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journal homepage: www.elsevier.com/locate/diagmicrobioAssessment of commercial chromogenic solid media for the detection of non-O157 Shiga toxin-producing *Escherichia coli* (STEC)Nathan Zelyas^a, Alan Poon^b, Laura Patterson-Fortin^b, Roger P. Johnson^c, Winki Lee^b, Linda Chui^{b,d,*}^a Department of Medical Microbiology and Immunology, University of Alberta, Edmonton, AB, Canada^b Alberta Provincial Laboratory for Public Health, Edmonton, AB, Canada^c Public Health Agency of Canada, National Microbiology Laboratory@Guelph, Guelph, ON, Canada^d Department of Laboratory Medicine and Pathology, University of Alberta, Edmonton, AB, Canada

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ABSTRACT

Detection of Shiga toxin-producing *Escherichia coli* (STEC) has evolved significantly since the introduction of sorbitol-MacConkey agar. This study compares four chromogenic media (CHROMagar™ STEC, Rainbow® O157 agar, CHROMagar™ O157, and Colorex® O157) in their identification of non-O157 STEC. When 161 non-O157 STEC were directly inoculated onto each medium, detection rates on CHROMagar™ STEC, Rainbow® O157 agar, CHROMagar™ O157 and Colorex® O157 were 90%, 70%, 3.7% and 6.8%, respectively. Tellurite minimal inhibitory concentrations (MICs) correlated with growth on CHROMagar™ STEC as 20 of 22 isolates with poor or no growth had MICs ≤1 µg/mL. Stool spiking experiments revealed that CHROMagar™ STEC had the highest recovery of the six most common non-O157 STEC, ranging from 30% (in mucoid stool) to 98% (in watery stool). When using clinical stool samples, CHROMagar™ STEC had a sensitivity, specificity, positive predictive value, and negative predictive value of 84.6%, 87%, 13.9%, and 99.6%, respectively.

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1. Introduction

Escherichia coli O157:H7 is a well-known cause of hemorrhagic colitis and the potentially fatal hemolytic uremic syndrome (HUS; Karmali et al., 1983). Although *E. coli* O157:H7 is the most common agent in Shiga toxin (Stx)-producing *E. coli* (STEC) outbreaks, other serotypes of STEC also cause sporadic disease and outbreaks. There have been numerous outbreaks involving serotypes such as O26, O100, O103, O104, O111, and O121 (Brooks et al., 2004; Buchholz et al., 2011; Lienemann et al., 2011; Lindstedt et al., 2007; McCarthy et al., 2001; Miyajima et al., 2007; Vojdani et al., 2008). As well, several non-O157 serotypes, including O26, O103, O111, and O145, have an apparent predilection for causing HUS (Johnson et al., 2006). In 2012, 486 human cases of *E. coli* O157:H7 were reported from the Canadian National Enteric Surveillance Program, representing an incidence rate of 1.40 per 100,000 (National Enteric Surveillance Program, 2012).

Currently, the Centers for Disease Control and Prevention recommend that all stool specimens submitted from patients with acute community-acquired diarrhea should be cultured for O157 STEC and assayed for Shiga toxin 1 and Shiga toxin 2 or the genes encoding these toxins (Gould et al., 2009). Such recommendations would allow for the detection of O157 as well as non-O157 STEC, aid in the prompt diagnosis of those infected with STEC, and permit STEC isolation and

typing for an early response to outbreaks. This does not usually pose a problem for O157 STEC isolates, but non-O157 STEC can be difficult to identify and isolate.

The detection of most O157 STEC can be done easily using sorbitol-MacConkey agar or CHROMagar™ O157. Neither of these media is capable of differentiating non-O157 STEC from other strains of *E. coli*; until recently, the detection of non-O157 STEC was based on either the detection of Stx using the Vero cell cytotoxicity assay (Karmali, 1989; Konowalchuk et al., 1977), enzyme immunoassay (Milley and Sekla, 1993), or immunochromatography (Chui et al., 2013; Chui et al., 2015), or detection of the *stx*₁ and *stx*₂ genes encoding Stx by PCR (Chui et al., 2010; Feng et al., 2011; Gannon et al., 1992).

A new chromogenic medium, CHROMagar™ STEC (CHROMagar Microbiology, Paris, France) has recently been designed which shows promise for detecting both O157 and non-O157 STEC from clinical samples. On CHROMagar™ STEC both O157 and non-O157 STEC strains typically produce mauve colonies while other *E. coli* strains grow as blue colonies. An added benefit of the medium is that non-O157 colonies can be further differentiated by their ability to fluoresce under UV light. Recent studies have examined the ability of CHROMagar™ STEC to detect STEC from food and clinical stool specimens (Gouali et al., 2013; Hirvonen et al., 2012; McCallum et al., 2013; Tzschoppe et al., 2012; Wylie et al., 2013). While the medium exhibits favourable sensitivity, specificity, and negative predictive value from clinical stool specimens, it has been found to have a positive predictive value of only 13.6–60% (Gouali et al., 2013; McCallum et al., 2013; Wylie et al., 2013).

