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Evaluation of CHROMagar STEC and STEC O104 Chromogenic Agar Media for Detection of Shiga Toxin-Producing *Escherichia coli* in Stool Specimens

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The performance of CHROMagar STEC and CHROMagar STEC O104 (CHROMagar Microbiology, Paris, France) media for the detection of Shiga toxin-producing *Escherichia coli* (STEC) was assessed with 329 stool specimens collected over 14 months from patients with suspected STEC infections (June 2011 to August 2012). The CHROMagar STEC medium, after an enrichment broth step, allowed the recovery of the STEC strain from 32 of the 39 (82.1%) Shiga toxin-positive stool specimens, whereas the standard procedure involving Drigalski agar allowed the recovery of only three additional STEC strains. The isolates that grew on CHROMagar STEC medium belonged to 15 serotypes, including the prevalent non-sorbitol-fermenting (NSF) O157:H7, O26:H11, and O104:H4 serotypes. The sensitivity, specificity, and positive and negative predictive values for the CHROMagar STEC medium were between 89.1% and 91.4%, 83.7% and 86.7%, 40% and 51.3%, and 98% and 98.8%, respectively, depending on whether or not *stx*-negative *eae*-positive *E. coli* was considered atypical enteropathogenic *E. coli* (EPEC) or STEC that had lost Shiga toxin genes during infection. In conclusion, the good performance of CHROMagar STEC agar medium, in particular, the high negative predictive value, and its capacity to identify NSF O157:H7 as well as common non-O157 STEC may be useful for clinical bacteriology, public health, and reference laboratories; it could be used in addition to a method targeting Shiga toxins (detection of *stx* genes by PCR, immunodetection of Shiga toxins in stool specimens, or Vero cell cytotoxicity assay) as an alternative to O157 culture medium. This combined approach should allow rapid visualization of both putative O157 and non-O157 STEC colonies for subsequent characterization, essential for real-time surveillance of STEC infections and investigations of outbreaks.

Certain strains of Shiga toxin-producing *Escherichia coli* (STEC) are important causes of food-borne disease in industrialized countries. The clinical manifestations of STEC infections range from mild diarrhea to severe and specific complications, such as hemolytic-uremic syndrome (HUS), which occurs primarily in young children (1, 2). These STEC strains associated with human infections are also called enterohemorrhagic *E. coli* (EHEC). Animals, and especially cattle, serve as reservoirs for STEC. Transmission occurs via ingestion of contaminated food or water, person-to-person contact, direct animal contact, and exposure to the environment. STEC strains are characterized by their ability to produce toxins related to those of *Shigella dysenteriae* type 1 (3): two types have been described among STEC isolates, Shiga toxin 1 and Shiga toxin 2, respectively, encoded by the stx_1 and stx_2 genes carried on temperate bacteriophages (4, 5). Most STEC isolates also carry the chromosomally located locus of enterocyte effacement (LEE), a pathogenicity island, first described in enteropathogenic *E. coli* (EPEC). LEE promotes the development of attaching-and-effacing lesions in the host intestinal mucosa cells (6). One of the LEE genes, *eae* (for EPEC attaching and effacing), encodes intimin, an outer membrane adhesin essential for the intimate attachment of the bacteria to enterocytes. Other adherence and colonization factors, such as adhesins and pili, are present in LEE-negative STEC strains. The STEC O104:H4 strain responsible for a large outbreak of HUS in Germany and other European countries in 2011 displays a characteristic aggregative adhesion (AA) pattern caused by an enteroaggregative *E. coli* (EAEC) genetic background (7–11).

The laboratory identification of STEC requires screening for Shiga toxin genes or proteins in stool specimens, followed by culture, serotyping, and confirmation of the presence of the virulence genes (at least stx_1 and stx_2 and then *eae* and *aggR*) in isolated colonies. Since the first reported STEC outbreak in 1982 (12), various methods for the detection of STEC, especially *E. coli* O157:H7, which is the most prevalent group of STEC, have been developed (13). STEC O157:H7 had been found to be non-sorbitol fermenting (NSF), and consequently, culture media containing sorbitol have been marketed and widely used. However, the identification of sorbitol-fermenting (SF) STEC O157:H7 (SF O157) strains, mainly in Germany (14–16), and the general increase of non-O157 STEC (generally SF) strains in clinical practice (17) have limited the use of sorbitol fermentation as a screening test for STEC. The development of a universal medium for STEC is difficult for many reasons, including the low STEC density and potential inhibitors in stool specimens and the absence of culture characteristics common to all the various unrelated *E. coli* lineages that have acquired *stx*-harboring bacteriophages (18). Recently, new chromogenic media not based on sorbitol fermentation were developed to improve the detection of O157 (19) or the most prevalent EHEC serogroups (20, 21). CHROMagar STEC medium (CHROMagar Microbiology, Paris, France) allows the growth and presumptive identification (mauve colonies) of ≈75% of STEC isolates in a vast collection of isolates encompassing 20 to 40 dif-

