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# Preliminary evaluation of a procedure for improved detection of Shiga toxin-producing *Escherichia coli* in fecal specimens

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**Abstract**

Culture confirmation of Shiga toxin-producing *Escherichia coli* (STEC) is very important for epidemiologic analysis. However, isolation of non-O157 STEC on conventional selective media such as sorbitol–MacConkey agar (SMAC) can be difficult because of heavy growth of competing bacteria and its phenotypical similarity to commensal nonpathogenic *E. coli*. An acid enrichment procedure was introduced in this study to facilitate detection of STEC from patients who were symptomatic. Forty-seven clinical fecal broths, which tested positive for Shiga toxin by commercial immunoassay, were processed for the isolation of STEC by both conventional and the acid enrichment methods. The acid enrichment method and conventional culture recovered STEC from 91% (43/47) and 70% (33/47) of the fecal broths, respectively. Neither method retrieved STEC in 3 specimens. Thirty-six STEC were successfully serogrouped, which included O26 ($n = 11$), O157 ($n = 9$), O103 ($n = 7$), O121 ($n = 3$), O111 ($n = 2$ each), O28AC, O146, O76, and O undetermined ($n = 1$ each). The analysis of STEC isolates by real-time PCR indicated that all 9 *E. coli* O157 contained *stx2* gene alone or in combination with *stx1*. Non-O157 STEC more frequently contained *stx1* only, and about one-third possessed *stx2*. The novel acid enrichment protocol greatly reduced the growth of competitor colonies on RTN and TCSMAC. The study demonstrated that incorporation of an acid enrichment procedure in clinical testing improved the isolation of STEC in fecal specimens.
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*Keywords:* Shiga-Toxin Producing *E. Coli* (STEC); Acid Enrichment; Clinical fecal specimens; Rainbow agar plus tellurite and novobiocin (RTN); Sorbitol MacConkey agar plus cefixime and tellurite (TCSMAC); Enzyme Immunoassay (EIA)

## 1. Introduction

Culture confirmation of Shiga toxin-producing *Escherichia coli* (STEC) is very important for epidemiologic purposes. Non-O157 STEC have been nationally notifiable since 2000 and O157:H7 STEC since 1987 (Anonymous, 2006). Historically, fewer non-O157 STEC infections are reported due in part to a lack of effective cultural methods to detect the multiple serotypes comprising the STEC group. Although detection of non-O157 serotypes is difficult, some studies have shown non-O157 STEC infections represent over 50% of laboratory-confirmed STEC cases and caused illnesses that range from mild diarrhea to bloody diarrhea, hemorrhagic colitis, and hemolytic–uremic syndrome (HUS) (Anonymous, 2007; Brooks et al., 2005; Fey et al., 2000; Jelacic et al., 2003).

Clinical laboratories typically use sorbitol–MacConkey (SMAC) agar, a culture method, to identify STEC O157, which cannot ferment sorbitol and therefore forms colorless colonies. However, most non-O157 STEC strains ferment sorbitol and form pink colonies like intestinal flora. Therefore, SMAC cannot be used to readily differentiate between sorbitol-fermenting non-O157 STEC strains and other sorbitol-fermenting intestinal flora growing on the plate. Typically, the identification of O157 and non-O157 has been facilitated by enzyme immunoassay (EIA) and/or optical immunoassay (Kehl et al., 1997; Stapp et al., 2000; Teel et al., 2007). However, recent studies have highlighted the importance of culture confirmation in STEC infections (Anonymous, 2007; Brooks et al., 2005; Fey et al., 2000; Jelacic et al., 2003). Procedures to improve the culture

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confirmation of both O157 and non-O157 STEC would be important to speed the outbreak investigations.

No single selective agar is effective for all STEC. Klein et al. (2002) reported that sole reliance on SMAC screening would have resulted in 28% underreporting of STEC, and sole reliance on EIA would have underdetected *E. coli* O157:H7 by 11%. TCSMAC, Rainbow agar, CHROMagar, and O157:H7ID agars are all designed primarily for detection of O157:H7 (Bettleheim, 2005). Even when only O157:H7 is being sought, Pao et al. (2005) reported successful recovery from spiked feces only when inoculation levels were $>10^5$ CFU/g using SMAC and $>10^3$ CFU/g using TCSMAC. Isolation failures at lower levels were attributed to overgrowth by nontarget competitor microorganisms.