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This study aimed to determine the growth characteristics of a panel of non-O157 STEC isolates on CHROMagar™ STEC as well as on three other commercially-available STEC agar media. The ability of the different media to detect STEC from stools of varying consistency and from clinical stool samples was also evaluated. Furthermore, the correlation between an isolate's resistance to tellurite and its growth on CHROMagar™ STEC was examined.

2. Materials and methods

2.1. Bacterial strains

A panel of 161 non-O157 STEC strains previously serotyped by the National Microbiology Laboratory, Winnipeg, Manitoba, Canada, and the National Microbiology Laboratory at Guelph, Ontario, Canada, was included in this study (Table 1). STEC O157:H7 strain EDL933 and Stx-negative DH5α were used as positive and negative controls, respectively, in experiments for determining tellurite minimal inhibitory concentrations (MICs) of different STEC isolates. An exclusivity panel consisting of the following bacteria was tested on each chromogenic medium: *Staphylococcus aureus* (ATCC 25923), *Staphylococcus epidermidis* (ATCC 12228), *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), *Enterobacter cloacae* (ATCC 13047), six Stx-negative *E. coli* isolates (*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* O111:B4 [clinical isolate A300], and *E. coli* [ATCC 25922]), and the Stx-positive strain *E. coli* EDL933.

2.2. Culture media

The following supplied solid media plates were used throughout this study: CHROMagar™ STEC (Alere, Inc., Ottawa, ON, Canada), Rainbow® O157 (Biolog, Hayward, CA, USA), CHROMagar™ O157 (Becton, Dickinson and Company, Franklin Lakes, NJ, USA), Colorex® O157 (Alere, Inc., Ottawa, ON, Canada) and MacConkey agar (Dalynn Biologicals, Calgary, AB, Canada). Inoculation on MacConkey agar served as a positive

control. Rainbow® O157 agar plates were prepared from powdered medium as per the manufacturer's instructions. The final concentrations of potassium tellurite (Sigma-Aldrich, St. Louis, MO, USA) and novobiocin (Sigma-Aldrich, St. Louis, MO, USA) in Rainbow® O157 agar were 0.15 mg/L and 5 mg/L, respectively.

2.3. Culture of STEC isolates inoculated directly on agar media

All 161 non-O157 STEC strains were inoculated from frozen skim milk cultures onto sheep blood agar plates (BAPs; Dalynn Biologicals, Calgary, AB, Canada) and incubated overnight at 37 °C. A single colony was picked from each BAP and inoculated onto the solid culture media described in 2.2. After incubating at 37 °C for 24 hours, these cultures were examined for growth characteristics.

2.4. Tellurite MIC determination

A total of 50 isolates from the non-O157 STEC panel, representing each O serogroup and including isolates which did not grow on CHROMagar™ STEC, were grown for 24 hours at 37 °C in Trypticase Soy Broth and then were inoculated at 2×10^4 colony-forming units (CFU; as per routine established protocol in this laboratory correlating optical density with CFU) onto Luria Broth (LB) agar (Becton Dickinson, Franklin Lakes, NJ, USA) incorporating doubling dilution concentrations of potassium tellurite of 1–1024 µg/mL including a control without potassium tellurite added. After incubating these cultures at 37 °C for 24 hours, the presence of colonies was noted. MIC was defined as the lowest tellurite concentration at which no colonies could be observed.

2.5. Stool spiking experiments

Pools of watery, semi-formed, mucoid and bloody stools were prepared from two to six clinical specimens with these characteristics that did not yield STEC-like colonies on all four chromogenic media described in 2.2. The stool pools were made using stool specimens collected by the Alberta Provincial Laboratory for Public Health and DynaLIFE_{DX} Laboratory (both located in Edmonton, Alberta, Canada) from patients with diarrhea in 2012. Once the stool pools were made, they were inoculated on the chromogenic media to confirm the absence of STEC-like organisms. This was to further ensure that prior to spiking, the stool pools did not contain STEC detectable by these culture methods.

