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Usability and Performance of CHROMagar STEC Medium in Detection of Shiga Toxin-Producing *Escherichia coli* Strains

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The performance and usability of CHROMagar STEC medium (CHROMagar Microbiology, Paris, France) for routine detection of Shiga toxin-producing *Escherichia coli* (STEC) strains were examined. The ability of the medium to selectively propagate STEC strains differing by their serotypes and virulence genes was studied with a collection of diarrheagenic *E. coli* isolates ($n = 365$) consisting of 49 different serotypes and with non-STEC and other bacterial isolates ($n = 264$). A total of 272 diarrheagenic *E. coli* (75.0%) isolates covering 24 different serotypes grew on CHROMagar STEC. The highest detection sensitivities were observed within the STEC serogroups O26 (90.0%), O111 (100.0%), O121 (100.0%), O145 (100.0%), and O157 (84.9%), and growth on CHROMagar STEC was highly associated with the presence of the tellurite resistance gene (*terD*). The specificity of the medium was 98.9%. In addition, CHROMagar STEC was used in parallel with a Shiga toxin-detecting immunoassay (Ridaquick Verotoxin/O157 Combi; R-biopharm, Darmstadt, Germany) to screen fecal specimens ($n = 47$) collected from patients suffering from hemorrhagic diarrhea. Positive growth on CHROMagar STEC was confirmed by the Premier EHEC enzyme immunoassay (Meridian Bioscience, Inc., Cincinnati, OH), and discrepant results between the two screening methods were confirmed by *stx* gene-detecting PCR. All 16 of the 47 stool samples that showed positive growth on CHROMagar STEC were also positive in the confirmatory tests. CHROMagar STEC proved to be an interesting option for STEC screening, allowing good detection sensitivity and specificity and permitting strain isolation for further outbreak investigations when required.

Shiga toxin-producing *Escherichia coli* (STEC) strains are frequently identified as causative agents of a wide spectrum of diseases, ranging from mild gastroenteritis and hemorrhagic colitis to life-threatening diseases such as hemolytic-uremic syndrome (HUS) (14). Due to their pathogenic properties, particularly phage-encoded Shiga toxins (Stxs), these strains are also called enterohemorrhagic *E. coli* (EHEC) (22).

Although sorbitol-negative serotype O157:H7 has been the most common STEC serotype, the clinical significance of non-O157 STEC strains is on the rise worldwide (11, 12, 14, 15). STEC strains are known to be diverse, and in addition to classical EHEC strains, newly emerging strains have recently been associated with severe clinical illness and with outbreaks in Europe (7, 17, 18). Moreover, there are reports that non-O157 STEC strains have been found in foods retailed for consumption in raw form by humans (4, 5, 19).

The increasing prevalence of these intestinal pathogens has important public health implications. Therefore, rapid and reliable detection of STEC isolates in food and clinical samples is required. The direct molecular detection of Shiga toxin (Stx1 or Stx2) genes alone or in combination with other genes coding for major STEC virulence factors by nucleic acid-based techniques is known to be highly sensitive and specific in STEC detection (1, 2, 10, 25). Major disadvantages of these assays are, however, the labor-intensive sample preparation and the increase of the costs when used only for screening purposes. An alternative approach is the detection of Shiga toxins in stools by immunochromatographic antigen tests or enzyme immunoassays (EIAs), either directly or after enrichment (3, 16, 20, 21, 23). These assays are rapid and easy to perform, but in order to gain the optimal sensitivity, an enrichment step which extends the total turnaround time from hours to days is usually required. In addition, as neither PCR nor EIAs allow STEC strain isolation, the strain is not available for further analyses, e.g., in epidemiological surveillance studies or outbreak investigations, when required.