The reduction of background microflora with concomitant improvement in STEC detection has been reported in food and water samples using a 2-step acid enrichment protocol (Grant, 2004, 2005, 2008). A modification of this method was evaluated using overnight enrichments of fecal broths which tested Shiga toxin (Stx)-positive by EIA.

## 2. Materials and methods

### 2.1. Conventional analysis

Fecal broth culture specimens from clinical laboratories were sent to the Washington State Public Health Laboratories (WAPHL) if the culture tested positive for Stx by EIA at the submitting laboratories. At WAPHL, conventional analyses of these specimens include the qualitative detection of Stx1 and Stx2 using ImmunoCard STAT!EHEC (Meridian Diagnostics, Cincinnati, OH), and/or Premier™ EHEC (Meridian Diagnostics, Cincinnati, OH). The samples are also streaked onto SMAC agar and MacConkey agar (MAC) to screen for typical sorbitol-negative colonies. When only sorbitol-positive colonies are present on SMAC agar, sets of 10 additional colonies are analyzed until 20 total colonies from each specimen are tested for Stx using ImmunoCard STAT!EHEC. Premier™ EHEC kit would be used if there are many specimens. Stx-positive isolates are then serogrouped.

### 2.2. Shiga toxin test

The Stx test was performed following manufacturer's instructions. Briefly, for ImmunoCard STAT!EHEC, 150 μL of the diluted enrichment fecal broth was added to the sample port of the card with incubation of the test at 20–25 °C for 20 min. The results were then read within 1 min after incubation. For Premier™ EHEC, 100 μL of diluted enrichment broth was added to the appropriate microwell, and the plate then incubated at 20–25 °C for 1 hour. The wells were washed 5 times with washing buffer. Then, the detection antibody was added and mixed by shaking the plate for 30 s. The wash procedure was repeated, and 2 drops of enzyme conjugate were added and mixed by shaking for 30 s. Incubate the plate for 30 min at 20–25 °C. Repeat the wash procedure, add 2 drops of substrate solution II to each well, shake the plate for 30 s, incubate for 10 min at 20–25 °C; add 2 drops of Stop Solution II to each well, shake the plate for 30 s. Observe the reactions visually within 15 min after adding Stop Solution II.

### 2.3. Serotyping

The *E. coli* OK antisera were purchased from Statens Serum Institut/MiraVista Diagnostics (Indianapolis, IN). Serotyping was performed according to the manufacturer's instruction. Briefly, a single colony of overnight culture of *E. coli* was mixed with a drop of antiserum on a glass slide. The reaction was then read in 5–10 s. A positive reaction is seen as a visible agglutination. A negative reaction is the persistence of the homogeneous milky turbidity. Late or weak agglutination reaction is considered negative. The isolates that could not be typed using the available antisera were sent to CDC for further characterization.

### 2.4. Acid enrichment procedure

The experimental acid enrichment procedure used TSB/3.00 and TYTP. TSB/3.00 is the acid enrichment medium and was prepared by adding concentrated HCl to trypticase soy broth (TSB, Becton Dickinson, Sparks, MD) to a final pH of 3.0 (approximately 5.3 mL/L). TYTP is the neutralization/growth medium used in these experiments. TYTP contained trypticase soy broth plus 12 g/L yeast extract (Becton Dickinson), 12.5 g/L TRIS crystallized free base (Fisher, Fair Lawn, NJ), and 1 g/L sodium pyruvate (Sigma-Aldrich, St. Louis, MO). The pH of TYTP was approximately pH 8.7. TSB/3.00 was sterilized by autoclaving at 121 °C for 10 min and TYTP was sterilized by autoclaving at 121 °C for 15 min.

