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# Evaluation of an Immunoassay for Direct Detection of *Escherichia coli* O157 in Stool Specimens

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**An enzyme-linked immunosorbent assay (ELISA) produced by LMD Laboratories, Inc., Carlsbad, Calif., was compared with culture for the detection of *Escherichia coli* O157. Nine of 185 stool specimens evaluated had positive results by the LMD *E. coli* O157 ELISA and grew *E. coli* O157 on culture; 174 had negative LMD *E. coli* O157 ELISA results and did not grow *E. coli* O157 on culture. Of 174 specimens negative by LMD *E. coli* O157 ELISA, 117 specimens grew other enteric pathogens: *Campylobacter* spp. (46 isolates), *Salmonella* spp. (43 isolates), *Yersinia* spp. (20 isolates), and *Shigella* spp. (8 isolates). There were two indeterminant results by the LMD *E. coli* O157 ELISA. One stool specimen did not grow other enteric pathogens on culture, and one grew a *Campylobacter* sp. on culture. Both had negative LMD *E. coli* O157 ELISA results upon repeat testing. The LMD *E. coli* O157 ELISA is an accurate, easy-to-read screening method for the detection of *E. coli* O157 in fecal specimens.**

*Escherichia coli* O157:H7 causes hemorrhagic colitis and has been associated with hemolytic-uremic syndrome (4, 5). The rapid diagnosis of *E. coli* O157:H7 directly from stool samples is preferred to avoid unnecessary diagnostic procedures (3, 11) and inappropriate antimicrobial therapy (10, 13) and to identify common sources linked to transmission (1, 2).

Conventional testing methods for *E. coli* O157:H7 involve the isolation of the organism on culture from stool samples followed by biochemical and immunologic confirmation. This method usually requires 72 h for complete identification. Rapid, less labor-intensive methods are desirable. In this context, we evaluated the utility of a rapid enzyme-linked immunosorbent assay (ELISA) compared with that of our standard culture method for detecting *E. coli* O157 directly from stool samples.

(This study was presented in part at the 94th General Meeting of the American Society for Microbiology, Las Vegas, Nev., 23 to 27 May 1994.)

**Materials and methods.** Stool specimens (185) collected from 161 patients between 5 August 1992 and 14 April 1994 were referred to the Mayo Clinic Clinical Microbiology Laboratory for enteric pathogen culture and were included in the present study. All patients were evaluated in either the outpatient or inpatient facility associated with the Mayo Clinic in Rochester, Minn. Seventy percent of the patients were Minnesota residents; the remaining patients were from 11 other states and two other countries. The stool specimens were transported to the laboratory in sterile containers and cultured immediately. An aliquot of stool specimen was set aside for ELISA testing the same day (30 specimens) or frozen at −20°C for batch testing (155 specimens).

Stool specimens were inoculated onto 5% sheep blood agar (BBL, Cockeysville, Md.), eosin-methylene blue agar (BBL), Hektoen enteric agar, cefsulodin-irgasan-novobiocin agar, *Campylobacter* agar, and sorbitol-MacConkey agar and into selenite F broth. The above-mentioned media were incubated at 35°C in ambient air, with the exception of the *Campylobacter* agar, which was incubated at 42°C in an atmosphere of 5% $O_2$, 10% $CO_2$, and 85% $N_2$, and the cefsulodin-irgasan-novobiocin agar, which was incubated at 30°C in ambient air. Colonies that were sorbitol negative on sorbitol-MacConkey agar were subcultured to 5% sheep blood agar, incubated overnight, and tested with spot-indole reagent (*p*-dimethylaminocinnamaldehyde). Indole-positive isolates were tested for the enzyme β-glucuronidase with the substrate 4-methylumbelliferone-β-D-glucuronide. Agglutination with *E. coli* O157 antisera (Difco Laboratories, Detroit, Mich.) was performed on 4-methylumbelliferone-β-D-glucuronide-negative isolates. Isolates that were agglutinated by *E. coli* O157 antisera were tested for the presence of the H7 flagellar antigen by inoculating the organism into motility agar and into motility agar containing H7 antisera (Difco). *E. coli* cells possessing the H7 antigen are immobilized in the H7 antibody-containing motility agar but remain motile in the non-H7 antibody-containing motility agar. A confirmatory conventional biochemical set was also performed.

