157. Both the sensitivity and specificity of the assay were 99% for isolates in pure culture. The assay detected 1 CFU of *E. coli* O157:H7 g^{-1} in artificially inoculated bovine feces following enrichment.

Shiga-toxigenic *Escherichia coli* (STEC) includes *E. coli* serotypes whose genomes contain one or more Shiga toxin genes. STEC infections in humans can range from mild self-limiting diarrhea to more severe disease, including hemorrhagic colitis and hemolytic uremic syndrome (HUS) (18, 21). HUS is mainly seen in younger children and is the leading cause of renal failure for children under the age of 5 years (6). The major STEC serotype associated with infections in humans in the United States is O157:H7, which caused 69 outbreaks of *E. coli* O157:H7 infection in the United States during 2000, resulting in thousands of illnesses, 50 cases of HUS, and four deaths (http://www.cdc.gov/foodborneoutbreaks/ecoli/2000_summaryLetter.pdf). Many outbreaks of *E. coli* O157:H7 infection are the result of contaminated hamburger, produce, or water (1, 4, 5, 12, 13, 24). Person-to-person (2, 22) and direct animal-to-human transmission of *E. coli* O157:H7 have also been reported previously (3, 7, 11, 27).

A number of *E. coli* O157:H7 genes have been targeted for diagnostic amplification by PCR, including those encoding the Shiga toxins (stx_1 and stx_2), *eaeA*, *hlyA*, *fliC*, and several genes from the *E. coli* O157 O-antigen synthesis operon (9, 14, 16, 17, 19, 20, 23, 26). Real-time PCR allows for quantification of the target, and when combined with a rapid cycling platform, results can be generated in 30 min from the start of thermal cycling. Because of the advantages of real-time and rapid-cycle real-time PCR, many assays that perform better than the standard culture-based assays have been developed to detect pathogenic organisms (25). In this study, the ruggedized advanced pathogen identification device (RAPID) system *E. coli* O157 kit (Idaho Technology, Inc., Salt Lake City, Utah) was evaluated for detecting *E. coli* O157 in pure culture and in artificially and naturally contaminated bovine feces.

DNA was extracted from pure cultures by using the Generation Capture plate kit (Gentra Systems, Minneapolis, Minn.) according to the manufacturer's directions. Extractions were taken from 98 STEC O157:H7, 9 non-STEC O157, 16 STEC non-O157, and 86 non-O157 *E. coli* isolates (detailed list of the strains used is available at http://www.marc.usda.gov/AHRU/E.coli/AEM_Bono_Table_1.pdf). The DNA was diluted to 500 ng/μl, and 1 μl was added to the LightCycler capillary (Idaho Technology, Inc.). The RAPID system *E. coli* O157 detection kit (Idaho Technology, Inc.) containing the freeze-dried reagent was reconstituted by adding 38 μl of water, and 19 μl was added to the LightCycler capillary. The reactions were performed on the RAPID system with the cycling conditions of 94°C for 60 s for one cycle and then 45 cycles of a two-step cycle of 95°C for 0 s and 60°C for 20 s. PCR threshold cycle (C_T) values were determined by using the LightCycler data analysis module (Idaho Technology, Inc.).

The C_T values for the 107 *E. coli* O157 and 102 non-*E. coli* O157 isolates are shown in Fig. 1. The average C_T of the *E. coli* O157 isolates was 27 cycles (95% confidence interval [CI], 26.6 to 27.4). Eighty-seven of the 102 non-*E. coli* O157 isolates did not amplify after 45 cycles and thus had no C_T values. The mean C_T for the remaining 15 isolates was 38.1 (95% CI, 36.7 to 39.5). The optimum C_T cutoff value between O157 and non-O157 *E. coli* was determined by using two-graph receiver operating characteristic analysis (CMDT version 2.0; http://city.vetmed.fu-berlin.de/~mgreiner/CMDT/cmdt.htm) (10), resulting in a C_T cutoff value of 35. With this cutoff value, 106 of the 107 *E. coli* O157 isolates were positive using the RAPID test, while 101 of the 102 non-*E. coli* O157 isolates were classified as negative (sensitivity, 99.1% [95% CI, 94.9 to 99.9]; specificity, 99% [95% CI, 94.7 to 99.9]) (Fig. 1).