The acid enrichment procedure is a modification of method described by Grant (2005). Briefly, 1 mL of fecal broth culture was added to 9 mL TSB/3.00. This was held at 20–25 °C for 30 min and then combined with 10 mL of TYTP, which had been prewarmed to 42 °C. The contents were mixed thoroughly and incubated at 42 °C overnight (typically 20–22 h) without shaking. The resulting enrichment culture was then evaluated by spreading onto Rainbow agar (Biolog, Hayward, CA) with 0.8 mg $L^{-1}$ tellurite and 10 mg $L^{-1}$ novobiocin (RTN), in addition to TCSMAC, and these plates were incubated at 35–37 °C for 24 h. Sets of 5 gray, black, purple, or magenta colonies from RTN agar, or clear or pink colonies from TCSMAC agar plates, were selected for real-time PCR analysis of the Stx-encoding genes *stx1* and *stx2* (Sharma and Dean-Nystrom, 2003). For the experiments described in Table 2, a comparison of enrichments was made by placing 1-mL volumes of submitted GN broth cultures into 99 mL fresh GN broth and incubating overnight at 42 °C. To prepare DNA templates for real-time PCR analysis of *stx1* and *stx2* genes, 1 mL volume from each enrichment broth was pelleted by centrifugation, the supernatant was removed, and the cell pellets was resuspended in

100 μL of sterile nuclease free water and then boiled for 10 min. Viable counts of *stx*-positive colonies were determined by randomly selecting approximately 20 presumptive positive colonies from plates prepared from each fecal broth and analyzing each colony individually for *stx* by real-time PCR, and then multiplying the percent positive by total count.

### 2.5. Real-time PCR detection of STEC

Real-time PCR was performed using an ABI 7500 platform (Applied Biosystems, Foster City, CA) as described previously (Sharma and Dean-Nystrom, 2003). The primers used for *stx1* include stx1-forward (5′ GACTGCAAA-GACGTATGTAGATTCG 3′), stx1-reverse (5′ ATC-TATCCCTCTGACATCAACTGC 3′), and stx-1 probe (5′ FAM-TGAATGTCATTCGCTCTGCAATAGGTACTC- 3′). The primers used for *stx2* include stx-2 forward (5′ ATTAACCACACCCCACCG 3′), stx-2 reverse (5′ GTCATGGAAACCGTTGTCAC 3′), and stx-2 probe (5′ VIC-CAGTTATTTTGCTGTGGATATACGAGGGCTTG-3′). The quencher dye TAMRA (6-carboxytetramethyl-rhodamine) was attached to the 3′ ends of the probes. Primers and probes were synthesized by Integrated DNA technologies (Coralville, IA). Reaction was performed in a final volume of 25 μL consisting of 12.5 μL of 2× PCR Supermix (Invitrogen, Carlsbad, CA), 0.75 μL of each primers (10 μmol/L), 0.25 μL of each probe (10 μmol/L), 2 μL cell suspension, and 7 μL of nuclease-free water. The cycling conditions were 95 °C for 10 min followed by 40 cycles of 94 °C for 20 s, 55 °C for 30 s, and 72 °C for 45 s. Fluorescence data were collected during the 72 °C stages using the channels for ROX (the reference dye), FAM (*stx1*), and VIC (*stx2*).

## 3. Results

During the study period, 47 fecal broth enrichments that tested positive for Stx by EIA at clinical diagnostic laboratories in Washington state were sent to WAPHL for confirmation and isolation of STEC. Stx test was repeated at WAPHL using ImmunoCard STAT!EHEC to confirm the presence of either Stx 1 or 2, or both, in each specimen submitted. Subsequently, conventional culture and experimental acid enrichment methods were used in parallel for the isolation of STEC from the fecal broth cultures. A comparison of the results from the 2 methods is shown in Table 1. A total of 32 STEC isolates were identified from the 47 specimens tested using both methods. However, the acid enrichment method produced STEC from an additional 11 specimens. Conversely, STEC from one specimen was recovered by the conventional method but not by the acid enrichment protocol. The sample was determined as STEC negative when a total of 20 colonies were Stx-EIA negative and *stx1*/*stx2*-PCR negative. Overall, acid enrichment recovered STEC from 43 (91%) of 47 specimens, while conventional culture recovered STEC from 33 specimens (70%). Three specimens did not produce STEC isolates by either method. Direct real-time PCR analysis on the 3 fecal broth enrichments, as well as on the colony sweeps from MAC, SMAC, RTN, and TCSMAC agar plates, produced negative results for *stx1* and*stx2*.