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ferent serotypes, including the most common serotypes of EHEC (20, 21). This chromogenic medium has also been evaluated for use with stools, but on a very limited scale (47 stool specimens), such that definitive conclusions about the suitability of this medium could not be drawn (20).

The aim of this study was to analyze the performance and usability of the CHROMagar STEC and STEC O104 media for the routine detection of STEC and STEC O104:H4, respectively, in stool specimens. This study, carried out at the French National Reference Center for *E. coli* and *Shigella* (FNRC-EcS), lasted 14 months and involved 329 stool specimens from patients with suspected STEC infection.

MATERIALS AND METHODS

Human patients. Between 16 June 2011 and 30 August 2012, 329 stool samples from patients (56 children <15 years of age and 273 adults) were addressed to the FNRC-EcS, Institut Pasteur, Paris, France, because of a suspicion of STEC infection (diarrhea, bloody diarrhea, HUS). The stool specimens were collected in sterile (screw-cap) containers and were transported at +4°C to the FNRC-EcS, where they were immediately processed before being stored at −20°C.

Isolation and characterization of STEC by the standard procedure or with chromogenic agar media. Stool specimens (0.2 g of soft stools or 1 ml of liquid stools) ($n = 220$) or rectal swabs ($n = 9$) were homogenized in 10 ml of Trypto-casein-soy (TCS) broth (Bio-Rad, Marnes-La-Coquette, France) and incubated at 37°C for 3 to 4 h; 10-μl aliquots were plated on Drigalski lactose agar and on CHROMagar media and incubated at 37°C for 18 to 24 h in air (protocol 1). Drigalski lactose agar is a selective (crystal violet and sodium deoxycholate) and differential (lactose) medium used for the isolation of all enterobacteria and several nonfermenting Gram-negative bacteria. The overnight cultures were examined for bacterial growth and colony morphology. For the CHROMagar STEC and STEC O104 media, we also tested a second enrichment protocol that consisted of plating 100 μl of the TCS culture after 7 h of incubation (protocol 2).

The standard FNRC-EcS procedure for STEC detection was used, as follows. A loopful (10 μl) of confluent lactose-positive colonies was transferred from the first quadrant of the Drigalski agar into a 1.5-ml microtube containing 1 ml of molecular biology-grade water. The samples were centrifuged, the supernatant was discarded, and DNA was extracted from the pellet with an InstaGene matrix (Bio-Rad) kit. PCR was used to test for the stx_1, stx_2, *eae*, EHEC *hlyA*, and *aggR* (in case of *eae* negativity) genes in 2-μl aliquots of the DNA as described previously (9). In cases of positive results for one of these virulence genes, about 10 (or up to 20) lactose-positive colonies from the Drigalski agar were isolated, identified biochemically as *E. coli*, tested for the corresponding virulence factors, and in some cases serotyped.

Mauve colonies on the chromogenic medium were suspected of being STEC. DNA was extracted from one to five mauve colonies with the InstaGene matrix (Bio-Rad) kit and directly tested for the stx_1, stx_2, *eae*, and *aggR* genes by PCR. In case of positive results for one of these virulence genes, one or two colonies were isolated from the CHROMagar STEC plates, confirmed biochemically to be *E. coli*, tested for the corresponding virulence factors, and serotyped.

Serotype determination. The *E. coli* strains with virulence factors were serotyped (O- and H-antigen determination) by slide agglutination assays using appropriate antisera (Sifin, Berlin, Germany) or by a molecular method based on the analysis of the O-antigen gene cluster (*rfb* restriction fragment length polymorphism [*rfb*-RFLP]) and flagellin gene (*fliC*) sequencing (22, 23).

Antimicrobial susceptibility testing. Antimicrobial susceptibility testing was performed on all O104:H4 *E. coli* strains and the three *E. coli* strains that had grown on CHROMagar STEC O104, as described previously (9).