Table 1
Growth and characteristics of purified strains of STEC on four agar media.¹

Serogroup ²	Number of isolates	CHROMagar™ STEC			Rainbow® O157		CHROMagar™ O157		Colorex® O157	
		Growth	STEC indication ³	Fluorescence	Growth	STEC indication ³	Growth	O157 indication ³	Growth	O157 indication ³
O26	38	37	37	37	38	34	38	0	38	0
O45	2	2	2	2	2	0	2	0	2	0
O103	19	19	19	14	19	15	19	4	19	4
O111	29	29	29	20	29	29	29	1	29	3
O121	20	20	19	20	20	9	20	0	20	0
O145	11	11	9	11	11	11	10	0	11	0
Total for top 6 non-O157 STEC	119	118 (99%)	115 (97%)	104 (87%)	119 (100%)	98 (82%)	118 (99%)	5 (4.2%)	119 (100%)	7 (5.9%)
Others ⁴	42	30 (71%)	30 (71%)	27 (64%)	42 (100%)	14 (33%)	28 (67%)	1 (2.4%)	42 (100%)	4 (9.5%)
Total for all non-O157 STEC	161	148 (92%)	145 (90%)	131 (81%)	161 (100%)	112 (70%)	146 (91%)	6 (3.7%)	161 (100%)	11 (6.8%)

¹ The number of isolates for each serotype are shown; as mentioned in the Materials and Methods, isolates were also grown on MacConkey agar plates, but because all isolates grew on MacConkey agar and did not show STEC-specific characteristics, this data was not included in the table for comparison

² O26 included O26:H11 (n = 33) and O26:NM (n = 5); O45 included O45:H2 (n = 2); O103 included O103:H2 (n = 15) and O103:H25 (n = 4); O111 included O111:H8 (n = 13), O111:NM (n = 15), and O111:NT (n = 1); O121 included O121:H19 (n = 19) and O121:NM (n = 1); and O145 included O145:NM (n = 11)

³ STEC indication on CHROMagar™ STEC was mauve colonies; STEC indication on Rainbow® O157 was black, gray, purple, or blue colonies; O157 indication on CHROMagar™ O157 and Colorex® O157 was mauve colonies

⁴ Includes less common non-O157 STEC: O5:NM (n = 6), O6:H2 (n = 1), O6:H16 (n = 1), O8:H8 (n = 1), O8:H14 (n = 1), O18:H7 (n = 1), O22:H2 (n = 1), O22:H8 (n = 1), O38:H21 (n = 2), O48:H21 (n = 1), O55:H7 (n = 1), O69:H11 (n = 1), O78:NM (n = 1), O91:H21 (n = 2), O114:H4 (n = 1), O117:H4 (n = 5), O117:H7 (n = 2), O118:H12 (n = 1), O118:H16 (n = 1), O118:H30 (n = 1), O153:H25 (n = 1), O165:H25 (n = 2), O174:H2 (n = 1), O174:H8 (n = 1), OR:H7 (n = 2), OR:H11 (n = 1), OR:NM (n = 2)

MacConkey broths (3 ml) were inoculated with approximately 10^6 CFU of STEC isolates and then spiked with 200 μ L or a cotton-tipped swab amount of the stool pools of differing quality. These spiked liquid cultures were grown for 24 hours at 37 °C; 10 μ L of these cultures were inoculated onto the aforementioned five solid media and growth characteristics were noted. Fig. 1 illustrates this experimental design. In all, the four types of stool pools were tested with 83 isolates from among those listed in Table 1; 41 randomly chosen to represent serotypes of the top six serogroups (including 19 O103 isolates, eight O111 isolates, four O121 isolates, four O145 isolates, four O26 isolates, and two O45 isolates) and all 42 isolates belonging to the less common non-O157 STEC.

2.6. Use of clinical specimens for STEC detection

To evaluate STEC recovery directly from stool, 536 stool specimens collected in 2014 and 2015 from patients with diarrhea from the Chinnook Regional Laboratory in Lethbridge, Alberta, Canada, and the DynaLIFE_{DX} Laboratory were used. Clinical specimens were inoculated directly onto CHROMagarTM STEC and pea-sized volumes or 200 μ L of the same specimens were inoculated into 3 mL of MacConkey broth. All cultures were incubated for 24 hours at 37 °C in the dark. STEC-like colonies on CHROMagarTM STEC and all MacConkey broth cultures were subjected to lysis and real-time PCR as outlined in 2.7. This experimental design is demonstrated in Fig. 1. The Shiga Toxin Quik Chek lateral flow membrane immunoassay (TechLab®, Inc., Blacksburg, VA, USA) was used to resolve discrepancies between CHROMagarTM STEC and broth culture PCR results. This method uses immobilized antibodies against Stx1 and Stx2 on a chromatographic surface to detect the toxins. MacConkey broth cultures of stool or STEC-like colonies from CHROMagarTM STEC were grown at 37 °C for 24 hours and were tested according to the manufacturer's instructions.