The bacterial growth on SMAC, RTN, and TC-SMAC was compared between conventional culture and acid enrichment methods. It was noted that use of the acid method typically reduced the background population as shown in Fig. 1.

Thirty-six of the forty-four STEC were identified to specific serogroups. Eleven were O26, 9 were O157, and 7 were O103. The remaining 9 STEC represent 6 different serogroups (see Table 1). One STEC was identified by polyvalent antisera, which contained O44, O55, O125, O126, O146, and O166. Seven STEC were difficult to serogroup, and the results are still pending during the preparation of this manuscript. However, the laboratory testing indicated that they belonged to non-O157 STEC based on the appearance of the colonies on SMAC or TCSMAC agar plates (sorbitol-positive) and negative reaction against O157 antisera. In summary, of the 44 fecal broth cultures, 9 contained *E. coli* O157 and 35 contained non-O157 STEC.

Analysis of the STEC by real-time PCR indicated that all *E. coli* O157 (100%) contained *stx2* genes, 7 of them (78%) contained both *stx1* and *stx2*, and none contained *stx1* alone. Of the non-O157 STEC, 24 contained *stx1* only (69%), 6 contained *stx2* only (17%), and 5 contained both *stx1* and *stx2* (14%).

For quantitative comparison of enrichment protocols, 6 additional broth cultures that were Stx-positive by EIA were enriched overnight in GN broth and by the acid enrichment (Fig. 2). As shown in Table 2, greater numbers of *stx*-containing cells were generated in 4 of 6 specimens with the acid method, as indicated by lower Ct values and larger viable counts. In specimens 14666 and 14678, low Ct values, indicating large target populations, were obtained from both enrichment methods. An $stx_1$-containing *E. coli* was isolated from 14449 in addition to an $stx_1$-containing *Enterobacter* sp. This isolate was identified as *E. cloacae* by API20E (bioMerieux, Durham, NC), *E. hormaechei* by Biolog (Biolog Inc., Hayward, CA), and *E. sakazakii* by 16S DNA sequencing. *Enterobacter* sp. harboring Stx has been reported, though infrequently, and the lack of agreement by different identification procedures may reflect horizontal *stx* gene transfer among different species (Herold et al., 2004).

## 4. Discussion

The purpose of the study was to determine the utility of the acid enrichment method and to compare it with conventional culture methods for the isolation of STEC. The WAPHL protocol routinely picked 10 sorbitol/lactose-fermenting colonies from SMAC and/or MAC agars to