E. coli O157:H7 isolates EDL 933 and O157 Sakai were grown overnight in brain heart infusion broth and diluted 10-fold; 9 ml of each dilution was added to duplicate 50-ml disposable tubes, after which 1 g of *E. coli* O157-negative bovine feces was added. One milliliter of the bovine fecal slurry was removed before and after the 6-h enrichment incubation at 37°C. After centrifugation at 2,000 × *g* for 2 min, the supernatants were transferred into a new tube and centrifuged (10,000 × *g*, 3 min); the pellets were then washed two times with 1 ml of phosphate-buffered saline plus 5% Tween 20. The pellets were resuspended in 200 μl of Prepman reagent, and DNA was extracted according to the manufacturer's directions. Preenrichment ($R^2 = 0.958$) and postenrichment ($R^2 = 0.989$) C_T values showed a linear relationship with the number of *E. coli* O157:H7 organisms added, indicating a direct correlation

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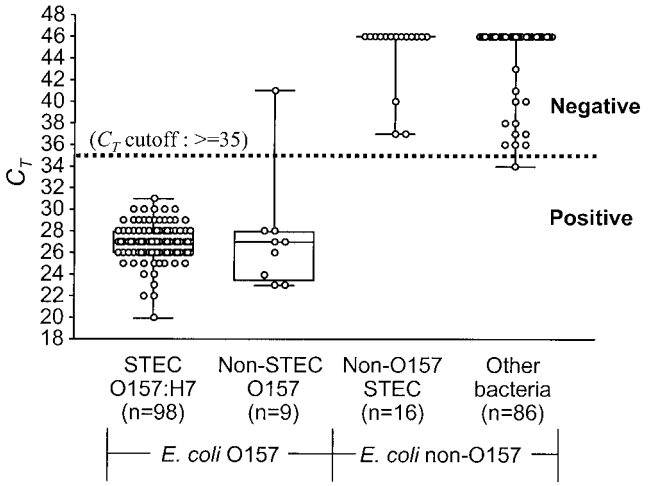

FIG. 1. Dot box plot of RAPID system *E. coli* O157 detection kit C_T values with bacterial isolates tested. Two hundred nine well-characterized bacterial isolates were assayed by using the RAPID system *E. coli* O157 detection kit. Dots represent the C_T value of each isolate. The bottom and top edges of the superimposed box plots are the 25th and 75th distribution percentiles, respectively; the central horizontal line represents the median (50th percentile), and the central vertical lines extend from the box as far as the data extend (range).

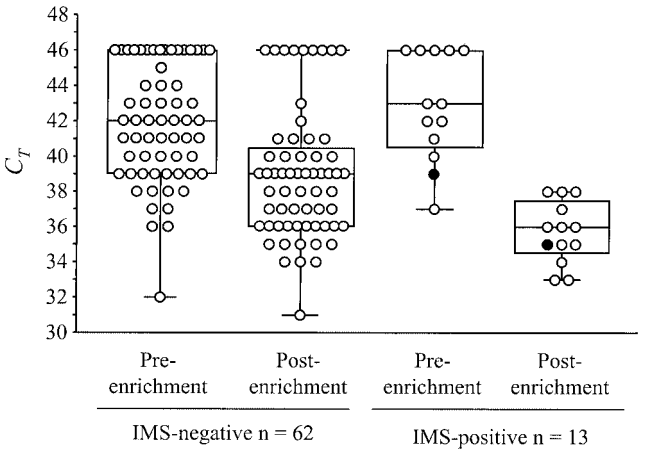

FIG. 3. Dot box plot of RAPID pre- and postenrichment C_T values from 75 bovine fecal samples. The pre- and postenrichment samples were divided into two groups: the 62 that were IMS culture negative and 13 that were IMS positive. Circles represent the C_T value of each sample. The filled circle represents a sample from which a non-STEC O157 strain was isolated. The bottom and top edges of the superimposed box plots are the 25th and 75th distribution percentiles, respectively; the central horizontal line represents the median (50th percentile), and the central vertical lines extend from the box as far as the data extend (range).

between the C_T and the number of *E. coli* O157:H7 CFU g of feces^{-1} (Fig. 2). The detection limit of the assay was generated by averaging the geometric mean of the last C_T from the linear curve. The detection limit of the assay was 512 CFU g^{-1} (95% CI, 34 to 7,798) preenrichment and 1 CFU g^{-1} (95% CI, 0.5 to 2) postenrichment.

