RESEARCH LETTER

Novel differential and confirmation plating media for Shiga toxin-producing *Escherichia coli* serotypes O26, O103, O111, O145 and sorbitol-positive and -negative O157

Björn Possé[1], Lieven De Zutter[1], Marc Heyndrickx[2] & Lieve Herman[2]

[1]Laboratory of Hygiene and Technology, Department of Veterinary Public Health and Food Safety, Faculty of Veterinary Medicine, University of Ghent, Merelbeke, Belgium; and [2]Ministry of the Flemish Community, Institute of Agricultural and Fisheries Research (ILVO), Unit Technology and Food, Melle, Belgium

Correspondence: Lieven De Zutter, Laboratory of Hygiene and Technology, Department of Veterinary Public Health and Food Safety, Faculty of Veterinary Medicine, University of Ghent, Salisburylaan 133, 9820 Merelbeke, Belgium. Tel.: +32 09 264 74 51; fax: +32 9 264 74 91; e-mail: lieven.dezutter@ugent.be

Received 2 August 2007; accepted 1 February 2008.
First published online 19 March 2008.

DOI:10.1111/j.1574-6968.2008.01121.x

Editor: Bruce Ward

Keywords
STEC; differential medium; O157; non-O157.

Abstract

This study reports two novel selective differential media. A first differential medium can be applied in methods for the isolation of non-O157 Shiga toxin-producing *Escherichia coli* (STEC) serotypes (O26, O103, O111 and O145) from food or faeces. A second differential medium was designed for both sorbitol-positive and -negative O157 STEC strains. Selective differential media are based on a chromogenic compound to signal β-galactosidase activity and one or more fermentative carbon sources. The chromogenic marker and carbohydrates were combined with a pH indicator and several inhibitory components, which resulted in highly specific differentiation media. Consecutive use of a serotype-dependent choice of confirmation media resulted in a very low incidence of false-positive isolates when comparing clinical STEC strains with a collection of commensal *E. coli* strains.

Introduction

Escherichia coli is widely recognized as an enteric commensal, but the species also includes a significant pathogenic group frequently associated with severe human enteric infection and characterized by the production of virulence factors, among which Shiga toxins are always present (Shiga toxin-producing *E. coli* or STEC). Although *E. coli* O157 is currently most widely recognized, more than 100 other STEC serotypes have been implicated in cases of human disease. Among these other serotypes, O26, O103, O111 and O145 have been increasingly isolated from clinical cases and outbreaks as well as from animals and environmental sources (Eklund *et al.*, 2001; Bettelheim, 2003, 2007; Sonntag *et al.*, 2004; Brooks *et al.*, 2005; Guth *et al.*, 2005). Together with serotype O157, these serotypes are referred to as 'the gang of five' (Beutin, 2006). Global testing of beef cattle faeces revealed prevalence rates ranging from 2.1% to 70.1% for non-O157 STEC and from 0.2% to 27.8% for O157 STEC (Hussein & Bollinger, 2005). European data indicated that the proportion of clinical cases linked to non-STEC serotypes ranged between 6% and 84% (Enter-net, 2005). Only a few food laboratories screen for these non-O157 STEC, mainly because of the absence of a specific International Organisation for Standardisation (ISO) method (ISO, 2001). Non-O157 STEC strains are a heterogeneous group that display a broad range of both genotypic and phenotypic differences (Schmidt *et al.*, 1999; Bettelheim, 2000, 2003, 2007).

O157:H7 serotyped strains have a unique phenotypic feature, facilitating their isolation from food and faeces: they fail to ferment sorbitol within 24 h of incubation. Sorbitol MacConkey agar was developed to exploit this unique phenotypic feature (March & Ratnam, 1986). More recently, however, sorbitol-positive strains have been increasingly isolated from clinical cases (Gunzer *et al.*, 1992; Bielaszewska *et al.*, 1998; Karch & Bielaszewska, 2001), stressing the importance of screening food products for both sorbitol-positive and -negative O157.

This study describes a new selective differential agar medium for STEC serotypes O26, O103, O111 and O145, based on

a mixture of carbohydrate sources, β-D-galactosidase activity and selective components, which allows colour-based separation of these serotypes. In addition, we describe a modified selective differential agar medium suitable for both sorbitol-positive and -negative O157 STEC. The incidence of false-positive results is reduced by consecutive serotype-dependent use of one or more confirmation agar media.