Table 1
Recovery of STEC from fecal swabs

| Specimen | Color of colonies on MS | Isolation by conventional method[a] | Isolation by acid enrichment at 42 °C | Color of colony on Rainbow agar | Color of colony on TCSMAC | *stx* testing by PCR[b] | Serotype[c] |
|---|---|---|---|---|---|---|---|
| 1 | Pale | + | + | Gray | Pink | 1 | Poly 2[d] |
| 2 | Pink | − | + | Gray/black | Pink | 1 | O26 |
| 3 | Pink | + | − | Light gray | Pink | 2 | O28AC:H25 |
| 4 | Pink | − | + | Purple | Pink | 1 | O26 |
| 5 | Pink/pale | + | + | Gray/black | Pale | 2 | O157 |
| 6 | Pink | + | + | Purple | Pink | 1 | O26:H11 |
| 7 | Pink/pale | − | + | Purple | Pink | 1 | O111 |
| 8 | Pink | − | + | Magenta | Pink | 2 | Pending |
| 9 | Pale | + | + | Gray/black | Pink | 1 and 2 | O157:H7 |
| 10 | Pink | + | + | Purple | Pink | 1 | O111 |
| 11 | Pink | − | − | No growth | No growth | Negative | |
| 12 | Pink | + | Yes | Grayish | Pink | 2 | O121:H19 |
| 13 | Pink/pale | + | Yes | Blue-gray | Pale | 1&2 | O157 |
| 14 | Pink | − | − | No growth | No growth | Negative | |
| 15 | Pink/pale | − | − | No growth | No growth | Negative | |
| 16 | Pink | + | + | Magenta | Pink | 1 and 2 | O26 |
| 17 | Pink | + | + | Purple | Pink | 1 and 2 | O26 |
| 18 | Pink | + | + | Light pink | Pink | 1 | O103:H2 |
| 19 | Pink | + | + | No growth | Pink | 1 | O146 |
| 20 | Pale | + | + | Dark gray | Pale | 1 and 2 | O157 |
| 21 | Pink | + | + | Gray | Pink | 1 | O26:H11 |
| 22 | Pale | + | + | Gray-black | Pink | 2 | O157 |
| 23 | Pink | + | + | Pale | Pink | 2 | Pending |
| 24 | Pink | + | + | Pink/gray | Pink | 1 | O26 |
| 25 | Pink | + | + | Pink/gray | Pink | 1 | Pending |
| 26 | Pink | − | + | Gray | Pink | 1 and 2 | O26 |
| 27 | Pink | + | + | Gray | Pink | 1 | O26:H11 |
| 28 | Pink | + | + | Magenta | Pink | 1 and 2 | O:NM |
| 29 | Pink | + | + | Purple | Pink | 1 | O103:H11 |
| 30 | Pink | + | + | Purple | Pink | 1 | O26:H11 |
| 31 | Pink | + | + | Gray | Pink | 1 | O103:H2 |
| 32 | Pink/pale | + | + | Dark blue/black | Pink | 1 and 2 | O157:H7 |
| 33 | Pink | − | + | Purple | Pink | 1 | O26:H11 |
| 34 | Pink | + | + | Pink | Pink | 2 | O121:H19 |
| 35 | Pink | − | + | Purple | Pink | 1 | O103:H2 |
| 36 | Pink | + | + | Light purple | Pink | 1 | O103:H2 |
| 37 | Pink | + | + | Purple | Pink | 1 | O103:H2 |
| 38 | Pink | + | + | Pink | Pink | 2 | O121:H19 |
| 39 | Pink/pale | + | + | Dark blue/black | Pink | 1 and 2 | O157:H7 |
| 40 | Pink | + | + | Blue/gray | Pink | 1 | Pending |
| 41 | Pink | − | + | Dark blue/black | Pink | 1 and 2 | O157:H7 |
| 42 | Pink | − | + | Purple | Pink | 1 | O103:H2 |
| 43 | Pink/pale | + | + | Dark blue/black | Pink | 1 and 2 | O157:H7 |
| 44 | Pink | + | + | Purple | Pink | 1 | Pending |
| 45 | Pink | − | + | Magenta | Pink | 1 | O76:H19 |
| 46 | Pink | − | + | No growth | Pink | 1 and 2 | Pending |
| 47 | Pink | + | + | Purple | Pink | 1 | Pending |
| Total | | 33 | 43 | | | | |

Conventional versus acid enrichment with 42 °C incubation.

[a] Up to 20 colonies picked from MacConkey–sorbitol agar (MS) and tested for Stx activities using ImmunoCard STAT!HEHC or Premier ™ EHEC by Meridian. Biochemical tests were then performed on the STEC positives.

[b] PCR conducted on colonies picked from RTN, TCSMAC, or MacConkey–sorbitol.

[c] Serotypes of the *E. coli* isolates are determined either using antisera produced by the Statens Serum Institut/MiraVista Diagnostics in the Washington state lab or tested by CDC.

[d] Polyvalent 2 includes O44, O55, O125, O126, O146, O166.

screen for potential non-O157 STEC by ImmunoCard STAT! EHEC or Premier™ EHEC if a large number of specimens is to be tested. The number of Stx-positive colonies out of 10 varied depending on the samples. In many cases, the first set of 10 sorbitol/lactose-fermenting colonies was not sufficient to recover non-O157 STEC, and additional 10 colonies was needed to be tested for Stx. This isolation process is time-consuming and costly.

