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Comparison between ImmunoCard STAT!® and real-time PCR as screening tools for both O157:H7 and non-O157 Shiga toxin-producing *Escherichia coli* in Southern Alberta, Canada

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ABSTRACT

An increasing number of non-O157 Shiga toxin-producing *Escherichia coli* (STEC) infections and outbreaks have been reported. In this study, we evaluated the performance of ImmunoCard STAT!® (Meridian Bioscience, Inc., Cincinnati, OH, USA) as a method to screen stool specimens for STEC (O157 and non-O157). An in-house real-time PCR method was used as the "gold standard". We also evaluated the prevalence and clinical characteristics of STEC infections in the Alberta South West Zone. From July to November 2011, 819 stool specimens submitted for routine stool culture were tested. With our in-house real-time PCR, 7 O157:H7 and 10 non-O157 STEC isolates were identified for a total of 17 STECs. In comparison, ImmunoCard STAT!® identified a total of 6, resulting in a sensitivity and specificity of 35% and 99%, respectively ($P < 0.05$). Because of the low sensitivity, ImmunoCard STAT!® cannot be recommended as a routine screening test for STEC from enriched stool specimens. The rate of STEC positivity as detected by PCR was 2.08%, of which 0.86% was O157:H7 and 1.22% non-O157 STEC. Five of the 7 cases of STEC O157 infection experienced bloody diarrhea, and 1 developed hemolytic uremic syndrome.

1. Introduction

Shiga toxin-producing *Escherichia coli* (STEC) is an emerging pathogen responsible for sporadic cases of infection and outbreaks. The disease caused by STEC is characterized by abdominal pain and watery diarrhea, which may progress to bloody diarrhea and hemorrhagic colitis in some individuals (Bowles et al., 2011; Hettiarachchi et al., 2012; Rangel et al., 2005). Complications such as hemolytic uremic syndrome (HUS) (Matsell and White, 2009; Ray and Liu, 2001; Tozzi et al., 2003) will develop in 5–15% of these cases (Black, 2009). HUS is characterized by hemolytic anemia, thrombocytopenia, and acute renal failure (Banatvala et al., 2001). Although the kidney has been found to be the targeted organ, the heart, central nervous system, pancreas, and lungs can be affected in some cases (Clark et al., 2010; Hizo-Abes et al., 2013; Ray and Liu, 2001).

Modes of transmission of STEC include consumption of contaminated food (Berger et al., 2010; Buchholz et al., 2011; Grant et al., 2008; Miller et al., 2012; Pennington, 2010; Pu et al., 2009; Rivas et al., 2003) and water (Clark et al., 2010; Matsell and White, 2009; Salvadori et al., 2009), contact with animals and their environment (Caprioli et al., 2005; CDC, 2012; Crump et al., 2002; Evans et al., 2011; Mather et al., 2008; Meichtri et al., 2004), and person-to-person transmission (Aldabe et al., 2011; Brown et al., 2012; Diercke et al., 2013; Gilbert et al., 2008; Seto et al., 2007; Vaillant et al., 2009).

Epidemiologically, the most commonly recognized STEC serotype is O157:H7, as it has caused major outbreaks in the United States, Canada, Europe, and Japan in the past, highlighting the threat of this pathogen to public health. Consequently, the major focus on outbreaks and HUS caused by STEC has traditionally been on O157:H7. For many years, the diagnostic methods in microbiology laboratories have been centered on the detection of the O157:H7 serotype at the expense of other non-O157 serotypes. However, the non-O157 STEC serotypes have been increasingly recognized recently as emerging pathogens, and some of these, including O111 (CDC, 2012), O26 (Brown et al., 2012), O145 (Folster et al., 2011), and O103 serotypes (Schimmer et al., 2008; Sekse et al., 2009), have been linked to outbreaks and HUS cases in different parts of the world (Elliott et al., 2001; Gould et al., 2013). The downside of the above-mentioned single-focus approach centering solely on the detection of O157 STEC in clinical specimens was highlighted in a ProMED report issued on March 2, 2006 (ProMED-mail, 2006), and subsequently published by Schimmer et al. (2008), in which a community outbreak of O103

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STEC, consisting of numerous diarrhea-associated HUS cases in Norway, was completely missed by the primary diagnostic laboratories. More recently, on May 31, 2011, the World Health Organization reported a large outbreak of STEC O104:H4 originating in Germany that resulted in a total of 4075 diarrheal cases with 908 cases of HUS and 50 fatalities. This outbreak also affected 13 other European countries and North America (Beutin and Martin, 2012). Alternative molecular and other diagnostic methods had to be developed during the early and subsequent phases of the outbreak to allow for the initial and ongoing detection of the causative agent. This significant event highlighted once more the potential of non-O157 serotypes to cause severe infections and death in humans (Wu et al., 2011) and further stressed the need to integrate the detection of non-O157 STEC in the routine stool culture algorithm as per the 2009 guideline recommendation by the Centers for Disease Control and Prevention in Atlanta (http://www.cdc.gov/mmwr/preview/mmwrhtml/rr5812a1.htm).

In this study, we sought to compare the performance of the Immuno*Card* STAT!® (Meridian Bioscience, Inc., Cincinnati, OH, USA), a commercially available qualitative enzyme immunoassay capable of differentiating between Shiga toxins 1 and 2, with our in-house real-time PCR assay, thereby determining the utility of Immuno*Card* STAT!® in the routine screening of stool specimens for O157 and non-O157 STEC serotypes. In addition, we also sought to determine the prevalence of STEC in Southern Alberta and to evaluate the clinical characteristics of the STEC positive patients.