2.7. Confirmatory testing of STEC

Testing for the presence of STEC was performed on overnight MacConkey broth cultures inoculated with clinical stool specimens or with STEC-like colonies after growth on CHROMagarTM STEC as well as directly on STEC-like colonies taken from the agar plates. Both real-time PCR and the Shiga Toxin Quik Chek assay were used to detect STEC. Nucleic acid was prepared from MacConkey broth cultures by centrifuging 200 μ L of overnight culture for 3 minutes at 13,000 \times g, washing the pellet with 1 mL of 12 mmol/L Tris buffer, pH 7.4, and resuspending the cells 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). This suspension was boiled for 15 minutes and centrifuged at 13,000 \times g for 15 minutes (Holland et al., 2000). A 1/10 dilution of the suspension was used as template for the PCR amplification assay. For template preparation from colonies, STEC-like colonies on CHROMagarTM STEC were picked with wooden sticks and suspended in rapid lysis buffer, boiled for 15 minutes and then centrifuged for 15 minutes at 13,000 \times g. The resulting supernatant was used as the source of template for real-time PCR. Oligonucleotide primers and probes used for the real-time PCR assay detecting *stx*₁ and *stx*₂ have been described previously (Chui et al., 2010). Amplification was performed in separate reactions for *stx*₁ and *stx*₂ using the ABI Prism® 7500FAST sequence detection system (Life Technologies, Inc., Burlington, ON, Canada). A volume of 5 μ L of template was added to a PCR cocktail mixture containing 1X Taqman® FAST master mix (Life Technologies, Inc.) and 50 mmol/L primers and probes to a 25 μ L final volume. Cycling conditions were as follows: 95 °C for 10 minutes, 40 cycles of 95 °C for 3 seconds, and 60 °C for 30 seconds. DNA extracted from *E. coli* EDL933 and water were included in the amplification assays as positive and negative controls for all the assays.

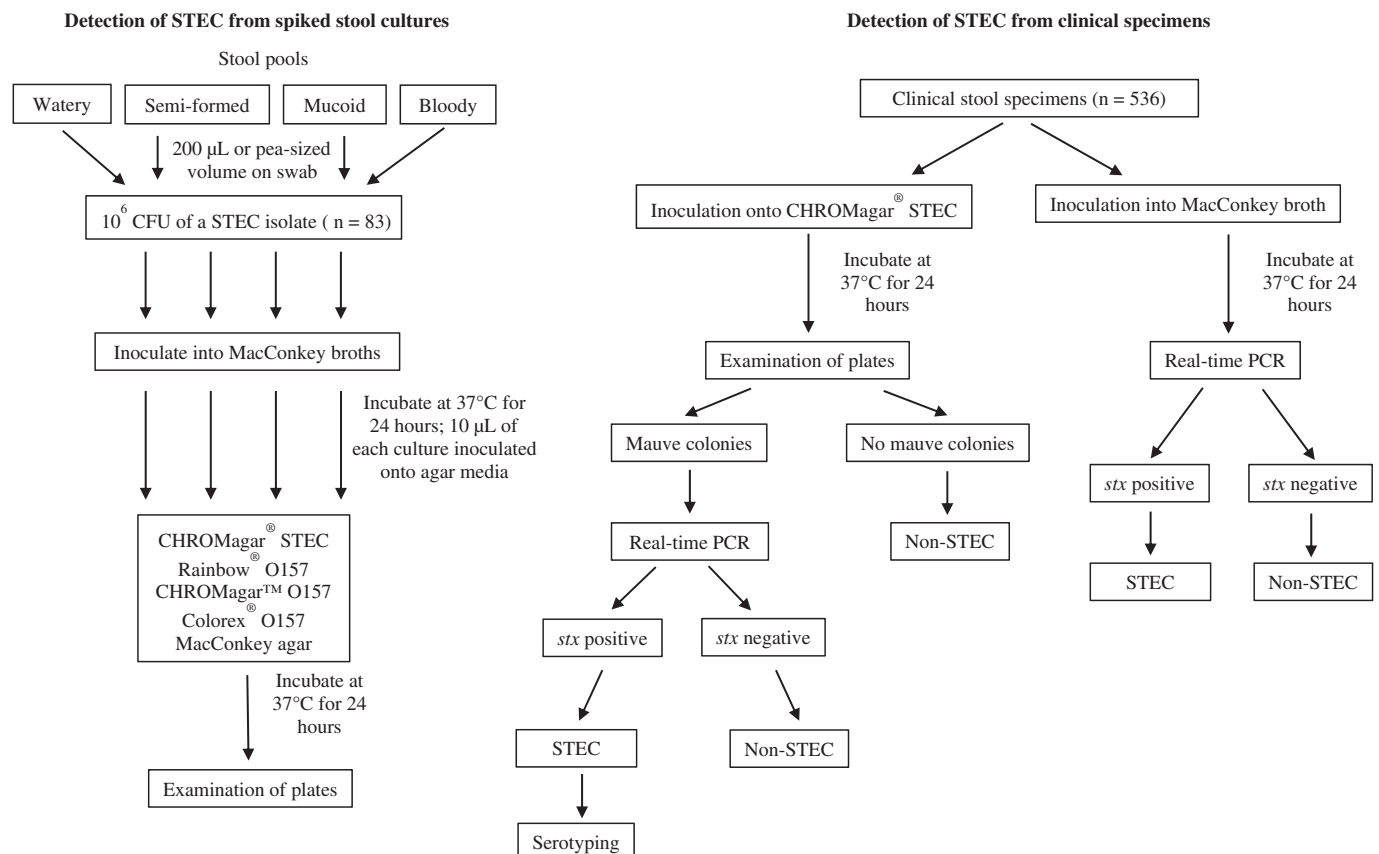


Fig. 1. Experimental designs for the detection of STEC from spiked stool cultures and clinical specimens.

