Evaluation of the Premier EHEC Assay for Detection of Shiga Toxin-Producing Escherichia coli

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An enzyme-linked immunosorbent assay for the detection of Shiga toxins (Premier EHEC assay; Meridian Diagnostics, Inc.) was compared to conventional sorbitol-MacConkey culture for the recovery of enterohemorrhagic Escherichia coli. A total of 74 enteric pathogens, including 8 E. coli O157:H7 isolates, were recovered from 974 stool specimens. Two of these specimens were not tested by Premier assaying due to insufficient sample and are not considered in the data analysis. The Premier EHEC assay detected the 6 evaluable specimens which were culture positive for E. coli O157:H7 and identified an additional 10 specimens as containing Shiga toxin. Seven isolates were recovered from these 10 specimens by an immunoblot assay and were confirmed as toxin producers by a cytotoxicity assay. Of these seven, four isolates were serotype O157:H7, one was O26:NM, one was O6:H-, and one was O untypeable:H untypeable. Three specimens contained Shiga toxin by both EHEC immunoassaying and cytotoxin testing; however, no cytoxin-producing E. coli could be recovered. The sorbitol-MacConkey method had a sensitivity and a specificity of 60 and 100%, respectively, while the Premier EHEC assay had a sensitivity and a specificity of 100 and 99.7%, respectively, for E. coli O157:H7 only. The Premier EHEC assay also detected an additional 20% of Shiga toxin-producing E. coli (STEC) that were non-O157:H7. Thus, the Premier EHEC assay is a sensitive and specific method for the detection of all STEC isolates. Routine use would improve the detection of E. coli O157:H7 and allow for determination of the true incidence of STEC other than O157:H7. The presence of blood in the stool and/or the ages of the patients were poor predictors of the presence of STEC. Criteria need to be determined which would allow for the cost-effective incorporation of this assay into the routine screen for enteric pathogens in high-risk individuals, especially children.

Shiga toxin-producing Escherichia coli (STEC) strains are an important cause of epidemic and endemic diarrhea, hemorrhagic colitis, and hemolytic-uremic syndrome (8). The most commonly reported serotype associated with outbreaks in the United States is O157:H7, but as many as 50 other serotypes of E. coli also produce Shiga toxins and have been reported to be associated with outbreaks and sporadic disease both within and outside the United States (6, 18, 20).

Since 1993, the Council of State and Territorial Epidemiologists has recommended the culture of all stools or, minimally, of bloody stools for E. coli O157:H7 (4). Since that time, the number of reported E. coli O157:H7 infections has risen steadily (2, 3). Most commercially available diagnostic tests are dependent on O157:H7 serotype-specific attributes, i.e., lack of sorbitol fermentation on sorbitol-MacConkey (sMac) agar or the lack of 4-methylumbelliferyl-β-D-glucuronidase activity. Tests which rely on reaction with specific O157:H7 antibody are also available. The number of documented infections with STEC other than O157:H7 is probably an underestimate, due to the use of these serotype-specific methods (1, 11, 17).

The virulence factor associated with severe gastrointestinal symptoms and the complications of hemolytic-uremic syndrome is the production of Shiga toxins (13). To reliably identify patients with STEC-associated diseases requires an assay which detects the Shiga toxins and not just one of the many serotypes of E. coli known to produce Shiga toxins. Cytotoxicity assays can detect Shiga toxin 1 (Stx1) and Shiga toxin 2 (Stx2) and are capable of detecting all serotypes of STEC (8). However, these assays are labor-intensive and cumbersome and, thus, not well suited for routine clinical laboratory use. Recently, an enzyme immunoassay, the Premier EHEC assay (Meridian Diagnostics Inc., Cincinnati, Ohio), which detects Shiga toxins by both EHEC immunoassaying and cytotoxin testing; however, no cytoxin-producing E. coli could be recovered. The sorbitol-MacConkey method had a sensitivity and a specificity of 60 and 100%, respectively, while the Premier EHEC assay had a sensitivity and a specificity of 100 and 99.7%, respectively, for E. coli O157:H7 only. The Premier EHEC assay also detected an additional 20% of Shiga toxin-producing E. coli (STEC) that were non-O157:H7. Thus, the Premier EHEC assay is a sensitive and specific method for the detection of all STEC isolates. Routine use would improve the detection of E. coli O157:H7 and allow for determination of the true incidence of STEC other than O157:H7. The presence of blood in the stool and/or the ages of the patients were poor predictors of the presence of STEC. Criteria need to be determined which would allow for the cost-effective incorporation of this assay into the routine screen for enteric pathogens in high-risk individuals, especially children.