Culture-based methods, e.g., culture on sorbitol-MacConkey agar, are known to be cost-effective options for STEC screening. Several drawbacks, however, limit the utility of culture, including the slow turnaround time and false-negative results due to emerging serotypes of sorbitol-fermenting non-O157 and O157 STEC (14, 15). Recent improvements include chromogenic media for STEC isolation with increased specificity and sensitivity (6, 24). These media contain antimicrobials, especially cefixime and tellurite, to inhibit the growth of fecal coliforms and other bacteria. Furthermore, presumptive species identification is achieved by coupling a chromogenic agent to substrates utilized by *E. coli* but not other microbes.

Here, we describe a performance study of the recently launched CHROMagar STEC medium (CHROMagar Microbiology, Paris, France), which has been shown to be an interesting option for STEC screening (24), by using a vast collection of isolates of different STEC sero- and genotypes and non-STEC isolates. In addition, the suitability of this new chromogenic medium for clinical purposes was investigated by screening human stool samples and comparing the results to those of a Shiga toxin-detecting immunochromatographic antigen test and EIA, as well as *stx*-detecting PCR.

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TABLE 1 Growth and virulence properties of Shiga toxin-producing and diarrheagenic *E. coli* strain collection[a]

Serotype[b]	Total no. of isolates	No. of isolates growing on CHROMagar STEC at:			Color of colonies	Fluorescence	stx_1 or stx_2 (no. of isolates)	Other virulence gene(s) (no. of isolates)
		16 h	24h	40 h				
O2:H27	1	0	0	0	−[c]	−	stx_2 (1)	*astA*(1), *estIa* (1), EHEC *hlyA* (1)
O2:H29	2	2	2	2	Mauve	Yes	stx_2 (2)	−
O5:H$^-$	3	3	3	3	Mauve	Yes	stx_1 (3)	*eae* (3), *ent* (3), *escV* (3), EHEC *hlyA* (3)
O8:H9	1	0	0	0	−	−	stx_2 (1)	−
O20:H7	2	0	0	0	−	−	stx_2 (2)	−
O26:H$^-$	2	2	2	2	Mauve	Yes	stx_1 (2)	*eae* (2), *ent* (1), *escV* (1), EHEC *hlyA* (1)
O26:H11	18	16	16	16	Mauve	Yes	stx_1 (12), stx_2 (6)	*eae* (18), *ent* (18), *escV* (18), EHEC *hlyA* (18)
O39:H40	1	0	0	0	−	−	stx_2 (1)	*eae* (1), *ent* (1), *escV* (1)
O43:H2	1	0	0	0	−	−	stx_1 (1), stx_2 (1)	EHEC *hlyA* (1)
O55:H− (EPEC)	1	1	1	1	Mauve	Yes	−	*eae* (1), *ent* (1), *escV* (1)
O55:H7	1	0	0	0	−	−	stx_2 (1)	*eae* (1)
O76:H19	1	0	0	0	−	−	stx_1 (1), stx_2 (1)	EHEC *hlyA* (1)
O78:H$^-$	6	0	0	0	−	−	stx_1 (6)	EHEC *hlyA* (6)
O91:H$^-$	1	0	0	0	−	−	stx_2 (1)	−
O91:H21	1	0	0	0	−	−	stx_2 (1)	EHEC *hlyA* (1)
O95:H10	1	1	1	1	Mauve	Yes	stx_2 (1)	*eae* (1), EHEC *hlyA* (1)
O101:H$^-$	1	1	1	1	Mauve	Yes	stx_2 (1)	*eae* (1), *escV* (1), *estIa* (1), EHEC *hlyA* (1)
O103:H$^-$	1	1	1	1	Mauve	Yes	stx_1 (1)	*eae* (1), EHEC *hlyA* (1)
O103:H2	30	0	0	10	Mauve	Yes	stx_1 (27), stx_2 (3)	*eae* (30), EHEC *hlyA* (30)
O104:H4	3	3	3	3	Mauve	Yes	stx_2 (3)	*aggR* (3), *pic* (3)
O107:H27	1	0	0	0	−	−	stx_2 (1)	EHEC *hlyA* (1)
O111:H$^-$	1	1	1	1	Mauve	No	stx_1 (1), stx_2 (1)	*eae* (1), EHEC *hlyA* (1)
O111:H8	6	6	6	6	Mauve	Yes (5), no (1)	stx_1 (6)	*eae* (6), EHEC *hlyA* (6)
O118:H16	1	1	1	1	Mauve	Yes	stx_1 (1)	*eae* (1), *ent* (1), *escV* (1), EHEC *hlyA* (1)
O121:H19	5	5	5	5	Mauve	Yes	stx_2 (5)	*eae* (5), *ent* (5), *escV* (5), EHEC *hlyA* (5)
O130:H38	2	2	2	2	Mauve	Yes	stx_1 (2), stx_2 (2)	EHEC *hlyA* (2)
O145:H$^-$	20	20	20	20	Mauve	Yes	stx_2 (20)	*eae* (20), *ent* (20), EHEC *hlyA* (20)
O145:H28	6	6	6	6	Mauve	Yes	stx_1 (3), stx_2 (3)	*eae* (6), *ent* (6), EHEC *hlyA* (6)
O146:H21	3	0	0	0	−	−	stx_1 (1)	−
O153:H33	1	0	0	0	−	−	stx_2 (1)	−
O156:H7	1	0	0	0	−	−	stx_1 (1)	−
O156:H25	2	2	2	2	Mauve	Yes	stx_1 (2)	*eae* (2), *ent* (2), *escV* (2), EHEC *hlyA* (2)
O157:H$^-$ (SF)	23	1	1	1	Mauve	No	stx_2 (23)	*eae* (23), *ent* (1), *escV* (1), EHEC *hlyA* (23)
O157:H$^-$ (NSF)	13	13	13	13	Mauve	No	stx_1 (13), stx_2 (13)	*eae* (13), *ent* (1), *escV* (1), EHEC *hlyA* (13)
O157:H7 (SF)	6	0	0	0	−	−	stx_1 (2), stx_2 (6)	*eae* (6), *ent* (4), *escV* (4), EHEC *hlyA* (6)
O157:H7 (NSF)	170	166	166	166	Mauve	No	stx_1 (48), stx_2 (176)	*eae* (176), *ent* (176), *escV* (176), EHEC *hlyA* (176)
O165:H25	2	0	0	0	−	−	stx_2 (2)	*eae* (2), *ent* (2), *escV* (2), EHEC *hlyA* (2)
O174:H2	1	0	0	0	−	−	stx_2 (1)	EHEC *hlyA* (1)
O174:H21	3	1	1	1	Mauve	Yes	stx_2 (3)	−
O177:H$^-$	1	1	1	1	Mauve	No	stx_2 (1)	*eae* (1), *ent* (1), *escV* (1), EHEC *hlyA* (1)