Serotyping of isolates obtained from clinical samples was performed using established serological agglutination protocols at the National Microbiology Laboratory, Winnipeg, Manitoba, Canada.

3. Results

3.1. Growth characteristics of non-O157 STEC isolates on different agar media

The growth and phenotypic indications for STEC are presented in Table 1. Among the 119 strains of serotypes of the top six serogroups of non-O157 STEC, 118 (99%) showed positive growth with 115 (97%) indicative of STEC and 104 (87%) exhibiting fluorescence on CHROMagar™ STEC. All but one (O26:NM) grew on CHROMagar™ STEC. A summary of the results not conforming to the manufacturer's description of typical STEC colony appearance or growth is shown in Table 2. Isolates belonging to the less common non-O157 serotypes were less likely to grow or be identified as STEC on CHROMagar™ STEC (Tables 1 and 2).

While Rainbow® O157 agar supported the growth of all the STEC isolates (Table 1), only 82% of isolates from the six most common serogroups yielded colonies with the appearance of non-O157 STEC. None of the O45 isolates and nine of 20 (45%) of the O121 isolates produced colonies characteristic of STEC. Among the 42 less common non-O157 STEC isolates, 14 (33%) were identified as STEC by Rainbow® O157 agar.

Although CHROMagar™ O157 and Colorex® O157 agar are meant for detection of O157 STEC, non-O157 STEC can occasionally be detected (Bettelheim, 1998a). On both of these media, all four O103:H25 isolates gave mauve colonies characteristic of O157 STEC (Table 1). Only one of 15 O111:NM isolates and one of two OR:H7 isolates produced mauve colonies on CHROMagar™ O157. On Colorex® O157, these same O111:NM and OR:H7 isolates, along with two more O111:NM isolates, an O118:H30 isolate (one isolate tested), an O165:H25 isolate (one of two isolates), and an OR:NM isolate (one of two isolates) generated mauve colonies. All other non-O157 STEC isolates that grew on these media produced blue colonies, which is characteristic of non-STEC *E. coli* strains.

To determine if colony colour or fluorescence under UV light when grown on CHROMagar™ STEC were dependent on Stx production,

strains which produced non-mauve colonies or ones that did not fluoresce were tested for Stx production. Testing by the Shiga Toxin Quik Chek assay revealed that all of the STEC strains without mauve coloured colonies or fluorescence on CHROMagar™ STEC (Table 2) still produced Shiga toxin.

3.2. Tellurite susceptibility of non-O157 STEC isolates

The isolates which did not grow on CHROMagar™ STEC after 24 hours at 37 °C (n = 13) were tested to determine their tellurite MICs. Tellurite MICs were also determined for representative isolates which grew well or poorly (producing only a small number of colonies at the site of inoculation after 24 hours of growth) on CHROMagar™ STEC. The positive control strain EDL933 had a tellurite MIC of 8 µg/mL while the negative control strain DH5α had an MIC ≤ 1 µg/mL. Among 22 isolates with poor or no growth on CHROMagar™ STEC, only two isolates (an O18:H7 and O26:NM isolate) exhibited tellurite MICs above 1 µg/mL. The O18:H7 isolate had a tellurite MIC of 2 µg/mL and that of the O26:NM isolate was 16 µg/mL. All tested isolates with robust growth on CHROMagar™ STEC (n = 28) had MICs between 2 and 512 µg/mL.

3.3. Exclusivity panel testing of the chromogenic media

Reference organisms in the exclusivity panel (listed in 2.1) were inoculated on each of the four agars. The only STEC strain tested (*E. coli* EDL933) produced mauve colonies on CHROMagar™ STEC; the six non-STEC *E. coli* strains tested did not grow on this medium. Seven of the ten non-*E. coli* Gram-negative organisms grew but did not yield mauve colonies on CHROMagar™ STEC; none of the five Gram-positive organisms grew. Rainbow® O157 allowed the growth of all 17 Gram-negative strains as well as *E. faecalis* (ATCC 29212). Additionally, four out of six Stx-negative *E. coli* strains and *E. cloacae* (ATCC 13047) appeared as STEC on Rainbow® O157. None of the non-STEC *E. coli* strains grew on CHROMagar™ O157; it only allowed the growth of *E. faecalis* (ATCC 29212) and *E. cloacae* (ATCC 13047), and neither produced mauve colonies. Colorex® O157 permitted the growth of all Gram-negative isolates and no Gram-positive isolates. *S. typhimurium* (ATCC 14028) was the only Gram-negative organism that generated mauve, O157-like colonies on Colorex® O157.