Materials and methods

Cultures

A total of 127 *E. coli* isolates were included in this study. Eighty-five strains belonging to several STEC serotypes had been isolated in different European countries from hospitalized patients diagnosed with (bloody) diarrhoea, haemorrhagic colitis (HC) or haemolytic uremic syndrome (HUS). A representative panel of strains containing between 11 and 15 strains of each of the group of serotypes was used (O26, O103, O111, O145, sorbitol-negative and sorbitol-positive O157). Forty-two commensal *E. coli* strains isolated from beef carcasses were also included. Commensal strains were isolated using Rapid *E. coli* medium (Bio-Rad). They were considered to be commensal because all tested PCR negative for both *stx1* and *stx2* and they did not belong to one of the serotypes mentioned above. The origin, metabolic and genetic characteristics of the strains were described elsewhere (Possé *et al.*, 2007).

A panel of non-*E. coli* control strains was used to evaluate the selectivity of the differential STEC medium for non-STEC serotypes. These strains are listed in Table 1. All strains were stored at −80 °C using Pro-Lab Microbank cryovials according to the manufacturer's instructions. Strains were cultured on tryptone soy agar (TSA) (Oxoid) at 37 °C for 24 h. From these plates, one colony was transferred for overnight incubation at 37 ° C in tryptone soy broth (TSB) (Oxoid): this culture was diluted appropriately prior to being used in the test described in this paper.

Set of media for *E. coli* O26, O103, O111 and O145 differentiation

A set of two different media, a selective differential medium and a confirmation medium, was used to consecutively differentiate and confirm strains belonging to serotypes O26, O103, O111 and O145. The composition of the selective differential medium was: MacConkey agar base (BD Biosciences) 40 g L^{-1}, sucrose (Sigma) 6.0 g L^{-1}, sorbose (Sigma) 6.0 g L^{-1}, bile salts No. 3 (Sigma) 3.5 g L^{-1}, 5-Bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-gal, Glycosynth) 0.05 g L^{-1}, isopropyl-β-D-thiogalactopyranoside (IPTG, Glycosynth) 0.05 g L^{-1}, novobiocin (Sigma) 8.0 mg L^{-1} and potassium tellurite (Sigma) 2.5 mg L^{-1}. All components, except X-gal, IPTG, novobiocin and bile salts No. 3, were added to the MacConkey agar base, suspended in distilled water and autoclaved for 15 min at 121 °C after

Table 1. Growth of control strains on the differential medium and CT-SMAC

		Growth on	
Species	Strain	Differential medium*	CT-SMAC
Enterobacteriaceae			
Citrobacter freundii	LMG 3246†	NG‡	NG‡
Enterobacter cloacae	LMG 3014†	NG‡	NG‡
Enterobacter cloacae	LMG 3008†	GG‡; Purple, slimy	GG‡; colourless
Klebsiella pneumoniae	LMG 3080†	NG‡	NG‡
Proteus mirabilis	LMG 3257†	MG‡; colourless	NG‡
Proteus vulgaris	LMG 2096†	NG‡	NG‡
Salmonella Typhimurium	MB 525§	NG‡	MG‡ purple
Salmonella Enteritidis	MB 478§	NG‡	GG‡; colourless
Serratia marcescens	LMG 2792†	NG‡	NG‡
Shigella flexneri	LMG 10472†	NG‡	GG‡ colourless
Yersinia enterocolitica	MB 818§	NG‡	NG‡
Hafnia alvei	LMG 2791†	NG‡	NG‡
Pseudomonas and Gram-positive bacteria			
Pseudomonas aeruginosa	LMG 6395†	NG‡	GG‡; colourless
Enterococcus faecalis	LMG 8222†	NG‡	GG‡; colourless
Staphylococcus aureus	LMG 8064†	NG‡	NG‡
Staphylococcus epidermidis	LMG 10273†	NG‡	NG‡
Listeria monocytogenes	MB 1916§	NG‡	NG‡

*Differential medium for selection of non-O157 serotypes, containing sucrose and sorbose.
†LMG, strain obtained from the 'Belgian Co-ordinated Collections of Micro-organisms' BCCM/LMG public collection.
‡NG, no growth, GG, good growth (comparable to growth rates on TSA), MG, moderate growth (reduced growth rate compared with growth on TSA).
§MB, strain collection 'Molecular Bacteriology' at ILVO-T&V laboratories, Melle, Belgium.

boiling. X-gal, IPTG, novobiocin and bile salts No. 3 were suspended in distilled water, filter sterilized (Millipore, 0.22 μm) and added to the modified MacConkey agar after autoclaving and cooling to 50 °C. The final pH was 7.4 at 25 °C. Differential plates were placed in an incubator at 37 °C for 24 h after inoculation of 100 μL of pure TSB culture diluted to 10^3 CFU mL^{-1} in sterile Pepton Water. After incubation, suspected colonies could be identified based on the general appearance and colour as explained further (Figs 1 and 2a–d). Suspected colonies were picked from the differential medium and streaked onto one or more specific confirmation media, composed of phenol red broth base (Sigma – 20 g L^{-1}) supplemented with 6 g L^{-1} of Dulcitol (Sigma), L-rhamnose (Sigma) or D-raffinose (Sigma) or 10 g L^{-1} of D-arabinose (Sigma) as a specific carbohydrate (Fig. 1 and Table 2). Confirmation media were autoclaved for 15 min at 121 °C. Plates were aerobically incubated for 24 h at 37 °C, except for confirmation media containing D-arabinose: these plates were anaerobically incubated at 37 °C for 48 h. After incubation, plates were scored based on the presence (yellow colonies) or absence (red colonies) of carbohydrate fermentation.