(Continued on following page)

TABLE 1 (Continued)

Serotype[b]	Total no. of isolates	No. of isolates growing on CHROMagar STEC at:			Color of colonies	Fluorescence	stx_1 or stx_2 (no. of isolates)	Other virulence gene(s) (no. of isolates)
		16 h	24h	40 h				
O178:H19	2	0	0	0	−	−	stx_1 (2), stx_2 (2)	EHEC *hlyA* (2)
O181:H49	1	0	0	0	−	−	stx_2 (1)	EHEC *hlyA* (1)
ONT:H$^-$ (EAEC)	1	1	1	1	Mauve	Yes	−	*aggR* (1), *astA* (1), *pic* (1)
OR:H$^-$	3	2	2	2	Mauve	Yes	stx_1 (3), stx_2 (1)	*eae* (3), EHEC *hlyA* (3)
OR:H2	1	0	0	0	−	−	stx_2 (1)	*eae* (1), EHEC *hlyA* (1)
OR:H4	3	0	0	0	−	−	stx_2 (3)	−
OR:H18	1	0	0	0	−	−	stx_2 (1)	−
OR:H21	1	0	0	0	−	−	stx_2 (1)	−
OR:H28	1	1	1	1	Mauve	Yes	stx_2 (1)	*eae* (1), EHEC *hlyA* (1)
OR:H49	1	0	0	0	−	−	stx_2 (1)	−
OX182:H25	2	2	2	2	Mauve	Yes	stx_1 (2)	*eae* (2), *ent* (2), *escV* (2), EHEC *hlyA* (2)
OX186:H16	1	0	0	0	−	−	stx_1 (1)	−
ND (ETEC)	1	1	1	1	Mauve	Yes	−	*astA* (1), *elt* (1), *estIb* (1)