Tests for non-STEC virulence determinants. The *E. coli* strains that were negative for the stx_1 and stx_2 genes but positive for the *eae* gene were tested for the presence of the bundle-forming pilus gene (*bfpA*) carried by the EPEC adherence factor (EAF) plasmid, to differentiate typical EPEC (EAF positive [EAF^+]) from atypical EPEC (EAF negative [EAF^-]) (24).

RESULTS

Prevalence and diversity of STEC in stool specimens. During the 14 months of the study period, 329 stool specimens were analyzed by the standard procedure. This identified stx_1 and/or stx_2 Shiga toxin genes in 39 (11.9%) specimens, and an STEC strain (two STEC strains from one stool specimen) was isolated from 35 (10.6%) (Tables 1 and 2). The 36 isolated STEC strains belonged to the following serotypes: O157:H7/NM (nonmotile) ($n = 8$), O104:H4 ($n = 7$), O26:H11/NM ($n = 6$), O103:H2/NM ($n = 2$), O148:H8 ($n = 2$), O113:H21 ($n = 1$), O118:H16 ($n = 1$), O121:H19 ($n = 1$), O91:H10 ($n = 1$), O171:H25 ($n = 1$), O174:H21 ($n = 1$), and O80:H2 ($n = 1$). Four O-nontypeable STEC strains displayed new *rfb*-RFLP patterns not present in our *E. coli*/*Shigella rfb*-RFLP molecular database, which currently contains 243 different patterns from 486 strains (22). None of the O157 strains fermented sorbitol.

Performance of CHROMagar STEC agar medium. Of the 39 Shiga toxin-positive stool specimens, STEC isolates from 26 (66.7%) grew on CHROMagar STEC medium after the initial enrichment protocol, giving typical mauve colonies. We tested whether the apparent false negativity of the remaining 13 stool specimens on CHROMagar STEC medium was due to an inability of the STEC strains to grow on this medium and produce a mauve color or to an insufficient sensitivity of the protocol before inoculation onto the chromogenic agar medium. The nine STEC strains isolated from these 13 samples by the standard procedure (no strain was isolated from 4 of the Shiga toxin gene-positive samples) were subcultured on CHROMagar STEC medium: six of the nine strains yielded mauve colonies. The three STEC strains for which no growth or mauve colonies were observed belonged to serotypes O148:H8, O80:H2, and O nontypeable:H19/NM. We then tested different conditions (enrichment of 3 to 4 h, 7 h, and 24 h and inoculation of 10 μl or 100 μl of the enrichment broth) to optimize the recovery of STEC from stool specimens. The best combination, here called protocol 2, for the frozen stool samples used was a time of enrichment of 7 h (3 to 4 h for the initial protocol) and 100 μl for inoculation (10 μl for the initial protocol). With protocol 2, we obtained growth on CHROMagar STEC with all the six stool specimens from which STEC strains were isolated by the standard procedure (i.e., on Drigalski agar). Thus, CHROMagar STEC medium and Drigalski agar identified 32 and 35 STEC isolates, respectively, among all samples included during the 14 months of the study.

Protocol 2 involves a larger inoculum, so we tested whether there was a higher rate of false-positive results (i.e., mauve colonies on CHROMagar STEC, despite no STEC or STEC virulence gene by the standard procedure). Thirty stool specimens that were negative both by the standard procedure and with CHROMagar STEC medium were retested by using protocol 2: none of the stool specimens yielded mauve colonies.

Of the 294 stool specimens from which no STEC was isolated by the standard procedure, 48 (16.3%) yielded mauve colonies on CHROMagar STEC. These mauve colonies did not contain the stx_1 or stx_2 gene. In 39 of the 48 cases, no other virulence genes, such as *eae*, EHEC *hlyA*, or *aggR*, were detected in the DNA isolated from the mauve colonies. However, in nine cases, the mauve colonies were found to contain one or both of the *eae* and EHEC