Set of media for differentiation of *E. coli* O157 strains

The composition of the differentiation medium for both sorbitol-negative and -positive O157 was: MacConkey agar base 40 g L^{-1}, sorbitol (Sigma) 10.0 g L^{-1}, bile salts No. 3 3.5 g L^{-1}, X-gal 0.05 g L^{-1}, IPTG 0.05 g L^{-1}, novobiocin 16.0 mg L^{-1} and potassium tellurite 2.5 mg L^{-1}. All components, except X-gal, IPTG, novobiocin and bile salts No. 3, were added to the MacConkey agar base, suspended in distilled water and autoclaved for 15 min at 121 °C after boiling. X-gal, IPTG, novobiocin and bile salts No. 3 were suspended in distilled water, filter sterilized (Millipore, 0.22 μm) and added to the modified MacConkey agar after autoclaving and cooling to 50 °C. The final pH was 7.4 at 25 °C. Differential plates were placed in an incubator at 37 °C for 24 h after inoculation of 100 μL of pure TSB culture diluted to 10^3 CFU mL^{-1} in sterile Pepton Water. After incubation, green colonies indicate sorbitol-negative colonies while purple colonies indicate sorbitol-positive colonies (Fig. 2e). To decrease the incidence of false-positive results, suspected sorbitol-positive colonies were streaked on the confirmation medium containing Phenol red broth base (20 g L^{-1}) and L-rhamnose (6 g L^{-1}) as indicated in Fig. 1, and incubated for 24 h at 37 °C. After incubation, plates were scored based on the presence (yellow colonies) or the absence (red colonies) of carbohydrate fermentation.

Evaluation of selectivity of the differential medium for non-O157 STEC serotypes using non-*E. coli* strains

The differential medium described above for non-O157 STEC serotypes was inoculated separately with clinical STEC strains or a selection of non-*E. coli* control strains (Table 1). From pure cultures, pregrown in TSB, 100 μL was streaked on the non-O157 differential medium, on sorbitol MacConkey agar (Oxoid) supplemented with cefixime-tellurite (Dynal) (CT-SMAC) and on TSA. After incubation for 24 h at 37 °C, morphological characteristics were evaluated and the growth rate (colony count) was compared using the

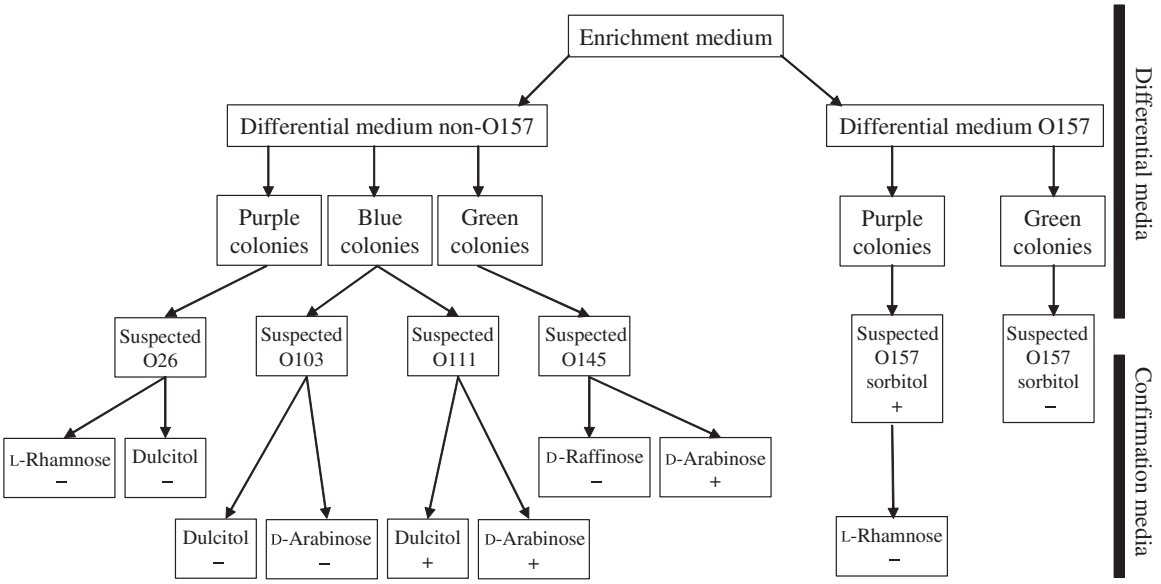

Fig. 1. Use of two sets of differential and confirmation media for the isolation of O26, O103, O111, O145 and O157 STEC strains.