[a] A total of 365 isolates were tested.
[b] ND, not determined; SF, sorbitol-fermenting; NSF, non-sorbitol fermenting; EPEC, enteropathogenic *E. coli*; EAEC, enteroaggregative *E. coli*; ETEC, enterotoxigenic *E. coli*.
[c] −, negative.

MATERIALS AND METHODS

The ability of the CHROMagar STEC medium to grow Shiga toxin-producing and other diarrheagenic *E. coli* strains was investigated by using a deep-frozen collection of STEC, enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), and enteroaggregative *E. coli* (EAEC) strains ($n = 365$) isolated from all parts of Finland from 1990 to 2011 (see Table 1). The culture collection consisted of strains of 49 different serotypes, including strains with 54 variations in *stx* and other genes coding for major virulence factors. The serotyping and the definition of virulence genes had been performed in the Bacteriology Unit of the National Institute for Health and Welfare (THL) as previously described (18), and the species identification had been performed by standard laboratory methods, e.g., with the API 20E system (bioMérieux, Marcy l'Etoile, France), when the strains were isolated. All strains were cultured from −70°C stocks on sorbitol-MacConkey agar (Lab M Ltd., Lancashire, United Kingdom) at 35°C for 16 to 24 h. After that, bacterial colonies were streaked onto CHROMagar STEC agar and incubated at 35°C for 40 h. Growth and colony morphology (color formation) were examined after 16 h, 24 h, and 40 h. In addition, the fluorescence of the colonies was observed after 16 h and 40 h under UV light. The association of growth on CHROMagar STEC with tellurite resistance was confirmed by analyzing for the presence of the *terD* gene as previously described (13). The specificity of the medium was investigated by analyzing a collection of non-Shiga toxin-producing *E. coli* strains from nonfecal origin and other microbes ($n = 264$) isolated from different clinical specimens (see Table 2). The species identification was performed as mentioned above.

The performance and diagnostic efficacy of the CHROMagar STEC medium for the routine detection of STEC isolates were assessed by analyzing stool specimens ($n = 47$) from patients suffering from hemorrhagic diarrhea (see Table 3). All specimens were collected from May to December 2011 in the Vaasa Hospital District (VHD), Finland, and cultured on sorbitol-MacConkey, cystine lactose electrolyte-deficient (Oxoid Limited, Hampshire, United Kingdom), and CHROMagar STEC agars for 16 to 24 h at 35°C. Presumed STEC strains forming mauve colonies on CHROMagar STEC were identified by the API 20E system and analyzed for fluorescence by using UV light. Strains were sent to the Helsinki University Hospital Laboratory (HUSLAB) for confirmation by the Premier EHEC EIA (Meridian Bioscience, Inc., Cincinnati, OH). In addition, the presence of Stxs in stool specimens was screened by using the immunochromatographic Ridaquick Verotoxin/O157 Combi antigen test (R-biopharm, Darmstadt, Germany) according to the manufacturer's instructions. In case of discrepant culture and Ridaquick test results, specimens were sent to HUSLAB for confirmation of the presence of the stx_1 and stx_2 genes by PCR (2).