TABLE 1 Data for the 39 stool specimens containing Shiga toxin genes

Stool specimen	Clinical symptom[a]	Results by standard procedure[b]						Result with the following chromogenic medium:	
		Presence of the following virulence factor:					STEC isolated	CHROMagar STEC[c]	CHROMagar STEC O104[d]
		stx$_1$	*stx*$_2$	*eae*	*hlyA*	*aggR*			
2011/04475	—	−	+	+	+		None	(+)	−
2011/04522	HUS	−	+	−	−	+	O104:H4	+	+
2011/04623	BD	−	+	−	−	+	O104:H4	+	+
2011/04632	—	−	+	−	−	+	O104:H4	+	+
2011/04709	Diarrhea	−	+	+	+	−	O157:H7	+	−
2011/04786 2011/04786	—	+	+	+	+	−	O26:F11	+	−
2011/04875	BD HUS	−	+	+	+		None	−‡	−
2011/04989	Abdominal cramps	−	+	−	−	+	O104:H4	+	+
2011/05004	BD	−	+	−	−	+	O104:H4	+	−
2011/05008	BD	+	+	+	+		O157:[F7]	+	−
2011/05017	BD, ulcerative colitis	+	+	+	+		O118:F16	+	−
2011/05049	—	+	−	+	+		O26:[F11]	+	−
2011/05050	BD	+	−	+	+		O103:F2	+	−
2011/05102	HUS	−	+	−	−	−	Rnew2:F14	+‡	−
2011/05105	BD	−	+	+	+		R121:F19	+	−
2011/05132 2011/05132	—	−	+	−	−	−	R148:F8	+	−
2011/05602	—	−	+	−	−	+	O104:H4	+	+
2011/05638	HUS	+	+	+	+		O26:F11, O157:H7	+	−
2011/06543	BD, HUS	−	+	−	−	−	None	(+)	−
2011/06667	BD, HUS	+	−	+	+		O103:[F2]	+	−
2011/06833	—	−	+	−	−	−	R113:F21	+‡	−
2011/08768	—	−	+	+	+		O26:[F11]	+	−
2011/09110	—	−	+	−	−	+	O104:H4	+	−
2011/09378	HUS	−	+	−	−	−	O91:F10	+‡	−
2011/10085	BD	+	+	+	+		O157:H7	+	−
2011/10600 2011/10600	—	+	+	+	+		Rnew1:F2	+	−
2011/11333	—	−	+	−	+	−	R171:F25	+‡	−
2011/11527	Diarrhea, HUS	−	+	+	+		R80:F2	−‡	−
2011/11551	Diarrhea, HUS	−	+	−	−	−	R174:F21	+‡	−
2011/11802	HUS	−	+	+	+		Rnew3:[F2]	+‡	−
2012/02395	—	+	+	−	+	−	Rnew4:[F19]	−‡	−
2012/02396	None	+	+	−	+	−	None	(+)	−
2012/02846	BD	+	+	+	+		O157:H7	+	−
2012/02998	—	−	+	−	−	−	R148:F8	−‡	−
2012/03065	—	+	−	+	+		O26:F11	+	−
2012/03794	Diarrhea, HUS	−	+	+	+		O157:[F7]	+	−
2012/03796	—	+	−	+	+		O26:F11	+	−
2012/05675	Diarrhea	+	+	+	+		O157:[F7]	+	−
2012/06881	Diarrhea, HUS	−	+	+	+		O157:[F7]	+	−

[a] BD, bloody diarrhea; HUS, hemolytic-uremic syndrome; —, no information.
[b] The virulence gene content reported is that obtained both for stool specimens and from isolated colonies, except for the four cases with no isolated STEC strain; *hlyA* refers to EHEC *hlyA*; the O type determined by *rfb*-RFLP and the H type determined by *fliC* sequencing are denoted R and F, respectively; an F type in brackets indicates the presence of nonmotile strains; R types not present in our *rfb*-RFLP database are denoted Rnew1 to Rnew4.
[c] + and +‡, mauve colonies with the same STEC isolated as that isolated by the standard procedure and obtained following enrichment protocols 1 and 2, respectively; (+), no virulence genes were detected in mauve colonies; −‡, no mauve colonies were detected following either enrichment protocol 1 or enrichment protocol 2.
[d] +, mauve colonies with the same STEC isolated as that isolated by the standard procedure and obtained following enrichment protocol 1; −, no mauve colonies following enrichment protocol 1.

hlyA genes (Table 2). None possessed the *bfpA* gene, carried by the EAF plasmid, so they were not typical EPEC strains. These nine *eae*-positive strains belonged to eight serotypes and may be either atypical EPEC or EHEC that have lost Shiga toxin genes during infection (EHEC-LST) (6, 25, 26).

If we consider these nine isolates to be false positives (atypical EPEC), then the sensitivity and specificity of the CHROMagar STEC medium for recovering STEC strains, relative to the reference standard method, were 91.4% (32/35), and 83.7% (246/294), respectively, with a positive predictive value (PPV) and a negative predictive value (NPV) of 40% (32/80) and 98.8% (246/249), respectively (Table 3).