The LMD Laboratories, Inc., *E. coli* O157 antigen detection kit provides plastic microwell test strips coated with anti-*E. coli* O157 polyclonal antibodies. The ELISA was performed according to the manufacturer's instructions. A slurry of each stool specimen was prepared by adding a minimal amount of diluted wash buffer, vortexing the mixture, and allowing particulate material to settle out. Two drops (100 μl) of stool supernatant and a positive and a negative control sample were added to the microwells, and the plates were incubated at room temperature (15 to 25°C) for 20 min. The plates were manually washed three times, after which 2 drops of peroxidase-labeled anti-*E. coli* O157 antibodies were added. The plates were incubated for 10 min at room temperature and were then washed manually three times. The wells were rinsed with deionized water. One drop each of substrate A (tetramethylbenzidine) and substrate B (peroxide) was added, and the plates were incubated at room temperature for 5 min. Two drops of stop solution (1 M phosphoric acid) were added, and the plates were read spectrophotometrically at 450 nm. The

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absorbance values provided by the manufacturer were used for interpretation of patient specimens and controls.

**Results.** A total of 9 of 185 stool specimens were positive by conventional culture for *E. coli* O157:H7; two of these specimens were obtained from the same patient. All nine specimens were also positive by the LMD *E. coli* O157 ELISA, yielding a sensitivity of the LMD ELISA of 100%. A total of 176 of 185 specimens were negative by conventional culture for *E. coli* O157:H7; two of these specimens were positive by the LMD *E. coli* O157 ELISA, while the remaining 174 specimens were negative by the LMD ELISA.

Stool specimens that were culture positive for other enteric pathogens with negative results by LMD *E. coli* O157 ELISA included *Campylobacter* spp. (45 isolates), *Salmonella* spp. (43 isolates), *Yersinia* spp. (20 isolates), and *Shigella* spp. (8 isolates). One exception was a specimen culture positive for *Campylobacter* spp. This specimen contained particulate material which resulted in a positive absorbance reading. Upon repeat testing, the specimen was negative.

Fifty-nine of 60 specimens with negative cultures for enteric pathogens had negative LMD *E. coli* O157 ELISA results. One was positive by the *E. coli* O157 ELISA and contained particulate material as described above. The specimen was negative upon repeat testing. If one counted the two stool specimens with particulate matter and initially positive results by LMD *E. coli* O157 ELISA as positives, the overall specificity for the LMD *E. coli* O157 ELISA was 98.9%.

**Discussion.** *E. coli* O157:H7 is the third most common cause of community-acquired bacterial diarrhea diagnosed at the Mayo-Rochester Medical Center. During the period from 1 January 1991 through 31 December 1993, the following relative frequencies of bacterial pathogens isolated from 14,699 stool specimens at the Mayo-Rochester Medical Center were noted: for *Campylobacter jejuni*, 40.9%; for *Salmonella* spp., 34.5%; for *E. coli* O157:H7, 10.6%; for *Yersinia enterocolitica*, 9.7%; for *Shigella* spp., 3.9%; and for *Vibrio* spp., 0.4% (unpublished data). Rapid methods for the detection of *E. coli* O157:H7 diarrhea are preferred to limit diagnostic evaluations including invasive procedures for hemorrhagic diarrhea in the clinical setting, decrease the time and expense associated with conventional culture techniques, speed public health evaluation of possible epidemics, and provide suitable isolation procedures for infected patients in the hospital setting (1–3, 6, 10, 11, 13).

Our laboratory standard culture method used to identify *E. coli* O157:H7 from stool specimens usually requires 72 h. Detection methods for *E. coli* O157:H7 disease other than culture have been reported, but because of special procedures (e.g., fluorescence microscopy and cell monolayer cultures, etc.), they are not easily adapted to most laboratories. Ritchie and colleagues (12) reported the utility of a direct fecal assay for the detection of verotoxins, or Shiga-like toxin I (SLT-I) and Shiga-like toxin II (SLT-II), associated with *E. coli* O157:H7 disease. This assay requires the observation of cytotoxicity of stool supernatants which are applied to a Vero cell monolayer. Neutralization of cytotoxicity with monoclonal antibodies directed against SLT-I and SLT-II is also required to confirm cytotoxicity related to these toxins. This assay requires a minimum of 48 h and is likely as labor-intensive as culture. Theoretically, this method has the advantage of detecting Shiga-like toxins from *E. coli* serotypes other than *E. coli* O157:H7 which have been shown to produce the same clinical syndrome and are not as easy to recover by conventional culturing methods (12). However, the frequency with which these *E. coli* serotypes cause hemorrhagic colitis or hemolytic-uremic syndrome appears to be low (12). Detection of Shiga-like toxins from *Shigella* isolates is also possible, as *Shigella* spp. possess them (12). Such a nonspecific assay may therefore preclude clinical usefulness, especially for *Shigella* spp., for which isolation of the organism is preferred so that susceptibility testing can be performed.