Twelve STEC isolates and one non-STEC O157 isolate were cultured from 75 bovine fecal grabs by using an immunomagnetic separation (IMS) and isolation procedure (Fig. 3). DNA was purified from the 75 bovine fecal samples as described above and assayed by using the detection kit. Fifty-two preenrichment and 68 postenrichment samples had C_T values of 44 or lower. The difference between the IMS isolation procedure and the PCR detection kit is significant and probably is a result of the increased sensitivity of PCR. However, false positivity or amplification of DNA from dead bacteria cells may also have had a role in the increased number of positive PCR samples compared to that found with the IMS procedure. Both IMS-positive and -negative samples had a decrease in their C_T values after enrichment. The median C_T of the IMS culture-negative samples was 42 (95% CI, 40 to 43) preenrichment and 39 (95% CI, 37 to 39) postenrichment. The median C_T of the IMS culture-positive preenrichment samples was 43 (95% CI, 40 to 46), whereas the IMS culture-positive postenrichment samples had a median of 36 (95% CI, 34 to 38) (Fig. 3). Only after enrichment did the assay identify all 13 IMS culture-positive samples (Fig. 3).

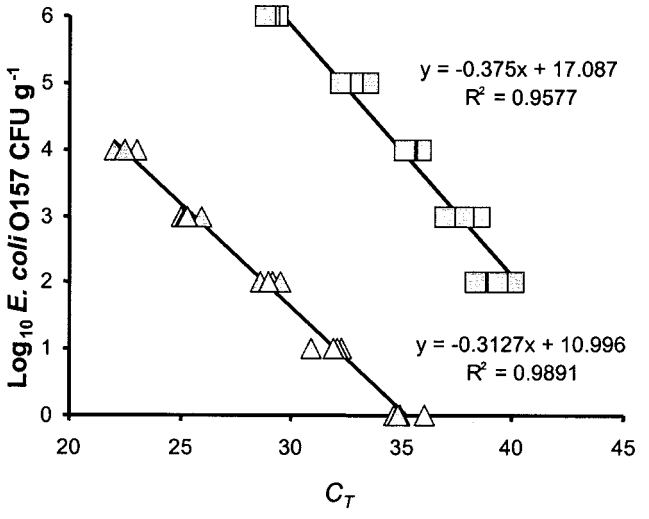

FIG. 2. Determination of the detection limit in bovine feces of the RAPID system *E. coli* O157 detection kit with C_T values. The C_T values are plotted against duplicate sets of *E. coli* O157 isolates EDL 933 and Sakai. DNA was extracted from a 10-fold dilution of inoculated slurry from preenrichment and postenrichment samples. The boxes and triangles represent the preenrichment and postenrichment samples, respectively.

The RAPID system *E. coli* O157 detection kit is a specific and sensitive assay for detecting *E. coli* O157 and has the added potential of detecting *E. coli* O157 in bovine feces. The detection range of 10^0 to 10^4 and minimum detection limit of $\leq$10 bacteria gram^{-1} of inoculated feces reported here for postenrichment samples are similar to other previously reported results (15, 23). The assay detects all *E. coli* O157 isolates whether they have Shiga toxin or not. Even though Shiga toxin-positive and -negative bacteria may share the O157 serotype, their virulence and genomes differed (8, 18, 21). STEC O157:H7 organisms have the H7 flagellar serotype, can be pathogenic in humans, and contain many virulence genes, including those encoding the Shiga toxins, the LEE locus, and HylA. Non-STEC O157 strains are usually not pathogenic in humans, generally have an H serotype other than H7, and lack

the virulence factors described above. A positive result with this assay would require further characterization to differentiate between *E. coli* O157:H7 and other *E. coli* O157 H serotypes.

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Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.

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