If these nine isolates are true positives (EHEC-LST), then the sensitivity and specificity of the CHROMagar STEC medium were 89.1% (41/46) and 86.7% (246/283), respectively, with a PPV of 51.3% (41/80) and an NPV of 98% (246/251) (Table 3).

TABLE 2 Data for the 15 Shiga toxin-negative, *eae*-positive stool specimens

Stool specimen	Clinical symptoms[a]	Results by standard procedure[b]				Result with the following chromogenic medium:	
		Presence of the following virulence factor:					
		eae	*hlyA*	*bfpA*	*E. coli* serotype isolated	CHROMagar STEC[c]	CHROMagar STEC O104[d]
2011/04625	BD	+	+	−	O145:F28	+	−
2011/05678	—	+	−	−	O111:F21	−‡	−
2011/07736	—	+	−	−	R38:[F9]	+	−
2011/07971	BD	+	−	−	R101/162:F33	+	−
2011/08324	—	+	−	−	Rnew5:F49	−‡	−
2011/08954	BD	+	+	−	O145:F28	+	−
2011/09320	Diarrhea	+	−	−	Rnew6:[F21]	+	−
2011/09604	—	+	−	−	None	(+)	−
2011/09777	—	+	+	−	O26:F11	+	−
2011/10026	BD	+	−	−	None	−‡	−
2012/00278	BD	+	−	−	None	−‡	−
2012/00781	Diarrhea	+	−	−	None[d]	+‡[e]	−
2012/00872	HUS	+	−	−	R76:F7	+	+
2012/06703	Diarrhea	+	+	−	R121:F19	+	−
2012/08776	—	+	−	−	None	−	−

[a] BD, bloody diarrhea; HUS, hemolytic-uremic syndrome; —, no information.
[b] The virulence gene content reported is that both for stool specimens and for isolated colonies, except for the four cases with no isolated *eae*-positive *E. coli* strain; the O type determined by *rfb*-RFLP and the H type determined by *fliC* sequencing are denoted R and F, respectively; an F type in brackets indicates the presence of nonmotile strains; R types not present in our *rfb*-RFLP database are denoted Rnew5 and Rnew6.
[c] + and +‡, mauve colonies with the same STEC isolated as that isolated by the standard procedure and obtained following enrichment protocols 1 and 2, respectively; (+), no virulence genes were detected in mauve colonies; − and −‡, no mauve colonies following enrichment protocols 1 and 2, respectively.
[d] +, mauve colonies with the same STEC isolated as that isolated by the standard procedure and obtained following enrichment protocol 1; −, no mauve colonies following enrichment protocol 1.
[e] An *eae*-positive R51:F40 strain was recovered only on CHROMagar STEC.

Performance of the CHROMagar STEC O104 agar medium. CHROMagar STEC O104 is a chromogenic agar medium supplemented with cephalosporins that was developed during the large outbreak of HUS in Germany in May 2011. The causative STEC agent, O104:H4, produced an extended-spectrum β-lactamase (ESBL). Of the 39 Shiga toxin-positive stool specimens in our study, 5 grew on CHROMagar STEC O104 medium and showed typical mauve colonies (Table 1). All five stool specimens were collected during the French O104:H4 outbreak and contained a CTX-M-15 ESBL-producing STEC O104:H4 strain, similar to the strain involved in the German outbreak (9, 11). However, there was no growth on CHROMagar STEC O104 for samples from two other cases of infection by an STEC O104:H4 strain; in both cases, the strains were penicillinase producers but not ESBL producers. One case (corresponding to stool specimen 2011/05004) was a secondary household transmission associated with the French outbreak (27). The other case (corresponding to stool specimen 2011/09110) was probably acquired during travel to Turkey in September 2011 (28). This strain was closely related to but not the same as the strains involved in the German and French outbreaks.

Of the 294 stool specimens from which no STEC was isolated by the standard procedure, only 3 yielded mauve colonies on CHROMagar STEC O104 (and on CHROMagar STEC). Each of these three strains produced an ESBL. Two strains did not contain any of the virulence genes tested, and one was positive for *eae* (not shown and Table 2).

TABLE 3 Results with CHROMagar STEC medium compared with those by the standard procedure on 329 stool specimens

CHROMagar STEC result	No. of stool specimens			
	STEC isolated by the standard procedure[a]		*eae*-positive *E. coli* isolated	
	Positive	Negative	Positive	Negative
Positive	32	48	41	39
Negative	3	246	5	244
Total (n = 329)	35	294	46	283

[a] Four stool specimens that were *stx* positive but from which STEC was not isolated were scored as negative.