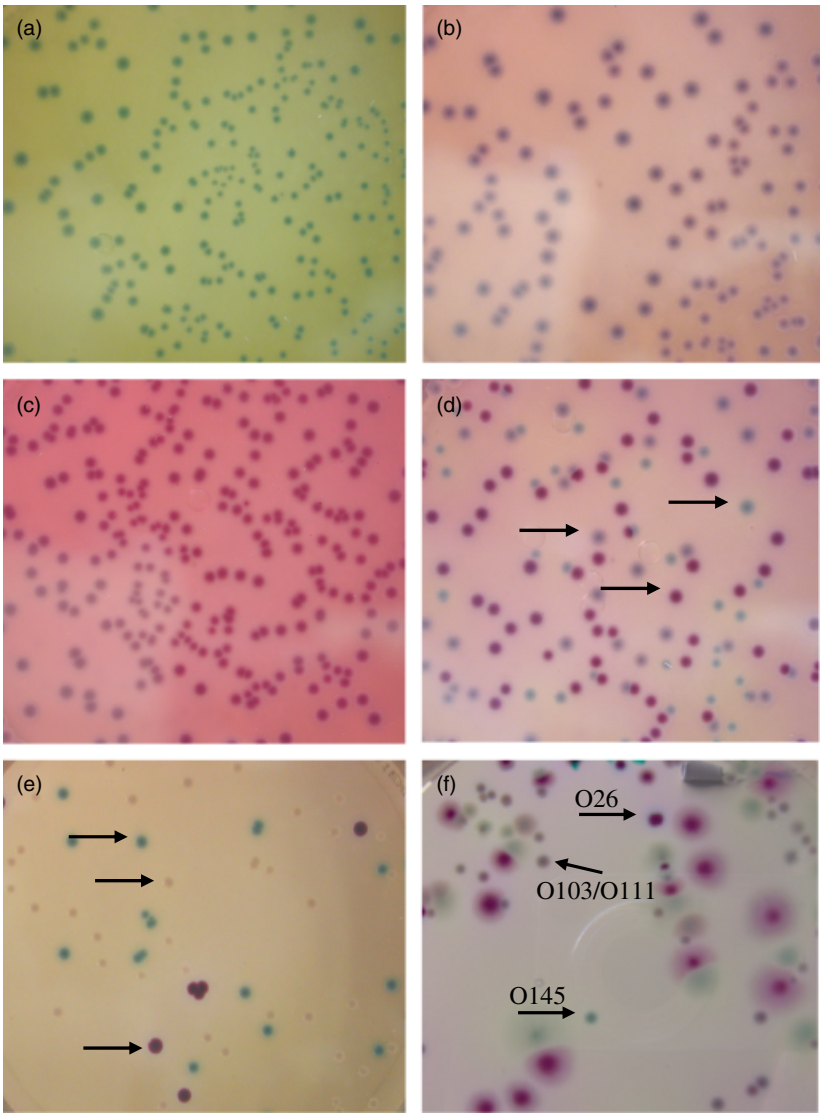

Fig. 2. (a–f) Growth of commensal *Escherichia coli* and clinical STEC strains on the differential media. Various enrichment procedures can be applied. No recommendation on this subject was given in this paper. O145 STEC strain, unable to ferment sucrose and sorbose: hydrolyzation of X-gal turns a yellow coloured colony into a light green morphology (a); Morphology of an O103 and O111 strain: 1 out of 2 carbohydrates was fermented resulting in a blue-purple colour when combined to X-gal hydrolyzation (b). O26 strains are able to ferment both sucrose and sorbose resulting in a pH shift changing neutral red from pale yellow to dark red. This dark colour is enhanced by X-gal hydrolyzation turning it into bright purple to red colonies (c). Picture d contains a mixture of three types of colonies (green O145/blue O103 & O111/purple O26): all serotypes can easily be distinguished based on colour properties (indicated by arrows). Sample of the isolation medium for O157 strains. Sorbitol positive (purple) and negative (green) colonies can easily be discriminated based on carbohydrate fermentation and X-gal hydrolyzation. The picture also contains background bacteria (colourless – no X-gal hydrolyzation) which were identified as *Hafnia alvei* (e). Mixture of random picked commensal strains grown on the non-O157 differentiation medium. This mixture also contained an O26, O103, O111 and O145 strain which could be morphologically identified (arrows) (f).