3.4. Detection of non-O157 STEC from spiked stool cultures

To compare the performance of the different agar media on stool samples, MacConkey broth cultures of representative isolates were spiked with stool pools of differing quality (watery, semi-formed, mucoid, or bloody) and grown overnight. The resulting cultures were inoculated on CHROMagar™ STEC, Rainbow® O157, CHROMagar™ O157, and Colorex® O157 and detection of STEC was recorded. As illustrated by Fig. 2, CHROMagar™ STEC consistently demonstrated the highest detection rates for non-O157 STEC from the four stool pools with different characteristics. The lowest rates of recovery on CHROMagar™ STEC occurred with mucoid stool (<30%). On CHROMagar™ STEC and Rainbow® O157, detection was superior in each stool quality for the six most common non-O157 serogroups than for the other non-O157 STEC serotypes. Although CHROMagar™ O157 and Colorex® O157 are meant for the detection of O157 STEC, they were both able to identify low numbers of non-O157 serotypes from stool belonging predominantly to the six most common non-O157 serogroups.

3.5. Detection of STEC from clinical specimens

Because of the apparent superiority of CHROMagar™ STEC in detecting higher numbers of STEC from previous experiments, it was evaluated using patient specimens. Five hundred and thirty-six stools collected from patients with diarrhea were inoculated onto CHROMagar™ STEC and into MacConkey broth. STEC-like colonies and all MacConkey

Table 2
Non-O157 STEC isolates with unexpected results on CHROMagar™ STEC.

Serotype	Atypical result ^{1,2}	Proportion of isolates of the same serotype with the unexpected result
O26:NM ³	No growth	1/5
O103:H2 ³	Not fluorescent	1/15
O103:H25 ³	Not fluorescent	4/4
O111:NM ³	Not fluorescent	8/15
O111:NT ³	Not fluorescent	1/1
O121:H19 ³	Gray colonies	1/19
O145:NM ³	Gray colonies	2/11
O6:H2	Not fluorescent	1/1
O8:H8	No growth	1/1
O8:H14	No growth	1/1
O55:H7	No growth	1/1
O91:H21	No growth	2/2
O114:H4	No growth	1/1
O117:H4	No growth	3/5
O117:H7	No growth	1/2
O118:H12	No growth	1/1
OR:H7	No growth	1/2
OR:H7	Not fluorescent	1/2
OR:NM	Not fluorescent	1/2

¹Atypical results refer to those that did not conform to the manufacturer's description of typical STEC colonies or growth (i.e., isolates which were not mauve, did not fluoresce, or did not grow)