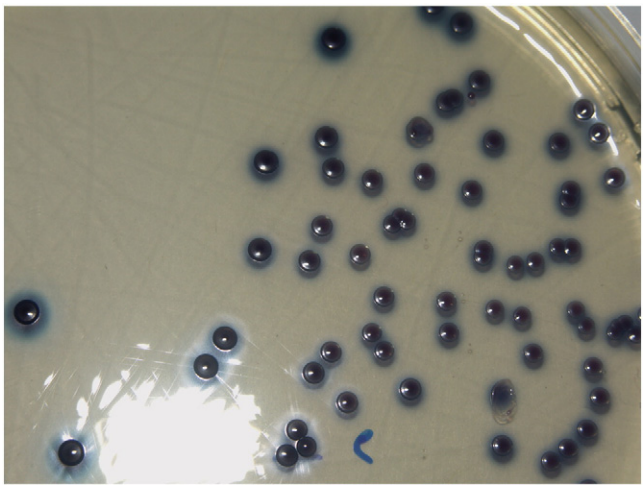

Fig. 1. Recovery of *E. coli* O157 on RTN agar from specimen 5 (Table 1) after acid enrichment.

The data indicated that the acid enrichment method increased the recovery of STEC by 21% increase in the recovery of STEC when compared with the conventional culture method. The lower recovery rate by conventional culture is probably due to the lower levels of STEC in the samples. The fecal broths included in the studies came from multiple clinical laboratories across the Washington state and represented a diverse patient population. In addition, many of the samples were already screened by the submitting laboratories for STEC, and the difficult-to-identify samples were then sent to the state lab for further analysis. Furthermore, the review of the sample submission forms revealed that the samples were delayed as long as up to 13 days in submitting to the WAPHL, which may reduce the number of viable STEC that were mixed within the common microflora and thus affect the isolation of STEC by conventional culture method. The acid shock/treatment combined with incubation at 42 °C can greatly reduced the competing microflora in fecal broths and therefore enhance the detection of STEC. In our parallel study, we compared the number of colonies on SMAC with those on RTN and TCSMAC and noticed that the selective agars streaked after the acid enrichment step often exhibit many more target colonies than with the conventional method. The decreased level of normal microflora and enhanced targeted colonies made the isolation of STEC much easier and more sensitive.

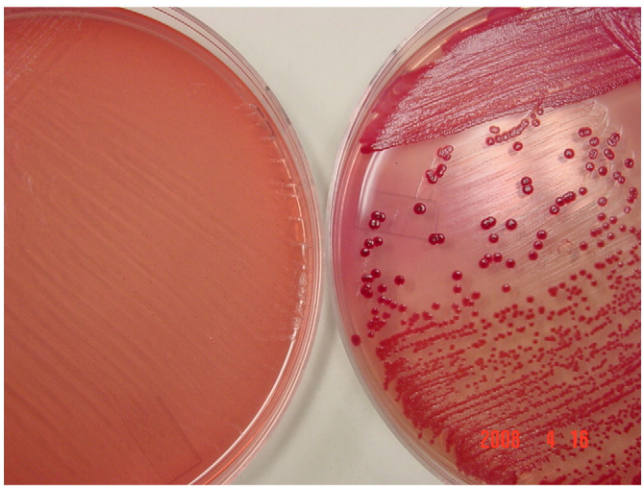

Fig. 2. Recovery of *E. coli* O26 on TCSMAC agar from 14644 (Table 2) after GN broth enrichment (L) or acid enrichment (R).