TSA plates as a reference, considering that all strains grew abundantly on the TSA plates.

Calculation of false-positive ratios (FP %)

The carbohydrate fermentation properties of all strains used in this study were determined using API50 test strips (Biomérieux, France). Detailed data on these carbohydrate fermentation profiles have been published previously (Possé *et al.*, 2007). The frequency of isolation of a commensal *E. coli* strain expressing the exact same fermentation pattern as clinical STEC strains, for carbohydrates used in the differential medium ('FP % D') or in both the differential and the confirmation media ('FP % D+C'), is referred to as the false-positive ratio in this study (Table 2). All data used for these calculations were generated by plating all commensal and STEC strains present in the collection of strains described above on the differential and confirmation media. Calculation of 'FP % D' can thus be formulated as: (frequency of isolation of a commensal *E. coli* with the same fermentation properties for sucrose) × (frequency of isolation of a commensal *E. coli* with the same fermentation properties for sorbose). Additional carbohydrates can be added to calculate 'FP % D+C'. Table 2 shows the range for this false-positive ratio, as identical fermentation properties for sucrose do not imply identical fermentation properties of commensal *E. coli* for sorbose or vice versa.

Evaluation of the differential media using *E. coli* strains

The differential medium described in this study was inoculated with different mixtures of STEC and commensal strains. For this purpose, 10 selected commensal *E. coli*

Table 2. Carbohydrate fermentation properties on differential and confirmation media of clinical STEC serotypes and commensal *Escherichia coli* strains and calculated false positive ratios

	Differentiation medium (D)			Confirmation medium (C)				
Serotype	D-Sucrose	L-Sorbose	FP % D (range) *	D-Arabinose	L-Rhamnose	Dulcitol	D-Raffinose	FP % D+C (range) †
O26	+	+	39 (32–47)		–	–		0.05 (0.04–0.06)
O103	+	–	45 (38–53)	–		–		0.3 (0.26–0.37)
O111	+	–	45 (38–53)	+		+		37.6 (31.8–44.3)
O145	–	–	8 (0–15)	+			–	1.008 (0.0–1.9)
PC‡	85%+	47%+		90%+	98%+	93%+	86%+	

*False Positive ratio using the differentiation medium only: this indicates the frequency of isolating a commensal strain from this medium presenting the exact same fermentation properties as the clinical serotype targeted. The range indicates the lower and upper limit of this frequency.

†False Positive ratio using both the differentiation and confirmation media: this indicates the frequency of isolating and confirming a commensal strain presenting the exact same fermentation properties as the clinical serotype targeted on both media. The range indicates the lower and upper limit of this frequency.

‡PC indicates the collection of 42 commensal strains: the fraction of strains from this collection able to ferment specific carbohydrates is indicated as a %.

Table 3. Mixtures of commensal and clinical STEC strains used for evaluation of the non-O157 STEC differential medium

Mixture	Commensal mixture (CFU mL^{-1})*	STEC strain (CFU mL^{-1})
M1	10 strains (10^2 each)	PH09, PH02, PH29, PH01 (10^2 each)
M2	10 strains (10^2 each)	PH09 (O26:H11) (10^2)
M3	10 strains (10^2 each)	PH02 (O103:H2) (55)
M4	10 strains (10^2 each)	PH29 (O111:H-) (55)
M5	10 strains (10^2 each)	PH01 (O145:H-) (71)

*The mixture of commensal strains was composed of 10 strains to ensure different carbohydrate fermentation properties: PC01, PC06, PC11, PC16, PC21, PC26, PC31, PC36, PC41, PC42 (Possé *et al.*, 2007).

strains and one randomly selected strain from serotypes O26, O103, O111 and O145 were grown overnight at 37 °C in TSB in single cultures. Fully grown cultures were diluted to 10^4 CFU mL^{-1}. From these dilutions, different mixtures were prepared, as shown in Table 3. From these mixtures, 100 μL each was used to inoculate (spread plating) the differential medium for non-O157 STEC and 100 μL was spread plated in parallel on the TSA plates. Both the differential plates and TSA plates were incubated for 24 h at 37 °C after inoculation. Following this incubation, colonies with a 'suspected' morphology were streaked onto the appropriate confirmation media based on the scheme shown in Fig. 1. Final confirmation of the isolates was performed by PCR-based detection of *stx1*, *stx2* and *eae* as described previously (Possé *et al.*, 2007).