²All colonies that grew produced fluorescence unless indicated otherwise

³Serotypes belonging to the top 6 non-O157 STEC

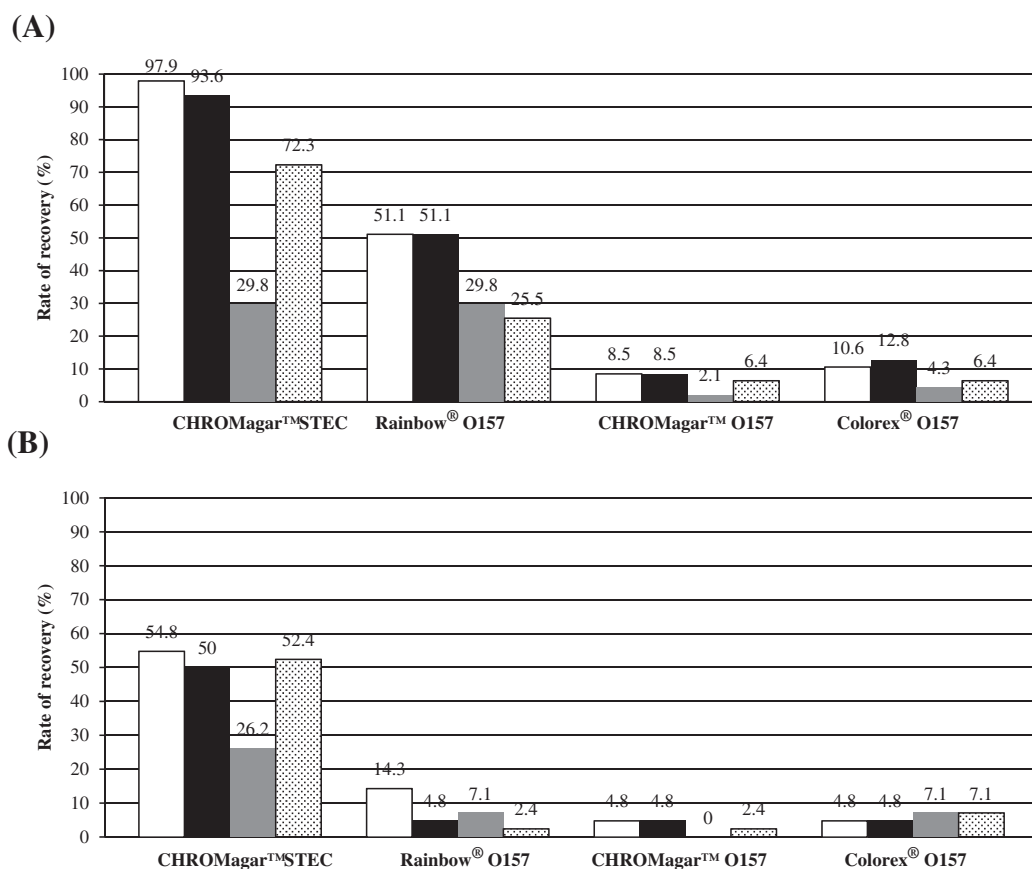


Fig. 2. Rates of detection of STEC isolates from different spiking stool pools on chromogenic agar media. Results are shown for the top six most common non-O157 STEC serotypes (A) and for the less common non-O157 STEC serotypes (B). All isolates were grown in broth with stool pools of watery (white bars), semi-formed (black bars), mucoid (gray bars), or bloody quality (stippled bars). Percentage of isolates detected as STEC is expressed on the y-axis and media used is expressed on the x-axis. Exact rates of detection are shown above each bar.

broth cultures were tested for the presence of the *stx* genes using real-time PCR (Table 3). In total, 13 specimens were confirmed to contain STEC and 68 yielded mauve colonies that were confirmed as non-STEC. CHROMagar™ STEC showed a sensitivity of 84.6%, a specificity of 87%, a positive predictive value of 13.9%, and a negative predictive value of 99.6%. A proportion of the specimens (30/68) that yielded STEC-like colonies which were negative for *stx*₁ and *stx*₂ by real-time PCR were tested for Shiga toxin production using the Shiga Toxin Quik Chek assay on MacConkey broth cultures of either the stool sample or the STEC-like colonies; all tested were negative for toxin production. Six of the 13 positive specimens carried O157:H7; two carried O26:H11 and the remaining carried O103:NT, O117:H7, O121:H19, O145:NM, or OR:NT. The two serotypes that were not detected on CHROMagar™ STEC were O117:H7 and OR:NT.

4. Discussion

The detection and isolation of STEC is severely hampered by the inability of most available culture media to differentiate non-O157 STEC from *Stx*-negative *E. coli*. Sorbitol- MacConkey agar and CHROMagar™ O157 have both been used extensively by clinical laboratories to detect

E. coli O157 but fail to identify most other STEC (Bettelheim 1998a; Church et al., 2007; March and Ratnam, 1986). Colorex® O157 is another medium developed for the detection of O157 STEC. It is not clear how Colorex® O157 differs in formulation from CHROMagar™ O157 and for this reason was included in this study. Rainbow® O157 is a medium that detects *E. coli* O157 as well as some other STEC serotypes, but has only been evaluated using food, water, and other non-human specimens (Bettelheim, 1998b; Ngwa et al., 2013; Radu et al., 2000; Tillman et al., 2012; Tutenel et al., 2003; Yoshitomi et al., 2012).

In the present study, 90% of non-O157 STEC isolates resembled STEC on CHROMagar™ STEC. This compares with 74.4–88.3% in other studies (Hirvonen et al., 2012; Tzschoppe et al., 2012; Wylie et al., 2013). While some European studies (Gouali et al., 2013; Hirvonen et al., 2012) have shown decreased detection of O103:H2 isolates on this medium, our work did not confirm their observation; this might be indicative of geographic variation in the growth characteristics of this serotype.

CHROMagar™ STEC was able to detect a higher proportion of non-O157 STEC isolates than the other three chromogenic media, including Rainbow® O157 agar. Gill et al. (2014) found that 28.1% of 96 STEC isolates did not grow on CHROMagar™ STEC while up to 29.2% did not grow on Rainbow® O157 agar supplemented with antibiotics. In our hands, Rainbow® O157 agar allowed the growth of all STEC isolates; this is likely a consequence of our use of lower antibiotic concentrations and fewer antibiotics than Gill et al. (2014) in our supplementation of Rainbow® O157 agar.