RESULTS

Seventy-five percent (272/365) of diarrheagenic *E. coli* culture collection isolates grew on CHROMagar STEC medium (Table 1). These isolates were classified into 24 different serotypes covering 49.0% of all the serotypes analyzed in this study. Strains belonging to the serogroups O2:[H29], O5, O26, O104, O111, O121, O130, O145, O157:[H7], and OX182, including the most prevalent O serogroups in Finland, grew well on CHROMagar STEC. However, in the case of two frequently identified serotypes, O157:H$^-$ and O103:H2, the performance of CHROMagar STEC was weaker, as only 38.9% (14/36) and 33.3% (10/30) of these isolates grew after 16 h. Nearly all nongrowing isolates within serogroup O157 were sorbitol-fermenting strains (28/29). On the contrary, non-sorbitol-fermenting strains of O157 grew well on CHROMagar STEC, as only 4 out of 183 isolates did not grow on the medium. Apart from other strains, isolates belonging to serotype O103:H2 formed only small or irregular colonies on the chromogenic medium. Interestingly, when these O103:H2 colonies were recultured on a CHROMagar STEC plate, abundant growth with large colonies was already seen within 16 h of incubation. Similar adaptation was not observed with other STEC strains. Moreover, these O103:H2 isolates did not carry the *terD* gene, although among all the other STEC strains, the growth on CHROMagar STEC correlated with the presence of *terD*.

The differentiation of isolates as non-O157 or O157 on the basis of the fluorescence around colonies on CHROMagar STEC under UV light proved to be highly specific. A total of 97.4% (149/153) of non-O157 isolates formed fluorescent colonies, whereas all strains of serogroup O157 growing on CHROMagar STEC ($n = 180$) were nonfluorescent. The four isolates which did not form fluorescent colonies belonged to serogroups O111 ($n = 2$), O177 ($n = 1$), and O95 ($n = 1$). Furthermore, in mixed cul-

TABLE 2 Results of growth of non-Shiga toxin-producing *E. coli* and other microbes on CHROMagar STEC[a]

Strain	No. of isolates with growth on CHROMagar STEC (total no. of isolates)	Color of colonies
Citrobacter freundii	0 (2)	–[b]
Citrobacter spp.	2 (4)	Blue (2)
Corynebacterium spp.	0 (2)	–
Escherichia coli	3 (186)	Mauve (3)
Enterobacter cloacae	0 (8)	–
Enterococcus faecalis	0 (6)	–
Enterococcus faecium	0 (6)	–
Klebsiella pneumoniae	2 (10)	Blue (2)
Klebsiella oxytoca	0 (2)	–
Pseudomonas aeruginosa	2 (4)	Colorless (2)
Proteus mirabilis	14 (14)	Colorless (14)
Proteus spp.	4 (4)	Colorless (4)
Providencia stuartii	2 (2)	Colorless (2)
Salmonella enterica serovar Virchow	0 (2)	–
Shigella sonnei	0 (2)	–
Staphylococcus aureus	0 (2)	–
Staphylococcus spp.	0 (2)	–
Stenotrophomonas maltophilia	0 (2)	–
Streptococcus agalactiae	0 (2)	–
Yersinia enterocolitica	0 (2)	–

[a] A total of 264 isolates were tested.
[b] –, negative.

tures, the strains of O157 and non-O157 were easily distinguishable under UV light.

The specificity of CHROMagar STEC proved to be high (98.9%), as only 3 non-toxin-producing isolates of 186 *E. coli* isolates of nonfecal origin grew as mauve and fluorescent colonies (Table 2). Other microbes were inhibited or their growth was colorless or blue.

Sixteen of the 47 stool specimens (34.0%) were STEC positive on CHROMagar STEC, and 14 were positive with the Ridaquick Verotoxin/O157 Combi assay (Table 3). All 16 samples were, however, confirmed to be positive by using the Premier EHEC EIA, and the 2 samples with discrepant results were also confirmed to be Shiga toxin positive by PCR. The isolated STEC strains were sorbitol positive on sorbitol-MacConkey agar and serotyped as O145:H$^-$ ($n = 15$) or O177:H$^-$ ($n = 1$).