Results and discussion

Non-O157 STEC serotype differentiation

The first set of differential and confirmation media was designed for serotypes O26, O103, O111 and O145 (Fig. 1). This set starts with a non-O157 differential medium containing two carbohydrates (sucrose and sorbose): the fermentative properties of these serotypes for these carbohydrates in the differential medium are listed in Table 2. IPTG was added to the differential medium to induce the activity of β-D-galactosidase, an enzyme that promotes lactose utilization, by binding and inhibiting the *lac* repressor. X-gal indicates β-D-galactosidase activity, resulting in a dark-coloured centre in a fully grown colony. As all coliforms (some rare exceptions not included) including *E. coli* STEC serotypes O26, O103 O111 and O145 showed β-D-galactosidase activity, this characteristic increased the ease of identification within the total microbiological background compared with the use of carbohydrate fermentation as the only discriminatory feature. Blending the colony colour originating from X-gal hydrolyzation (dark centre) and the colour change due to carbohydrate fermentation or non-fermentation resulted in seven different colours of the colonies on the differential medium after 24 h of incubation at 37 °C (Fig. 2a–f). O26 strains were able to ferment both sucrose and sorbose, resulting in a significant pH drop (6.1–6.4) below the neutral red colour change interval. This resulted in a bright red to dark purple colour for O26 colonies. O103 and O111 strains were recognized by their blue-purple colour: strains belonging to these serotypes failed to ferment sorbose while sucrose was fermented. The pH drop in the differential medium caused by this fermentation resulted in a final pH that is within the colour change interval of neutral red (6.7–6.8). O145 strains were unable to ferment sucrose and sorbose, presenting only the dark centre indicating β-D-galactosidase activity: these colonies show a typical green colour (pH 7.2–7.3). Figure 2a–c shows detailed images of these three different types of colonies on the differential medium. Three remaining colours on the differential medium comprised carbohydrate fermentation inducing the pH indicator to change colour, without X-gal hydrolyzation (pale yellow, dark orange and red colonies).

These colours were typical for noncoliforms. A seventh possible shade on this medium is a pale translucent colour that occurred for some *Proteus mirabilis* or *Hafnia alvei* strains. Combining both β-D-galactosidase activity and two carbohydrates in a single medium allowed differentiation of selected STEC serotypes from commensal strains, as the latter tended to be able to ferment more carbohydrates compared with STEC strains (Durso *et al.*, 2004; Possé *et al.*, 2007).

Reducing the false-positive ratio using confirmation media for non-O157 STEC

Colonies showing a 'suspected' phenotype on the differential medium for non-O157 STEC were streaked onto one or more confirmation media, as indicated in Fig. 1: for example a 'green' colony on the non-O157 differential medium, indicative of a putative O145 isolate, was transferred to the confirmation medium containing D-raffinose as well as to the confirmation medium containing D-arabinose using the same bacterial loop. This additional confirmation medium allowed strain purification, combined with an additional metabolic confirmation (based on acid production during carbohydrate fermentation: the yellow colony indicates positive fermentation, and the red colony indicates no fermentation) resulting in a dramatic decrease of false-positive ratios for three of the four serotypes (Table 2). Using only the differential medium, this false-positive ratio was estimated at 8% for O145, 39% for O26 and 45% for both O103 and O111. When using both the differential and the confirmation media, the average ratios declined to 0.05% (O26), 0.3% (O103), 1.008% (O145) and 37.6% (O111), respectively. For strains belonging to serotype O111, no additional discriminating carbohydrate could be identified, enabling a decrease of the occurrence of false-positive results to lower levels. Although applying this method on a much larger collection of STEC strains and non-STEC strains belonging to the selected serotypes (*stx* negative strains) will influence these false-positive ratios, the trends observed in this study are likely to prevail due to the lack of intraserotypic variation in the fermentation properties of the carbohydratesused, and the observation of the increased fermentation abilities of commensal strains (Hiramatsu *et al.*, 2002; Possé *et al.*, 2007). No false-negative isolates were obtained based on characterization of our collection of strains: i.e. strains not appearing as suspected on isolation and purification media, while being confirmed as O26, O103, O111, O145 or O157 by conventional or molecular serotyping methods. However, it is not unlikely that this type of strains can be present in naturally contaminated samples or among non-STEC strains belonging to the serotypes included.