Paton and colleagues (9) described a method for detecting the presence of nucleic acids which encode SLT-I and SLT-II directly from stool samples. This method incorporates PCR and DNA probe hybridization detection formats. Like both the cytotoxin cell culture and conventional culture methods, this method is not a rapid procedure and requires at least 24 h to complete. Furthermore, Paton and colleagues observed a significant number of positive results for patients who were asymptomatic. In this study, direct comparisons of PCR results with results from toxin cytotoxicity assays and *E. coli* O157:H7 culture isolation were not done and PCR sterilization procedures to prevent contamination were not used.

In another study, Yamada and colleagues (14) used an ELISA for the direct detection of SLT-I and SLT-II from stools from patients who were infected during an *E. coli* O157:H7 outbreak. Of 14 inpatients, 5 had fecal Shiga-like toxins in stools obtained within 5 days of the onset of symptoms. Among these five patients, three had *E. coli* O157:H7 recovered from culture whereas the other two did not. Of the 14 inpatients, 4 additional patients had *E. coli* O157:H7 recovered from stools but did not have Shiga-like toxins detected by ELISA. In summary, this Shiga-like toxin ELISA was relatively easy to perform but was less sensitive than culture.

Park and colleagues (8) recently described a rapid (<2-h turnaround time) direct immunofluorescent antibody staining method to detect *E. coli* O157:H7 directly from stool specimens. For 336 fecal samples evaluated, direct immunofluorescent antibody staining of the fecal smear detected all isolates of *E. coli* O157 that were recovered by culture, including nonmotile strains, strains possessing the H7 flagellar antigen, and one with a flagellar antigen other than H7.

Like the assay evaluated by Park and colleagues, the LMD *E. coli* O157 ELISA described herein appears to be an acceptably sensitive, specific, and rapid (~1-h turnaround time) method for directly screening stool samples for *E. coli* O157. According to the manufacturer, there was no demonstrated cross-reactivity with other *E. coli* organisms not possessing the O157 antigen, including the following serotypes: O26:H11, O55, O88:H49, O91:21, O111:NM, and O163:NM. Additionally, no cross-reactivity was demonstrated for the following gram-negative bacteria: *Escherichia hermanii*, *Aeromonas hydrophila*, *Brucella abortus*, *Citrobacter freundii*, *Enterobacter cloacae*, *Hafnia alvei*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Xanthomonas maltophilia*, and *Y. entero-colitica* O9. An advantage of the LMD *E. coli* O157 ELISA over the direct assay used by Park and colleagues is that it does not require fluorescent microscopy. For the LMD *E. coli* O157 ELISA, additional prospective studies are required to corroborate our results, as the total number of stools positive for *E. coli* O157:H7 was low (9 of 185 specimens). Nevertheless, our results suggest that this assay could be used to screen stool samples prior to culture for *E. coli* O157 and that negative LMD *E. coli* O157 ELISA results may not require confirmatory culture. The LMD *E. coli* O157 assay may detect nonmotile (negative H serotypes) as well as H serotypes other than H7 which may not produce Shiga-like toxins. Cultures performed with O157-positive specimens would allow for H-antigen confirmation. In this study, inoculation of 176 fecal specimens onto sorbitol-MacConkey agar could have been avoided, which could represent savings in materials and subsequent evaluation. In some clinical settings, especially when

recovery of the organism on culture is not required for additional study (e.g., strain analysis for epidemiologic purposes [7]), this assay could replace culture. As antimicrobic therapy is not currently recommended for *E. coli* O157:H7 disease, isolation of the organism for susceptibility testing is also not required (10, 13). The LMD *E. coli* O157 ELISA is a rapid direct test which is easy to perform and can be readily adapted to most laboratories. This assay may be particularly useful for large-scale batch screening of routine stool specimens submitted to reference laboratories or large-scale screening of stool specimens from exposed persons by public health laboratories during outbreaks of food-borne diarrhea.

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