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MATERIALS AND METHODS

Specimens. Consecutive stool specimens (974 specimens) from outpatients or from inpatients less than 3 days after admission were submitted to Children's Hospital of Wisconsin from 1 December 1993 to 30 November 1994 for routine enteric pathogen culture. Blood was recorded as present when it was visible to the technologist or was reported as such by the person submitting the sample. Stool specimens were processed for routine enteric pathogen culture. Blood was recorded as present when it was visible to the technologist or was reported as such by the person submitting the sample. Stool specimens were inoculated into 5 ml of sterile MacConkey or sMac broth (DIFCO, Detroit, Mich.). The specimens were vortexed for 10 to 15 s and allowed to stand for 10 to 15 s and incubated for 16 to 24 h at 37°C.

The first 500 specimens were tested for the presence of Shiga toxin by Premier EHEC assay and cytotoxicity assay. Of the remaining specimens, only those positive for Shiga toxin by Premier EHEC or positive for E. coli O157:H7 on sMac agar were confirmed by cytotoxicity testing. Specimens which were Premier EHEC positive and sMac negative were recultured to isolate STEC by the mitomycin immunoblot procedure (7), which is described below. The organisms were then tested directly in the cytotoxicity assay to demonstrate the production of cyto-
TABLE 1. Enteric pathogens identified from 974 stool samples tested at Children’s Hospital of Wisconsin from December 1993 to November 1994

<table>
<thead>
<tr>
<th>Enteric organism</th>
<th>Prevalence no. (%)</th>
<th>Blood* (%)</th>
<th>Median age (yr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shigella spp.</td>
<td>30 (3.1)</td>
<td>2/4 (14.3)</td>
<td>3.4</td>
</tr>
<tr>
<td>Salmonella spp.</td>
<td>27 (2.8)</td>
<td>2/15 (13.3)</td>
<td>1.1</td>
</tr>
<tr>
<td>Campylobacter jejuni</td>
<td>9 (0.9)</td>
<td>1/6 (16.7)</td>
<td>5.1</td>
</tr>
<tr>
<td>E. coli O157:H7†</td>
<td>12 (1.2)</td>
<td>3/12 (25)</td>
<td></td>
</tr>
<tr>
<td>STEC other than O157:H7</td>
<td>3 (0.3)</td>
<td>0/3 (0)</td>
<td>6.3</td>
</tr>
<tr>
<td>No bacterial pathogens</td>
<td>893</td>
<td>36/567 (6.3)</td>
<td></td>
</tr>
</tbody>
</table>

* Number of samples with visible blood/total number of samples with information supplied concerning presence or absence of visible blood.
† Detected by sMac culture or Premier EHEC.
‡ Detected by Premier EHEC and confirmed by immunoblot and cytotoxin assays.

RESULTS

A total of 74 enteric pathogens, including 8 E. coli O157:H7 isolates, were recovered from 974 stool specimens. Two of the 8 specimens were not tested by EHEC and cytotoxin assay due to an insufficient amount of sample and were not considered in the Premier EHEC data analysis. Table 1 lists the enteric pathogens identified during that period of time, the presence of blood in the stool specimen, and the median ages of the patients. The presence or absence of blood was reported in only 63% of the specimens tested. It was reported for all 15 specimens that were positive for STEC; however, only 3 of these (20%) were positive for blood. The median age of children with Salmonella, Shigella, and Campylobacter was 3.0 years (range, 0.1 to 18.5 years), while the median age of children with STEC was 6.3 years (0.1 to 17.1 years).

The Premier EHEC assay detected the 6 evaluable specimens which were sMac culture positive for E. coli O157:H7 and identified an additional 10 specimens as containing Shiga toxin. Seven isolates were recovered from these 10 specimens by the immunoblot assay and were confirmed as toxin producers by the cytotoxicity assay. Of these seven isolates, four were serotype O157:H7, one was O26:NM, one was O6:H1—, and one was O unknown:H unknown. Three specimens contained Shiga toxin by both EHEC and cytotoxicity assaying, but no cytotoxin-producing E. coli could be recovered by the immunoblot assay, and these were considered false-negative Premier EHEC tests.

The sMac culture method had a sensitivity of 60% for the detection of E. coli O157:H7. The Premier EHEC assay had a sensitivity of 100%, a specificity of 99.7%, and a positive predictive value of 81% for E. coli O157:H7 (Table 3). In addition to an improved sensitivity for the detection of E. coli O157:H7, the Premier EHEC assay also detected an additional 20% more STEC.

DISCUSSION

The purpose of the study was to determine the utility of the Premier EHEC assay and compare it to that of sMac agar
culture, which is the method most commonly used in routine clinical microbiology laboratories, for the detection of STEC. Cytotoxicity testing had been shown to be more sensitive than SMac culture (19); therefore, the first 500 specimens were tested for Shiga toxin by both Premier EHEC and cytotoxicity tests. The cytotoxicity test detected no specimens which contained Shiga toxin which were not identified as such by Premier EHEC. The cytotoxicity test, as performed here, and the Premier EHEC assay have comparable detection limits for Stx1 and Stx2. Thus, we expected them to have comparable sensitivities. In a separate study, an additional 30 specimens collected during a daycare outbreak of E. coli O157:H7 were tested, and, again, no cytotoxin-positive—Premier EHEC-negative specimens were found (data not shown). This confirms the manufacturer’s findings that the sensitivity of the Premier EHEC assay was comparable to that of the cytotoxicity assay.