Sorbitol-negative and -positive O157 STEC differentiation

A second set of differential and confirmation media was designed for O157 STEC. This set starts with a modified selective differential medium for O157 strains making use of sorbitol fermentation to distinguish sorbitol-positive and -negative colonies. This medium is a modification (addition of X-gal, IPTG, bile salts No. 3 and novobiocin) of the SMAC agar described previously (March & Ratnam, 1986). For sorbitol-positive O157 isolation, a very high number of false-positive colonies was obtained using sorbitol as a single discriminatory substrate as 98% of the tested commensal strains were able to ferment this carbohydrate. To eliminate this high false-positive ratio prior to final confirmation (using serotyping, PCR or other typing methods), sorbitol-positive colonies from the O157-specific isolation medium were streaked onto a confirmation medium containing L-rhamnose (Fig. 1). After 24 h of incubation at 37 °C L-rhamnose fermentation allowed an improved discrimination of sorbitol-positive O157 strains from commensal coliforms indicated by a decrease in the incidence of false positives from 98% down to 1.96%. Based on the sorbitol fermentation properties of the commensal strains, a false-positive ratio of 2% was obtained for sorbitol-negative O157 strains. This confirmed the effectiveness of the ISO 16654 (ISO, 2001) method for isolation of sorbitol-negative O157 STEC from food products.

Evaluation of the selectivity of the differential media

Non-*E. coli* control strains were streaked onto the non-O157 differential medium described in the paragraphs above to demonstrate the selectivity. A list of control strains and their growth properties on the non-O157 selective differential medium and on CT-SMAC are shown in Table 1. All non-*E. coli* control strains presented good growth on TSA (data not shown). All clinical STEC strains showed good growth on the differential agar described in this study, as well as on CT-SMAC and on TSA. Except for one of the tested *Enterobacter cloacae* strains and *Proteus mirabilis*, both showing different colony morphology compared with clinical STEC strains, none of the strains present in the control panel was able to grow on the differential medium. As control strains could be distinguished from STEC strains on the differential medium, isolates were not transferred to the subsequent confirmation media. Comparison of the novel differential medium with CT-SMAC indicated an increased selectivity: control strains of *Salmonella* Enteritidis, *Salmonella* Typhimurium, *Shigella flexneri, Pseudomonas aeruginosa* and *Enterococcus faecalis* were able to grow on CT-SMAC while no growth was observed on the STEC differential medium. The increased concentration of bile

salts No. 3 in the differential medium (5 g L^{-1} compared with 1.5 g L^{-1} in CT-SMAC) inhibits the growth of Gram-positive bacteria. Besides this selective advantage, the increased concentration of bile salts No. 3 also has another advantage: the acid produced during carbohydrate fermentation causes a deposition of bile salts No. 3 around the STEC colonies, which intensifies the colour of the colonies.

All STEC strains have a similar colony morphology: colonies are round and have a solid edge; due to the colour change induced by carbohydrate fermentation and X-gal hydrolyzation, typical colours were detected for all strains in the collection belonging to the different serotypes. Some commensal strains can have similar morphological traits, although many commensal strains have a branched or a slimy morphology: the images obtained of commensal strains used during this experiment are shown in Fig. 2f. To demonstrate the effectiveness of combining a differential medium and serotype-dependent confirmation media, mixed cultures containing both clinical STEC strains and randomly selected commensal strains (Table 3) were plated onto the non-O157 differential medium. From all mixtures of commensal and STEC strains (M1–M5 in Table 3), all isolates with a suspected morphology on the differential medium (green/blue/purple-coloured round colonies with a dark centre and a solid edge) were subsequently confirmed using the designated confirmation media, as shown in Fig. 1. All isolates suspected on both differential and confirmation media were examined using serotype-specific PCR and PCR-based virulence typing as described previously (Possé *et al.*, 2007): all STEC strains added to the mixtures could be isolated and identified using the combination of the differentiation and the confirmation media.

Conclusion

This study describes two sets of a combination of a differential medium with one or more confirmation media for some selected STEC serotypes. A first selective differential medium, containing sucrose, sorbose and X-gal, allows phenotypical discrimination of O26, O103, O111 and O145 STEC serotypes. A second selective differential medium targets sorbitol-positive and sorbitol-negative O157 STEC strains based on sorbitol fermentation and X-gal hydrolyzation. Subsequently, false-positive results were vastly reduced using a serotype-dependent selection of confirmation media (Fig. 1). Only for O111 strain isolation does a false-positive ratio of 37.6% remain due to the absence of an additional discriminatory substrate. However, subsequent final confirmation by conventional or PCR-based serotyping eliminates these false positives. In conclusion, the application of the novel media described in this paper allows selective isolation of a selection of important STEC serotypes based on the combination of a chromogenic compound and selected carbohydrates. The selectivity of the media described in this paper can significantly improve screening for these potentially important food pathogens. Consequently, the differential media described in this paper can be applied by routine diagnostic laboratories to screen for these severe pathogens in food products and faeces from animals and humans by combining these media with specific enrichment procedures.

Acknowledgements

We thank Annelies Wachtelaere, Daisy Alleman, Stefanie Vanbiesbrouck and Karen Albert for excellent technical assistance. This research was supported by the Belgian Science Policy grant CP/58.