DISCUSSION

Chromogenic media have been marketed in recent years for the detection of STEC O157:H7 in humans, food, and animal feedstuffs. These media contain a particular mixture of artificial chromogenic conjugates composed of a substrate for an *E. coli*-specific enzyme coupled to a chromophore. When the *E. coli* enzyme cleaves the colorless conjugate, one or more insoluble chromophores are released, giving a distinctive color to the *E. coli* colonies. *E. coli* O157:H7 can be distinguished from *E. coli* non-O157 with substrates specifically recognized by β-D-galactosidase and β-D-glucuronidase (13). β-D-Galactosidase is produced by all *E. coli* strains, whereas β-D-glucuronidase is produced by all *E. coli* strains except NSF STEC O157:H7. These media also contain various selective agents, such as antibiotics and potassium tellurite developed for sorbitol agar media, to inhibit the growth of the many sorbitol nonfermenters (13): cefixime has been used to inhibit *Proteus* spp., and tellurite has been used to inhibit *Aeromonas*

spp. and *Morganella* spp. Potassium tellurite at a concentration of 2.5 mg/liter also improves the selection of STEC NSF O157:H7, which displays higher MICs than *E. coli* isolates of the normal flora (29). Resistance to tellurite is encoded by the *terZABCDEF* gene cluster present in one or two pathogenicity islands of STEC NSF O157:H7 (21, 30–32).

In response to the increase in the number of infections caused by STEC other than NSF O157:H7, a new medium (CHROMagar STEC, whose composition has not been made publicly available) and a second medium derived from it (CHROMagar STEC O104) have been commercialized for the identification of the most common STEC strains and the O104:H4 outbreak strain, respectively. To be useful in the routine in clinical microbiology, these chromogenic media need to detect the prevalent STEC populations circulating in the geographic area covered by the laboratory with good sensitivity and specificity (not growing too many mauve colonies that are not STEC). The most common EHEC types, in particular, those containing the *eae* gene, in bacterial collections can be detected on CHROMagar STEC, and their growth correlated well with resistance to tellurite encoded by genes of the *terZABCDEF* cluster (20, 21). The performance of CHROMagar STEC for the most prevalent serogroup, O157, was variable and depended on the relative proportions of NSF O157:H7/NM and SF O157:[H7]. The failure of SF O157:[H7] to grow on CHROMagar STEC is associated with the absence of the *terABCDEF* cluster from this bacterial population (20, 21, 31, 33).

We report an evaluation of the suitability of CHROMagar STEC and CHROMagar STEC O104 agar media for routine diagnostic use with stool specimens. The study covered a relatively long period (14 months) and included a significant number of stool specimens (>300) from patients with suspected STEC infection (>10% positivity for various serotypes). Note that the number of laboratory-confirmed STEC infections, the proportion of positive stool specimens, and, to a lesser extent, the serotype distribution are not representative of all STEC infections in France: the laboratories of the FNRC-EcS network preferentially provide *E. coli* isolates rather than stool specimens, most samples were from adults, and O104:H4 isolates were overrepresented due to the outbreak caused by this pathogen in France in June 2011.