2. Methods

2.1. Specificity of the assays

A panel of organisms was used to determine the specificity of our in-house real-time PCR assay and the Immuno*Card* STAT!® test. This included *Staphylococcus aureus* (ATCC 25923), *Staphylococcus epidermidis* (ATCC 1228), *Enterococcus faecalis* (ATCC 29212), *Micrococcus luteus* (ATCC 49732), *Staphylococcus saprophyticus* (ATCC 15305), *Proteus mirabilis* (ATCC 43071), *Yersinia enterocolitica* (ATCC 9610), *Salmonella typhimurium* (ATCC 14028), *Serratia marcescens* (ATCC 8100), *Shigella sonnei* (clinical isolate A79), *Shigella flexneri* (ATCC 12022), *Klebsiella pneumoniae* (ATCC 13883), *Proteus vulgaris* (ATCC 13315), *Pseudomonas aeruginosa* (ATCC 27853), and *Enterobacter cloacae* (ATCC 13047). The following known negative controls were also included: *E. coli* O26:B6 (clinical isolate, A302), *E. coli* O55:B5 (clinical isolate A301), *E. coli* O86:B7 (clinical isolate A303), *E. coli* O128:B12 (clinical isolate A305), *E. coli* ATCC 25922, and *E. coli* O111:B4 (clinical isolate A300). *E. coli* EDL933, a known positive control for STEC, was part of this specificity panel.

2.2. Clinical samples, sample preparation, and testing

Stool samples collected from 819 patients during the period of July 13 to November 12, 2011, and submitted to the Chinook Regional Laboratory in Lethbridge, Alberta, Canada, for routine stool culture were included in this study. Duplicate patient samples were excluded. The algorithm for stool screening for the presence of STEC is shown in Fig. 1.

2.2.1. Routine stool culture

Submitted stool samples were subjected to routine stool culture for the detection of enteric bacterial pathogens such as *E. coli* O157, *Salmonella* spp., *Shigella* spp., *Campylobacter* spp., *Listeria* spp., and *Yersinia* spp. with direct inoculation of the stool specimen to agar plate media. BBL™ CHROMagar™ O157 (Becton, Dickinson, Inc., Mississauga, ON, Canada) agar plates were used for the detection of O157 STEC. All culture plates were examined after 18 to 24 hours of incubation. Mauve colonies on the BBL™ CHROMagar™ O157 plate were tested for O157 by direct antibody agglutination (BD Difco, Burlington, ON, Canada). There was no attempt made to identify non-O157 STEC on the routine stool culture.

2.2.2. Immunoassay

From each sample, 200 μL of watery stool or a pea-sized solid stool was inoculated into 4.5 mL of MacConkey broth and incubated at 37 °C overnight for culture enrichment and an aliquot of the enriched broth culture was used for the Immuno*Card* STAT!® (Fig. 1). The procedure was carried out as per manufacturer's instructions.

2.2.3. DNA extraction and real-time PCR

In preparation for the DNA template, an aliquot of the above-referenced enriched broth culture was transferred from the middle of the culture tube into a 1.5-mL screw cap centrifuge tube and centrifuged for 3 minutes at 13,000*g*, after which the supernatant was discarded and the pellet was washed with 1 mL of 12 mmol/L Tris buffer, pH 7.4. Following decanting of the Tris buffer, the pellet was re-suspended in rapid lysis buffer (100 mmol/L NaCl; 10 mmol/L Tris-HCl, pH 8.3; 1 mmol/L EDTA, pH 9.0; 1% Triton X-100), boiled for 15 minutes and centrifuged at 13,000*g* for 15 minutes (Holland et al., 2000). The supernatant of this preparation was then used as a source of template in the real-time PCR assay (Fig. 1). Positive and negative STEC stool samples were included in every run for monitoring the extraction procedure.

Oligonucleotide primers and fluorescent probes for stx_1 and stx_2 were described previously (Chui et al., 2010). The probe for stx_2 was modified from the original (STX2-TM-P) by replacing the VIC® dye with the FAM™ dye. Real-time nucleic acid amplification testing was performed as a separate reaction for stx_1 and stx_2 on an ABI Prism® 7500FAST sequence detection system (Life Technologies, Inc., Burlington, ON, Canada) using the following amplification conditions:

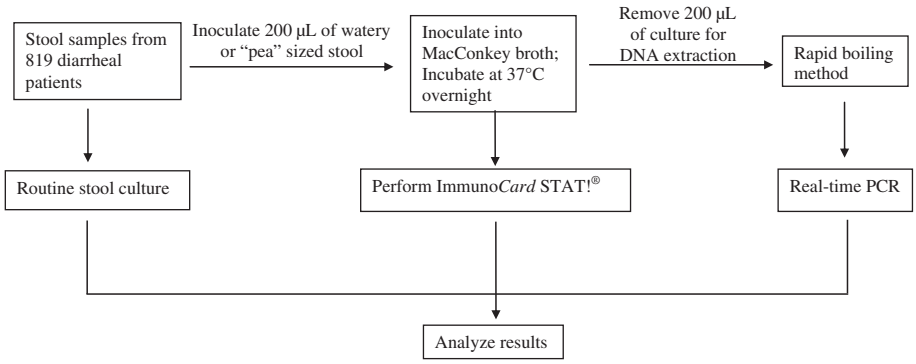

Fig. 1. Algorithm for STEC screening using routine culture, Immuno*Card* STAT!® and real-time PCR.