The ability of CHROMagar™ STEC to support the growth of non-O157 STEC was greater for the more common non-O157 STEC than for the less common serotypes. Additionally, growth of isolates on CHROMagar™ STEC correlated highly with MICs to tellurite of 2 µg/mL or higher. This is in agreement with previous studies which found that

Table 3
Detection of STEC from clinical stool samples using CHROMagar™ STEC.

CHROMagar™ STEC result	Real-time PCR result		
	Positive	Negative	Total
Positive	11	68	79
Negative	2	455	457
Total	13	523	536

most STEC capable of growth on CHROMagar™ STEC had genetic determinants for tellurite resistance while isolates lacking the same genes did not grow (Hirvonen et al. 2012; Tzschoppe et al. 2012). Considering this correlation between growth on CHROMagar™ STEC and tellurite MIC, tellurite is likely a selective ingredient in CHROMagar™ STEC as is the case with CHROMagar™ O157 (Becton, Dickinson and Company). The two isolates with elevated tellurite MICs that did not grow on CHROMagar™ STEC were likely inhibited by an unknown selective agent in the agar.

Differences in colony colour and fluorescence under UV light did not correlate with Stx production, as non-mauve and non-fluorescent strains still produced Stx1 and/or Stx2. Therefore, the appearance of strains must be governed by some other determinant.

Among the strains used to determine the exclusivity of the chromogenic media, none of the Stx-negative strains of *E. coli* produced STEC-appearing colonies on CHROMagar™ STEC. Rainbow® O157 agar was less specific in that all but two of the Stx-negative strains of *E. coli* produced STEC-like colonies as did the tested *E. cloacae* strain. A further advantage of CHROMagar™ STEC is that it permitted the growth of fewer genera of organisms, suggesting that it may be better able to effectively screen out fecal flora.

Using stool to spike broth cultures of multiple STEC isolates revealed that the lowest rates of detection on any media were usually seen with mucoid stool. This may indicate the presence of growth inhibitors in mucoid stool or may simply reflect the difficulty in inoculating a homogeneous mixture of the broth culture onto the agar medium when using mucoid stool. While carrying out these experiments, we tested the ability of real-time PCR to detect STEC from differing types of stool and found no obvious inhibition (Chui et al., 2013).

Enrichment in MacConkey broth for the spiking experiments prior to plating onto the tested agar media was chosen for two reasons. Firstly, it was noticed that stool specimens often produced fluorescence on CHROMagar™ STEC in the absence of STEC-like colonies (and sometimes in the absence of any growth), and this apparent non-specific fluorescence disappeared when MacConkey broth cultures were used. Secondly, MacConkey broth is often used in other diagnostic procedures for STEC, such as immunoassays and DNA extraction for PCR (Chui et al., 2011; Chui et al., 2013).

Detection of STEC strains from spiked stool cultures on CHROMagar™ STEC was variable and dependent upon the STEC serotype as well. The top six non-O157 STEC serotypes were detected more frequently than the less common serotypes on CHROMagar™ STEC. This was generally seen with the other media tested and it is encouraging that the more common STEC serotypes are more easily detected by culture methods. CHROMagar™ STEC was superior to Rainbow® O157 agar and the other media in detecting STEC in spiked stool cultures.

Using clinical specimens, CHROMagar™ STEC demonstrated acceptable specificity and sensitivity (84.6% and 87%, respectively) and a high negative predictive value (99.6%). However, the medium exhibited a low positive predictive value of 13.9%. Previous studies evaluating CHROMagar™ STEC using clinical specimens are in general agreement with these results, showing a sensitivity, specificity, positive predictive value, and negative predictive value of 50–91.4%, 83.7–95.8%, 13.6–60%, and 98.8–98.9%, respectively (Gouali et al., 2013; McCallum et al., 2013; Wylie et al., 2013). Such a low positive predictive value indicates that a high number of false positive results from truly negative specimens would need to undergo confirmatory testing if the medium was implemented for screening in a clinical microbiology laboratory.

Consistent with other studies performed in Alberta, over half of the STEC isolates detected from clinical specimens belonged to non-O157 serotypes (Chui et al., 2011; Chui et al., 2013; Chui et al., 2015; Couturier et al., 2011; Gilmour et al., 2009). The two isolates not detected on CHROMagar™ STEC belonged to serogroups O117 and OR, two serogroups that were not always detected by the medium in initial experiments (Table 2). This highlights the importance of local variations

in STEC serotype prevalence when using CHROMagar™ STEC as a primary screening agar.

In summary, among the chromogenic media tested, CHROMagar™ STEC is the most suitable for detecting the widest range of STEC serotypes. Its low positive predictive value limits its utility and makes it necessary for non-culture methods such as real-time PCR or lateral flow immunoassay to confirm positive results. The usefulness of CHROMagar™ STEC may therefore lie in its ability to recover isolates from specimens that have already tested positive for STEC by other methods rather than as a primary medium in stool cultures.

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