DISCUSSION

This study demonstrated that the performance of CHROMagar STEC for the selection of isolates belonging to major STEC types is good. In our material, the major proportion of diarrheagenic *E. coli* isolates were *eae* and *stx* positive (321/362), and in this population, the percentage of growth on CHROMagar STEC was 81.6. In addition, 20.5% (8/39) of *eae*-negative and *stx*-positive STEC isolates but only 1.6% of the common *E. coli* strains from nonfecal origin grew on the chromogenic medium. Thus, CHROMagar STEC showed a high specificity but a weaker sensitivity, as only one-fifth of the diarrheagenic *eae*-negative and *stx*-positive STEC isolates were detected. A similar limitation was observed among sorbitol-fermenting O157 strains, as only 3.4% (1/29) of these isolates grew on this chromogenic medium. Growth on CHROMagar STEC was highly associated with the presence of the *terD* gene, and only a few isolates, belonging to serotype O103:H2, grew on the medium, even though they lacked the tellurite resistance gene. Similar results were reported in a recent study of STEC detection and isolation by Tzschoppe et al. (24). According to Tzschoppe et al. (24), the growth on CHROMagar STEC was closely associated with the major STEC serotypes and with the presence of the tellurite resistance (*terB*) gene. They discovered that the distribution of *terB* was high (87.2%) within the diarrheagenic *eae*- and *stx*-positive *E. coli* strains but significantly lower among the *eae*-negative and *stx*-positive strains (13.5%), sorbitol-fermenting nonmotile O157 strains (0.0%), and apathogenic *E. coli* strains (12.0%). These results suggest that one of the selective components of CHROMagar STEC is tellurite and that differences in sensitivity and specificity may vary due to the regional differences in the distribution of tellurite resistance among diarrheagenic and nondiarrheagenic *E. coli* isolates.

As the presence of the tellurite resistance gene complex (genes *terZABCDEF*) is well correlated with the resistance of *E. coli* strains to tellurite-containing media (22), it was of general interest to observe the enhanced growth behavior of O103:H2 isolates lacking the *terD* gene after reculturing. It has previously been indicated that the expression of the *ter* genes may vary in *E. coli* strains depending on the surrounding concentration of tellurite, and some isolates have even shown inducible resistance when growing in a subinhibitory concentration of potassium tellurite (22). However, all of these resistant or inducibly resistant strains have been shown to contain *ter* genes, a finding which suggests that there may be protective mechanisms other than the *ter* gene complex causing the inducible tolerance of O103:H2 isolates against the growth inhibition of tellurite. In addition, when these isolates grown on sorbitol-MacConkey agar were plated on CHROMagar

TABLE 3 Results of STEC screening from fecal specimens by using CHROMagar STEC, Shiga toxin-detecting immunoassays, and reference STEC gene-detecting PCR assay

CHROMagar STEC result ($n = 47$)	No. of isolates with the indicated result by:					
	Ridaquick Verotoxin/O157 Combi assay ($n = 47$)		Premier EHEC assay[a] ($n = 16$)		Reference PCR assays[b] ($n = 2$)	
	Negative	Positive	Negative	Positive	Negative	Positive
Negative ($n = 31$)	31	0	ND[c]	ND	ND	ND
Suspected STEC ($n = 16$)	2	14	0	16	0	2

[a] Positive growth on CHROMagar STEC (CHROMagar Microbiology, Paris, France) was confirmed by the Premier EHEC EIA (Meridian Bioscience, Inc., Cincinnati, OH).
[b] In-house diarrheagenic multiplex PCR assay (2). Only specimens positive on CHROMagar STEC but negative on Ridaquick Verotoxin/O157 Combi assay (R-biopharm, Darmstadt, Germany) were confirmed by the reference PCR method.
[c] ND, not determined.

STEC from a dense cell suspension (>6 × 10^8 CFU/ml in 0.9% NaCl), growth similar to that seen with reculturing was directly observed (data not shown). This suggests that tolerance against tellurite among these O103:H2 isolates becomes more evident as the cell density in the inoculum increases. Accordingly, the detection of serotype O103:H2 in fecal samples with tellurite-containing selective medium may vary significantly depending on the cell concentration. Cell enrichment prior to plating may enhance detection, but at the same time, it significantly extends the total turnaround time. Examining the mechanism of tellurite tolerance in O103:H2 isolates requires additional studies.

