PCR-Based Assay Using Occult Blood Detection Cards for Detection of Diarrheagenic *Escherichia coli* in Specimens from U.S. Travelers to Mexico with Acute Diarrhea

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Travelers’ diarrhea (TD) is one of the most common illnesses affecting individuals from industrialized countries who visit developing countries. Bacterial pathogens account for about 60 to 85% of diarrheal cases. Worldwide, diarrheagenic *Escherichia coli*, especially enteropathogenic *E. coli* (EAEC) and enterotoxigenic *E. coli* (ETEC), account for the majority of the cases of TD (1, 5, 12). Individuals who live in developed countries such as the United States do not have acquired immunity to these organisms and are at high risk for developing symptomatic infection.

Large epidemiologic field studies of travelers’ diarrhea for multiple destinations are limited by the need to perform stool cultures on site in a timely manner. A method for the collection, transport, and storage of fecal specimens that does not require immediate processing and refrigeration and that is stable for months would be advantageous. This study was designed to determine if enterotoxigenic *Escherichia coli* (ETEC) and enteropathogenic *E. coli* (EAEC) DNA could be identified from cards that were processed for the evaluation of fecal occult blood. U.S. students traveling to Mexico during 2005 to 2007 were monitored for the occurrence of diarrheal illness. When ill, students provided a stool specimen for culture and occult blood by the standard methods. Cards then were stored at room temperature prior to DNA extraction. Fecal PCR was performed to identify ETEC and EAEC in DNA extracted from stools and from occult blood cards. Significantly more EAEC cases were identified by PCR that was performed on DNA that was extracted from cards (49%) or from frozen feces (40%) than from culture methods that used HEp-2 adherence assays (13%) (P < 0.001). Similarly, more ETEC cases were detected from card DNA (38%) than from fecal DNA (30%) or by culture that was followed by hybridization (10%) (P < 0.001). The sensitivity and specificity of the card test were 75 and 62%, respectively, compared to those for EAEC by culture and were 50 and 63%, respectively, compared to those for ETEC. DNA extracted from fecal cards that was used for the detection of occult blood is of use in identifying diarrheagenic *E. coli*.

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**TABLE 1. PCR primers used in this study**

<table>
<thead>
<tr>
<th>Organism and target gene</th>
<th>Amplicon size (bp)</th>
<th>Primer sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>EAEC aggR&lt;sup&gt;a&lt;/sup&gt;</td>
<td>324</td>
<td>5'-CTAAATGTGACAATCGATGTA-3'</td>
</tr>
<tr>
<td>EAEC aggR&lt;sup&gt;b&lt;/sup&gt;</td>
<td>433</td>
<td>5'-CTGGAATGATTCTAATCTG-3'</td>
</tr>
<tr>
<td>EAEC attA&lt;sup&gt;a&lt;/sup&gt;</td>
<td>630</td>
<td>5'-CTGCGCAAGAAGCTGTACAT-3'</td>
</tr>
<tr>
<td>ETEC est&lt;sup&gt;a&lt;/sup&gt;</td>
<td>190</td>
<td>5'-ATTITITCTCTTGTATTGTC-3'</td>
</tr>
<tr>
<td>ETEC est&lt;sup&gt;b&lt;/sup&gt;</td>
<td>450</td>
<td>5'-GCCGACAGTTATACCGTGCC-3'</td>
</tr>
<tr>
<td>Enterococcus faecalis ace&lt;sup&gt;c&lt;/sup&gt;</td>
<td>327</td>
<td>5'-ACCAGAGCGACGCTTGAATG-3'</td>
</tr>
</tbody>
</table>

<sup>a</sup> Used for card PCR and multiplex PCR.
<sup>b</sup> Used for card PCR only.

**Guaiacum officinale** (to which 80% ethanol and 4% H<sub>2</sub>O<sub>2</sub> has been added), we also sought to determine if the occult blood assay interfaced with PCR.

*(This study was presented in part at the 44th Annual Meeting of Infectious Diseases Society of America, Toronto, Canada, 12 to 15 October 2006.)*

**MATERIALS AND METHODS**

**Study population.** U.S. students, aged 18 years and older or between 16 and 18 with parental consent, traveling to Mexico from 2005 to 2007 were enrolled in a parent study to assess genetic polymorphisms that lead to susceptibility to TD in U.S. students, aged 18 years and older or between 16 and 18 (mean age, 57; mean age, 31). Overall, the microbiology findings were compared to those for the culture method for the detection of ETEC (38, 35, and 10%), for culture) (Tables 2 and 3). Card PCR also was superior to PCR that was performed on DNA cards were estimated by comparing the results to the gold standard, which in this case was routine stool culture, and to the results of multiplex fecal PCR that was performed directly on stool samples.