In our study, CHROMagar STEC medium allowed the recovery of the STEC strain from 32 of the 39 (82.1%) Shiga toxin-positive stool specimens. These results were good, as the standard procedure involving Drigalski agar allowed the recovery of only three additional STEC strains. The isolates that grew on CHROMagar STEC medium belonged to the most prevalent EHEC serogroups, including O157, O26, and O103, but also less common serogroups, such as O118, O148, and O121. The O104:H4 outbreak strain was also detected in 100% of the cases in which it was suspected. This good sensitivity (82.1%), however, required modifications of the enrichment step (longer incubation and larger inoculum; protocol 2), as only 26/39 (66.7%) of the STEC positive-stool specimens yielded mauve colonies with the original enrichment protocol. It has been reported that the *ter* cluster is less widespread in *eae*-negative STEC strains than in *eae*-positive strains (31), and consequently, *eae*-negative STEC strains are significantly less well detected on CHROMagar STEC (20). However, five of the six STEC strains recovered after enrichment protocol 2 were *eae* negative. This higher inoculum may allow *ter*-negative, *eae*-negative strains to grow on CHROMagar STEC, as observed for O103:H2 by Hirvonen et al. (20). These *eae*-negative O103:H2 strains did not contain *terD*, but plating a dense cell suspension led to the growth of normal-sized mauve colonies on the chromogenic medium. They possibly express a mechanism, unrelated to the *ter* cluster, conferring tolerance to tellurite, but this remains to be elucidated. For the three strains not growing on CHROMagar STEC, the problem did not appear to be associated with the enrichment step, as they were not able to grow on the medium even after reculturing from Drigalski agar. Two of the three strains that did not grow on this chromogenic medium were *eae* positive. Two of these strains belonged to serotypes with a low or very low prevalence (O80 and O148) (17), and the third one belonged to a previously undescribed serotype. One reason for the good sensitivity that we observed was that all our STEC O157 strains were NSF and not SF. This particular SF O157:[H7] population had not been detected in France before June 2011, when there was an outbreak of 18 pediatric cases of HUS due to the consumption of contaminated frozen ground beef in the north of France (34). Several STEC strains, including SF O157:[H7], were found both in patients and in the imported meat. Although the strains were isolated during the study period, they were not referred to us, but it is very likely that they would not have grown on CHROMagar STEC medium, such that their inclusion would have decreased the sensitivity value calculated for this medium.

For a chromogenic medium to be suitable for routine STEC screening, it is important that it has good specificity to minimize false positives, which generate an additional workload. We found a false-positive rate of 16.3% (48/294). This is slightly better than the 18.3% (13/71) observed by Tzschoppe et al. (21) with their collection of strains but considerably higher than the 1.1% (3/186) observed by Hirvonen et al. (20) with their strain collection. The collection used by Hirvonen et al. (20) consisted of nonfecal *E. coli* isolates and thus did not represent the *E. coli* populations of the normal flora. As tellurite resistance determinants have been reported on various plasmids (IncHI1, IncHI2, IncP) that also carry multiple antibiotic resistance determinants in *Enterobacteriaceae* (32, 35), it is possible that such plasmids and *ter* genomic islands are more prevalent in *E. coli* from the intestinal flora due to horizontal transfer.

The specificity rate that we calculated was higher (up to 86.7%) if all or some of the nine cases with mauve *stx*-negative, *eae*-positive *E. coli* colonies were considered to be EHEC-LST rather than atypical EPEC. Two studies on patients with HUS or bloody diarrhea in Germany concluded that most of the *stx*-negative, *eae*-positive, and EAF-negative *E. coli* strains recovered from such cases were actually EHEC-LST (25, 26). The authors estimated that for 1 in every 10 patients with EHEC-mediated HUS, the strain isolated is an EHEC-LST strain (25). Various stimuli may lead to *stx*-phage excision, including UV light and antimicrobial therapy (25, 26). In our study, the *stx*-negative, *eae*-positive, and *bfpA*-negative strains belonged to common EHEC serotypes such as O145:H28 ($n = 2$) and O26:H11 ($n = 1$) and/or were associated with classic EHEC infection symptoms (three with bloody diarrhea, one with HUS).

In addition to the good performance of CHROMagar STEC medium, it is also rapid: putative STEC colonies can be clearly visualized the day after receipt of the stool specimen, at the same time that the results of PCR tests for *stx* genes or immunodetection of Shiga toxins are available. This is very convenient, as otherwise, about 10 and even up to 20 *E. coli*-like colonies have to be sampled from each plate of less selective medium and tested by

PCR for virulence genes to identify STEC-positive colonies and the representative isolate(s) must be typed.

While the manuscript was in the submission process, Wylie et al. (36) published a study evaluating CHROMagar STEC in comparison to a cytotoxin assay involving 205 routine stool samples sent to a provincial laboratory in Manitoba, Canada, over a 10-month period. The authors also tested 111 STEC isolates (belonging to 29 serotypes) from their collection. Of the 111 STEC isolates, 96 (86.5%) grew on the chromogenic medium. Together with the studies by Hirvonen et al. (20) and Tzschoppe et al. (21) (≈75% of the STEC isolates in these two collections grew), this third, Canadian study confirms that most STEC isolates are able to grow on CHROMagar STEC: of the 205 routine stool specimens, 14 were found to be true positive for STEC. The sensitivity, specificity, PPV, and NPV of CHROMagar STEC for stools were 85.7%, 95.8%, 60%, and 98.9%, respectively. The NPV was therefore similar to that in our study. However, we found a higher sensitivity (≈90%) but a lower specificity (≈85%) and a lower PPV (≈46%). This might be explained by a methodological difference: we used an enrichment step with the stool samples, whereas Wylie et al. (36) used direct inoculation. Also, the distribution of STEC populations may differ between Canada and France, resulting in apparent differences in performance.