Although rare in occurrence, coinfections with different STEC strain types may be possible (8, 9). Thus, by showing good differentiation performance in mixed cultures, CHROMagar STEC may provide useful assistance in outbreak investigations in such cases. Differentiation is, however, limited to a rough assortment of strains into non-O157 and O157 groups. The performance of CHROMagar STEC in detecting newly emerging strains may be inadequate, as less than half of the different STEC serotypes were detected in the present study. On the other hand, the overall sensitivity in screening for STEC in fecal samples proved to be higher than that of the Stx-detecting immunochromatographic test used. A limitation of this research frame due to the low number of the primary specimens and the bias introduced by the occurrence of only two O serogroups (mainly O145, with only one O177 strain) in 47 screening specimens is apparent. However, serotypes O145:H$^-$ and O103:H2 belong to the most common non-O157 serotypes in Finland.

In conclusion, CHROMagar STEC proved to be a promising new culture medium for the screening and detection of STEC isolates, allowing positive results for major STEC groups within 24 h. It was found to improve significantly screening for STEC compared to the conventional culture method with sorbitol-MacConkey agar, providing assistance, based on its fluorescence property, with detection of STEC coinfections and enabling strain isolation for further investigations, when required. CHROMagar STEC can substantially enhance STEC detection when combined with Stx-detecting immunoassays, especially in laboratories lacking PCR facilities and experts in molecular biology. This combined screening practice is highly recommended, as it also reduces the risk for underdetection of the diarrheagenic *E. coli* strains such as *eae*-negative and *stx*-positive isolates and sorbitol-fermenting O157 isolates not growing on CHROMagar STEC.