References

Bettelheim KA (2000) Role of non-O157 VTEC. *Symp Ser Soc Appl Microbiol* 38S–50S.

Bettelheim KA (2003) Non-O157 verotoxin-producing *Escherichia coli*: a problem, paradox, and paradigm. *Exp Biol Med (Maywood)* **228**: 333–344.

Bettelheim KA (2007) The non-O157 shiga-toxigenic (verocytotoxigenic) *Escherichia coli*; under-rated pathogens. *Crit Rev Microbiol* **33**: 67–87.

Beutin L (2006) Emerging enterohaemorrhagic *Escherichia coli*, causes and effects of the rise of a human pathogen. *J Vet Med B Infect Dis Vet Public Health* **53**: 299–305.

Bielaszewska M, Schmidt H, Karmali MA, Khakhria R, Janda J, Blahova K & Karch H (1998) Isolation and characterization of sorbitol-fermenting Shiga toxin (Verocytotoxin)-producing *Escherichia coli* O157: H- strains in the Czech Republic. *J Clin Microbiol* **36**: 2135–2137.

Brooks JT, Sowers EG, Wells JG, Greene KD, Griffin PM, Hoekstra RM & Strockbine NA (2005) Non-O157 Shiga toxin-producing *Escherichia coli* infections in the United States, 1983–2002. *J Infect Dis* **192**: 1422–1429.

Durso LM, Smith D & Hutkins RW (2004) Measurements of fitness and competition in commensal *Escherichia coli* and *E. coli* O157:H7 strains. *Appl Environ Microbiol* **70**: 6466–6472.

Eklund M, Scheutz F & Siitonen A (2001) Clinical isolates of non-O157 Shiga toxin-producing *Escherichia coli*: serotypes, virulence characteristics, and molecular profiles of strains of the same serotype. *J Clin Microbiol* **39**: 2829–2834.

Enter-net (2005) Enter-net Annual Report: Surveillance of enteric pathogens in Europe and beyond. http://www.hpa.org.uk/hpa/inter/enter-net/Enter-net%20annual%20report%202005%20final.pdf.

Gunzer F, Bohm H, Russmann H, Bitzan M, Aleksic S & Karch H (1992) Molecular detection of sorbitol-fermenting *Escherichia coli* O157 in patients with hemolytic-uremic syndrome. *J Clin Microbiol* **30**: 1807–1810.

Guth BE, Vaz TM, Gomes TA, Chinarelli SH, Rocha MM, de Castro AF & Irino K (2005) Re-emergence of O103: H2 Shiga toxin-producing *Escherichia coli* infections in Sao Paulo, Brazil. *J Med Microbiol* **54**: 805–806.

Hiramatsu R, Matsumoto M, Miwa Y, Suzuki Y, Saito M & Miyazaki Y (2002) Characterization of Shiga toxin-producing *Escherichia coli* O26 strains and establishment of selective isolation media for these strains. *J Clin Microbiol* **40**: 922–925.

Hussein HS & Bollinger LM (2005) Prevalence of Shiga toxin-producing *Escherichia coli* in beef cattle. *J Food Prot* **68**: 2224–2241.

ISO (2001) Microbiological methods, ISO 16654: microbiology of food and animal feeding stuffs – horizontal method for the detection of *Escherichia coli* O15. First Edition.

Karch H & Bielaszewska M (2001) Sorbitol-fermenting Shiga toxin-producing *Escherichia coli* O157:H(-) strains: epidemiology, phenotypic and molecular characteristics, and microbiological diagnosis. *J Clin Microbiol* **39**: 2043–2049.

March SB & Ratnam S (1986) Sorbitol-MacConkey medium for detection of *Escherichia coli* O157:H7 associated with hemorrhagic colitis. *J Clin Microbiol* **23**: 869–872.

Possé B, De Zutter L, Heyndrickx M & Herman L (2007) Metabolic and genetic profiling of clinical O157 and non-O157 Shiga-toxin-producing *Escherichia coli*. *Res Microbiol* **158**: 591–599.

Schmidt H, Geitz C, Tarr PI, Frosch M & Karch H (1999) Non-O157:H7 pathogenic Shiga toxin-producing *Escherichia coli*: phenotypic and genetic profiling of virulence traits and evidence for clonality. *J Infect Dis* **179**: 115–123.

Sonntag AK, Prager R, Bielaszewska M, Zhang W, Fruth A, Tschape H & Karch H (2004) Phenotypic and genotypic analyses of enterohemorrhagic *Escherichia coli* O145 strains from patients in Germany. *J Clin Microbiol* **42**: 954–962.