We show that the second chromogenic medium, CHROMagar STEC O104, could identify the STEC O104:H4 strain involved in the small French outbreak; this strain produces a CTX-M-15 ESBL, as described for the German strain, which also grows on this medium (11, 21). However, other sporadic O104:H4 isolates identified after the summer of 2011 and not epidemiologically linked with these two outbreaks did not produce an ESBL and consequently could not be detected by CHROMagar STEC O104 (28, 37). Furthermore, the marker used by this medium, based on resistance to extended-spectrum cephalosporins encoded by an IncI1 plasmid, is not stable, and indeed, the resistance was lost during transmission within a household during the French outbreak (27). Therefore, the CHROMagar STEC O104 medium developed during the German outbreak is not suitable for detecting all STEC O104:H4 strains and is useful only for detecting STEC strains having acquired resistance to extended-spectrum cephalosporins.

In conclusion, this study carried out on >300 stool specimens from patients with suspected STEC infection shows that CHROMagar STEC agar medium, after an enrichment step, performed well in comparison with our "gold standard" method. The good performance of CHROMagar STEC agar medium, in particular, the high negative predictive value and its capacity to identify NSF O157 and common non-O157 STEC strains, makes this medium potentially useful for clinical bacteriology laboratories. Its place in the STEC diagnostic arsenal needs to be considered in the light of new trends in the epidemiology of EHEC. Focusing on only historical (NSF) O157 strains with selective and differential or chromogenic culture media (sorbitol-MacConkey agar [SMAC], cefixime tellurite-sorbitol MacConkey agar [CT-SMAC], or CHROMagar O157) seems to be inappropriate because non-O157 EHEC strains are becoming more common (e.g., in France during the period from 2006 to 2010, only 42% of the 439 EHEC strains isolated by the National Reference Center for *E. coli*, *Shigella*, and *Salmonella* were O157; F.-X. Weill, unpublished data). CHROMagar STEC failed to detect only 5/249 (2%) NSF O157 isolates when combining the data from the three published studies (20, 21, 36) and ours, and furthermore, it covers most non-O157 STEC strains. Therefore, the cost/benefit ratio does not argue in favor of using CHROMagar STEC as a supplemental medium, in addition to an O157 medium. Indeed, CHROMagar STEC might be a true alternative to O157 culture media if culture is associated with the use of a method targeting Shiga toxins (detection of *stx* genes by PCR, immunodetection of Shiga toxins in stool specimens, or Vero cell cytotoxicity assay). This type of combined approach is similar to the methodology recommended by the CDC for routine screening of stool specimens from cases of community-acquired gastroenteritis, except that the culture media are oriented toward O157 (38). Most clinical laboratories that are not reference laboratories do not use nucleic acid amplification tests or Vero cell cytotoxicity assays but are able to perform methods for direct antigen detection for Shiga toxins 1 and 2. Immunochromatographic cartridge assays (differentiating Stx1 from Stx2) designed for single-specimen testing and microwell immunoassays (not differentiating between Stx1 and Stx2) designed for batch testing are now available commercially for use with stool specimens (39). By this combined approach, STEC colonies can be clearly visualized the day after receipt of the stool specimen, at the same time that the results of immunodetection of Shiga toxins (generally performed on stool enrichment) are available. The limitations of CHROMagar STEC (not all STEC isolates grow) and those of immunoassays (not all Stx subtypes are recognized) may counterbalance, allowing optimal detection of STEC. This approach would allow selective referral to a reference laboratory of mauve colonies in cases of positive immunoassay results (and in cases of negative immunoassay results, when STEC infection is strongly suspected) and of stool specimens in cases of positive immunoassay results without growth of mauve colonies. However, this proposed combined approach needs to be validated by a prospective trial, and such a study should begin soon at the FNRC-EcS.

Another advantage of using CHROMagar STEC compared with an O157 culture medium, whatever the nature of the second method targeting Shiga toxins and whatever the type of laboratory (clinical or reference laboratories), is that CHROMagar STEC allows rapid isolation of putative non-O157 STEC colonies for subsequent characterization. This is essential for both real-time surveillance of STEC infections and investigation of outbreaks.

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