The Premier EHEC assay was more sensitive than SMac culture for the detection of E. coli O157:H7 and detected an additional four cases. In all of these cases, E. coli O157:H7 was subsequently detected by the immunoblot colony assay. There were no Premier EHEC-negative—SMac-positive specimens. SMac culture relies on the identification and serotyping of isolated non-sorbitol-fermenting colonies. Of the specimens tested by both methods, SMac culture detected only 60% of the E. coli O157:H7 isolates. The low level of sensitivity of this method compared to those of a colony-sieving method, an EIA, and an immunofluorescence method (14, 15), as well as that of cytotoxin testing (19), has also been demonstrated. Since an ever increasing number of laboratories have implemented testing for E. coli O157:H7, the reported incidence of E. coli O157:H7 has increased (2, 3). The low sensitivity of this culture method suggests a much higher incidence of E. coli O157:H7 disease than what has been previously reported.

In addition to the improved detection of E. coli O157:H7, the Premier EHEC assay also detected three non-O157:H7 isolates of STEC. None of these non-O157:H7 specimens was reported to contain blood; however, all three patients were diagnosed with gastroenteritis. Since this clinical presentation is consistent with E. coli infection and since no other enteric pathogens were detected, we assume that these are significant findings. Thus, 3 of 15 (20%) of the STEC isolates would not have been detected by serotype-specific methodology. In a similar study employing a free verotoxin test, an additional 30% of the STEC isolates were detected (17). There are many reports worldwide of non-O157:H7 STEC causing disease and outbreaks, as well as hemolytic-uremic syndrome (16). If the prevalence of non-O157:H7 organisms is similar nationwide, there is also a much higher incidence of E. coli non-O157:H7 disease than what has been previously reported.

There were three specimens that were positive by the Premier EHEC assay and cytotoxicity testing; however, no STEC could be detected even with the use of the immunoblot colony assay. Edwardsiella tarda was isolated from one specimen, and one patient was admitted for respiratory infection and possible sepsis. No information on the clinical presentation of the third patient was available. The immunoblot method may have been too insensitive to detect low numbers of organisms present in these specimens, or the toxin test may be able to detect toxin persisting in the absence of viable organism, as was suggested by Ramotar et al. (17). However, for the purposes of our analysis, these were considered false-positive Premier EHEC results.

The prevalence of STEC (both O157:H7 and non-O157:H7) in this study was 1.3%, which is higher than that of Campylobacter species at our institution. This high prevalence would argue for the routine testing for STEC as part of an enteric-pathogen evaluation. However, this additional testing would add to the cost of enteric pathogen screening.

It has been suggested that the presence of blood be used to select specimens for E. coli O157:H7 culture (4). In our study, the presence or absence of blood was reported for only 65% of the specimens tested. Of the 15 STEC specimens for which the presence or absence of blood was reported, only 27% were positive for blood, resulting in a positive predictive value of only 7%. As shown in Table 1, there appears to be a higher association between the presence of blood and STEC; however, this is a poor predictor of STEC and is thus not useful as an initial screen.

Because of the high risk of hemolytic-uremic syndrome in children with STEC infections, it has also been suggested to limit STEC testing to children. Since this study was performed in a children’s hospital, we have data only for individuals less than 18 years old. The age-specific incidence of hemolytic-uremic syndrome in Minnesota in 1988 varied from 0.41 to 1.15 per 100,000 children who were 5 to 15 years old to 5.8 per 100,000 children less than 5 years old (12). The median age of children with STEC in our study was 6.3 years, with the 75th percentile being 12 years and a range of <1 to 17 years. From these data, it appears that STEC infection is common in older children as well. If we had limited testing to <5-year-old children, more than 50% of our patients with STEC would not have been detected. Although there is an increased risk of hemolytic-uremic syndrome in young children, the development of age-specific criteria can be controversial and problematic.

The Premier EHEC assay detected 40% more STEC than did conventional SMac culture. STEC other than O157:H7 accounted for 20% of STEC disease in children. Use of this assay would allow us to determine the true incidence of non-O157:H7 disease as well as improve detection of STEC O157:H7. However, the cost of the assay may limit its widespread use. Good criteria which would allow for the cost-effective utilization of this assay need to be determined. Previously suggested screening criteria, such as bloody stools and patient age, are poor predictors of the presence of STEC. Based on the high prevalence of STEC disease in children and the risk of hemolytic-uremic syndrome, the Premier EHEC assay should be considered part of routine enteric-pathogen evaluation in children.

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