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REFERENCES

1. **Anklam KS, Kanankege KS, Gonzales TK, Kaspar CW, Döpfer D.** 2012. Rapid and reliable detection of Shiga toxin-producing *Escherichia coli* by real-time multiplex PCR. J. Food Prot. **75**:643–650.
2. **Antikainen J, et al.** 2009. New 16-plex PCR method for rapid detection of diarrheagenic *Escherichia coli* directly from stool samples. Eur. J. Clin. Microbiol. Infect. Dis. **28**:899–908.
3. **Beutin L, et al.** 2006. Comparative evaluation of the Ridascreen® Verotoxin enzyme immunoassay for detection of Shiga-toxin producing strains of *Escherichia coli* (STEC) from food and other sources. J. Appl. Microbiol. **102**:630–639.
4. **Buchholz U, et al.** 2011. German outbreak of *Escherichia coli* O104:H4 associated with sprouts. N. Engl. J. Med. **365**:1763–1770.
5. **Castro-Rosas J, et al.** 2012. Presence of faecal coliforms, *Escherichia coli* and diarrheagenic *E. coli* pathotypes in ready-to-eat salads, from an area where crops are irrigated with untreated sewage water. Int. J. Food Microbiol. **156**:176–180.
6. **Church DL, Emshey D, Semeniuk H, Lloyd T, Pitout JD.** 2007. Evaluation of BBL CHROMagar O157 versus sorbitol-MacConkey medium for routine detection of *Escherichia coli* O157 in a centralized regional clinical microbiology laboratory. J. Clin. Microbiol. **45**:3098–3100.
7. **Frank C, et al.** 2011. Epidemic profile of Shiga-toxin-producing *Escherichia coli* O104:H4 outbreak in Germany. N. Engl. J. Med. **365**:1771–1780.
8. **Gilmour MW, et al.** 2009. Isolation and detection of Shiga toxin-producing *Escherichia coli* in clinical stool samples using conventional and molecular methods. J. Med. Microbiol. **58**:905–911.
9. **Gilmour MW, et al.** 2007. Isolation and genetic characterization of a coinfection of non-O157 Shiga toxin-producing *Escherichia coli*. J. Clin. Microbiol. **45**:3771–3773.
10. **Grys TE, Sloan LM, Rosenblatt JE, Patel R.** 2009. Rapid and sensitive detection of Shiga toxin-producing *Escherichia coli* from nonenriched stool specimens by real-time PCR in comparison to enzyme immunoassay and culture. J. Clin. Microbiol. **47**:2008–2012.
11. **Hadler JL, et al.** 2011. Ten-year trends and risk factors for non-O157 Shiga toxin-producing *Escherichia coli* found through Shiga toxin testing, Connecticut, 2000-2009. Clin. Infect. Dis. **53**:269–276.
12. **Käppeli U, Hächler H, Giezendanner N, Beutin L, Stephan R.** 2011. Human infections with non-O157 Shiga toxin-producing *Escherichia coli*, Switzerland, 2000-2009. Emerg. Infect. Dis. **17**:180–185.
13. **Karch H.** 2011. Laborinformationen zum EHEC Ausbruchsstamm (Stand 01.06.2011). Institut für Hygiene der Universität Münster, Münster, Germany. http://www.ehec.org.
14. **Karch H, Tarr PI, Bielaszewska M.** 2005. Enterohaemorrhagic *Escherichia coli* in human medicine. Int. J. Med. Microbiol. **295**:405–418.
15. **Kehl SC.** 2002. Role of the laboratory in the diagnosis of enterohemorrhagic *Escherichia coli* infections. J. Clin. Microbiol. **40**:2711–2715.
16. **Kehl KS, Havens P, Behnke CE, Acheson DWK.** 1997. Evaluation of the Premier EHEC assay for detection of Shiga toxin-producing *Escherichia coli*. J. Clin. Microbiol. **35**:2051–2054.
17. **Lienemann T, et al.** 2011. Shiga toxin-producing *Escherichia coli* O100:H$^-$:stx_{2e} in drinking water contaminated by waste water in Finland. Curr. Microbiol. **62**:1239–1244.
18. **Lienemann T, et al.** 2012. Shiga toxin-producing *Escherichia coli* serotype O78:H$^-$ in family, Finland, 2009. Emerg. Infect. Dis. **18**:577–581.
19. **Manna SK, et al.** 2010. Serogroup distribution and virulence characteristics of sorbitol-negative *Escherichia coli* from food and cattle stool. J. Appl. Microbiol. **108**:658–665.
20. **Nakasone N, Toma C, Lu Y, Iwanaga M.** 2007. Development of a rapid immunochromatographic test to identify enteropathogenic and enterohemorrhagic *Escherichia coli* by detecting EspB. Diagn. Microbiol. Infect. Dis. **57**:21–25.
21. **Park CH, Kim HJ, Hixon DL, Bubert A.** 2003. Evaluation of the Duopath verotoxin test for detection of Shiga toxins in cultures of human stools. J. Clin. Microbiol. **41**:2650–2653.
22. **Taylor DE, et al.** 2002. Genomic variability of O islands encoding tellurite resistance in enterohemorrhagic *Escherichia coli* O157:H7 isolates. J. Bacteriol. **184**:4690–4698.
23. **Teel LD, et al.** 2007. Rapid detection of Shiga toxin-producing *Escherichia coli* by optical immunoassay. J. Clin. Microbiol. **45**:3377–3380.
24. **Tzschoppe M, Martin A, Beutin L.** 2012. A rapid procedure for the detection and isolation of enterohaemorrhagic *Escherichia coli* (EHEC) serogroup O26, O103, O111, O118, O121, O145 and O157 strains and the aggregative EHEC O104:H4 strain from ready-to-eat vegetables. Int. J. Food Microbiol. **152**:19–30.
25. **Wang F, Jiang L, Ge B.** 2011. Loop-mediated isothermal amplification assays for detecting Shiga toxin-producing *Escherichia coli* in ground beef and human stools. J. Clin. Microbiol. **50**:91–97.