Table 2
Real-time PCR detection of *stx*1 and *stx*2 after overnight enrichment of presumptive positive fecal specimens by acid or GN broth methods

| Specimen | *stx*1 | *stx*2 | CFU $mL^{-1}$ *stx*-positives | API20E |
|---|---|---|---|---|
| 14025 | | | | |
| Acid enriched | 28.1[a] | 28.4 | $2.0 \times 10^7$ | *E. coli* 5144172 |
| GN enriched | 34.7 | 34.6 | $<5 \times 10^6$ | ND |
| 14449 | | | | |
| Acid enriched | 23.3 | ND | $1.3 \times 10^7$ | *E. coli* 5144572[b] |
| GN enriched | 30.5 | ND | $<5 \times 10^6$ | ND |
| 14556 | | | | |
| Acid enriched | 22.4 | ND | $2.7 \times 10^8$ | *E. coli* 5144562 |
| GN enriched | 34.5 | ND | $<5 \times 10^6$ | ND |
| 14644 | | | | |
| Acid enriched | 22.9 | ND | $8.6 \times 10^7$ | *E. coli* 5144562 |
| GN enriched | ND | ND | $<5 \times 10^5$ | ND |
| 14666 | | | | |
| Acid enriched | ND | 20.6 | $1.8 \times 10^8$ | *E. coli* 5144572 |
| GN enriched | ND | 23.2 | $2.6 \times 10^8$ | *E. coli* 5144572 |
| 14678 | | | | |
| Acid enriched | 23.2 | 23.1 | $1.2 \times 10^7$ | *E. coli* 5144542 |
| GN enriched | 23.2 | 22.4 | $2.7 \times 10^8$ | *E. coli* 5144542 |

ND = not detected.

[a] Ct values are means of duplicate analyses. Ct values of 35–22 correspond to viable counts of $1 \times 10^5$–$5 \times 10^8$.

[b] In addition to *E. coli*, a *stx*-positive *Enterobacter* sp. (API 3305573) was isolated from this sample.

These results are in agreement with earlier studies indicating that acid enrichment is comparable or better than several standard STEC enrichment procedures using food and wastewater samples. Earlier and recent work also demonstrated that variants of the acid enrichment protocol were effective with numerous STEC strains. From those studies, 53 of 53 STEC including O157 were enriched from approximately 1 CFU $mL^{-1}$ to approximately $1 \times 10^9$ CFU $mL^{-1}$ after overnight incubation (Grant, 2004, 2005; and unpublished data). These data would suggest that the acid enrichment procedure (Grant, 2005), as described in this study, followed by neutralization with TYTP and incubation at 42 °C, is an effective strategy with which to improve the culture and subsequent detection of STEC from clinical specimens.

Three positive Stx EIA results from fecal broths without STEC present a dilemma. STEC may have been present in these 3 EIA-positive broths, but alternatively, the EIA signal might have represented a false-positive test, as has been reported (Anonymous, 2006).

Of the 44 STEC culture, 9 were *E. coli* O157 (20%) and 35 belonged to non-O157 STEC (79%). The number of non-

O157 STEC is significantly higher than *E. coli* O157. However, the ratio of the non-O157 versus O157 STEC in this study does not necessarily reflect the prevalence of non-O157 STEC in Washington state due to the inadequate number of samples analyzed. Among the non-O157 STEC identified in this report, the most common serogroups was O26 (30.6%), consistent with the national data as reported by Brooks et al. (2005).

It is reported that the virulence gene *stx2* was significantly associated with an increased risk of HUS in persons with non-O157 STEC (Brooks et al., 2005; Jenkins et al., 2003). About one-third (31%) of the non-O157 STEC identified in this study contained either *stx2* gene alone or in combination with *stx1*. The association of *stx2*-containing STEC with the severity of diseases remains unknown since limited clinical information is available.

The findings in this study demonstrated that the use of an acid enrichment protocol in conjunction with incubation at 42 °C increased STEC detection in fecal specimens. The most beneficial effect of the acid protocol with 42 °C incubation was to lower colony background on selective agars and therefore reduce the possibility of false-negative results due to low numbers of target cells being obscured by the heavy growth of competitors.

The acid enrichment step when combined with the use of TCSMAC agar is a simple and cost-effective method that can be easily applied in clinical laboratories to improve the detection and isolation of STEC. The reduced background of normal flora greatly enhances the recovery of lower levels of STEC in fecal specimens. Use of sensitive and efficient culture confirmation can expedite serotyping and subtyping of isolates and detection of both O157 and non-O157 SETC outbreaks.

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