**Statistical analysis.** The proportions of positive test results from each one of the extraction methods were compared by chi square tests to determine significant differences between methods. Statistical analyses between groups were performed with STATA 9.0. The sensitivity, specificity, and kappa statistics of the PCR that was performed on DNA cards were estimated by comparing the results to the gold standard, which in this case was routine stool culture, and to the results of multiplex fecal PCR that was performed directly on stool samples.

**RESULTS**

There were 236 study participants from whom stored occult blood cards, which had been obtained from 2005 to 2007, were available for PCR. A total of 54 study participants had to be excluded; 39 of them did not experience diarrhea and 15 participants provided duplicate samples, leaving a total of 182 subjects for study. Fecal culture data was available from 39 individuals for ETEC and from 70 subjects for EAEC.

Travelers with diarrhea predominantly were female (71%), white (91%), and between the ages of 20 and 29 (maximum age, 57; mean age, 31). Overall, the microbiology findings were consistent with the previously reported frequency of diarrheagenic *E. coli* in U.S. adults traveling to Mexico (5, 12).

EAEC was identified more frequently by card PCR (49%) than by PCR done on fecal DNA (40%) or fecal cultures (13%) (*P* < 0.001 for the result for card PCR compared to that for culture) (Tables 2 and 3). Card PCR also was superior to fecal PCR and culture in detecting ETEC (38, 35, and 10%, respectively; *P* < 0.001 for the result for card PCR compared to that for culture) (Tables 2 and 3).

The sensitivity and specificity of the card DNA method, compared to those for the culture method for the detection of EAEC, were 75 and 62%, respectively. In the case of ETEC,
the card DNA demonstrated a sensitivity and specificity of 50 and 63%, respectively (Table 2). Compared to those for fecal PCR, the card DNA had a sensitivity and specificity of 53 and 51%, respectively, for EAEC and 56 and 70%, respectively, for ETEC. Kappa statistics indicated slight to fair concordance for EAEC card DNA test results and those from culture (57%) between EAEC card DNA test results and those from culture (57%) or fecal PCR and ETEC card DNA test results (57%). The highest kappa value was observed between the ETEC card PCR and fecal PCR tests (Table 3).

### DISCUSSION

The guaiac-based card method is an established and effective means of identifying small amounts of blood in fecal specimens. We sought to determine whether, once cards were fixed and developed, DNA from enteric pathogens could still be identified by PCR. This would provide an easy and economical means of collecting and transporting bacterial fecal DNA for the PCR identification of diarrheagenic *E. coli*. In our pilot study, we demonstrated that DNA from *Enterococcus faecalis*, an organism that is representative of fecal flora, could be identified by PCR after card fixation. Similarly, we demonstrated that DNA for the detection of EAEC and ETEC (using spiked samples) could be extracted from the cards after many days of storage. The use of the developer/fixative might have helped to stabilize the DNA and inactivate DNAses due to the high content of alcohol. The card and fixatives also may have inactivated other enzymes that could degrade DNA.

In this study, we demonstrate that fecal DNA that has been fixed on cards and stored at room temperature for up to 14 months was stable enough for PCR amplification. The potential advantages of this method include its use as a backup for the identification of enteropathogens, the elimination for the need for cold chain for transportation, the elimination of on-site culture processing, and the stabilization of DNA for long periods of time prior to processing. A possible commercial and clinical application includes the ability to detect pathogens from a rectal exam after examination for occult blood without the need for the collection of a stool specimen. A disadvantage of this method is that it does not yield viable organisms for phenotypic analysis, such as antimicrobial susceptibility testing, adherence patterns, or studies that deal with the molecular epidemiology of the pathogen(s).

Although the PCR performed on card DNA was the method that detected the highest number of *E. coli* pathogens, the correlation between results of card PCR and culture or fecal multiplex PCR was modest. This may be explained by several reasons. (i) Stool cultures were done from samples that had been transported in Cary-Blair vials for several days prior to being processed, and this may have decreased the viability of some of the bacteria. It is plausible that if cultures had been performed on site, the yield would have been higher. (ii) Fecal PCR was done on specimens that were frozen and then thawed. The presence of DNases in intestinal secretions may have decreased the amount of DNA present. (iii) The conditions used for the card PCR and the multiplex fecal PCR could have affected the concordance of the two tests. (iv) Different parts and amounts of the fecal specimen were sampled for each one of the tests. Additional studies that take these aspects into consideration are warranted.

We conclude that after prolonged storage, fixed cards that are used for the detection of occult blood are a source of DNA for the detection of diarrheagenic *E. coli*. Our study emphasizes the need for alternative means of the collection, transport, and storage of enteric pathogens for epidemiologic purposes that can replace or complement current methods. We plan studies to examine the detection of other non-*E. coli* enteropathogens by using this technique. We do not know how much longer than 14 months the Hemoccult card can be maintained and still reliably be used for DNA studies.

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### REFERENCES


