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Evaluation of test-kits for the detection of *Escherichia coli* O157 in raw meats and cattle faeces.

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Abstract

Escherichia coli O157 detection limits in artificially contaminated beef and cattle faeces samples were determined using Dynabeads anti *E. coli* O157 immunomagnetic beads, VIDAS-UP, VIDAS-ICE, and real-time PCR (GeneDisc and LightCycler) systems. Dynabeads anti-*E. coli* O157 immunomagnetic separation (IMS) and the GeneDisc cycler were the most sensitive methods, and could detect an initial 1 CFU in 25g beef samples after 6h of incubation in modified tryptone soya broth with novobiocin (mTSB+n) or buffered peptone water (BPW). The VIDAS-UP method could detect an initial 10 CFU, while VIDAS-ICE and the LightCycler methods could only detect an initial 100 CFU. Higher detection rates were achieved with 18 hour incubations, where an initial 1 CFU in a 25g sample could be detected with all five methods. For cattle faeces enrichments, Dynabeads anti-*E. coli* O157 IMS could detect an initial 1 CFU after a 6 h incubation in mTSB+n, while the VIDAS-UP and VIDAS-ICE methods could detect an initial 10 CFU and both PCR methods could only detect an initial 100 CFU. Detection rates were lower in BPW, compared to mTSB+n, with thresholds of 100 CFU for VIDAS-ICE, VIDAS-UP and GeneDisc methods, and >100 CFU for the LightCycler method.

Keywords: *E. coli* O157; Dynabeads; VIDAS- ICE; VIDAS-UP; GeneDisc ; LightCycler

Short title: Evaluation of *E. coli* O157 detection methods.

1 Introduction

Escherichia coli was for many years considered to be a non-pathogenic inhabitant of human and animal intestinal tracts, and was only recognized as a cause of infantile gastroenteritis in the late 1940s (Smith & Fratamico, 2005; Fratamico, 2006). Pathogenic strains of *Escherichia coli* have since emerged as a major cause of documented outbreaks and sporadic cases of diarrhoea world-wide, and are reported by the Centers for Disease Control and Prevention to be one of the three most common food-borne infections (CDC, 2005, 2008).

Strains of *E. coli* inducing mild to serious gastrointestinal diseases in humans have been classified into six major categories; enterohaemorrhagic (EHEC), enterotoxigenic (ETEC), enteroinvasive (EIEC), enteroaggregative (EAEC), enteropathogenic (EPEC), and diffusely adherent (DAEC) *E. coli*. However, enterohaemorrhagic *E. coli* are the most significant group of

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diarrhoeagenic *E. coli*, based on frequency and severity of illness. The group includes O157:H7, O26, O111 and sorbitol-fermenting O157:NM serotypes associated with cases of hemorrhagic colitis (HC) and haemolytic uremic syndrome (HUS). All EHEC strains produce Shiga-like toxins (*Stx*), also known as Verotoxins (VT), but require the presence of additional virulence markers to be fully pathogenic, such as the *eae* chromosomal gene associated with attachment and effacement, plasmid encoded enterohemolysin (*ehx*), and an enterocyte effacement pathogenicity island (Buchanan & Doyle, 1997; Lindstedt, Heir, Gjernes, Vardund, & Kapperud, 2003).

E. coli O157:H7 is the serotype accounting for the greatest proportion of EHEC disease cases, and is responsible for more than 73 000 cases of illness and 61 deaths each year in the U.S.A, in comparison to the 37 000 cases and 30 deaths each year attributed to non-O157 EHEC serotypes (CDC, 2008). While undercooked ground beef has been the food most often associated with *E. coli* O157 (Rangel, Sparling, Crowe, Griffin, & Swerdlow, 2005; Ateba & Bezuidenhout, 2008), outbreaks have also been linked to other food vehicles such as apple cider, mayonnaise, pea salad, cantaloupe, lettuce, hard salami, raw milk, goat cheese, and alfalfa and radish sprouts (Buchanan & Doyle, 1997; Meng, Doyle, Zhao, & Zhao, 2007; Stirling, Mc Cartney, Ahmed, & Cowden, 2007; Fonseca, Fallon, Sanchez, & Nolte, 2011). Fruit and vegetables implicated in outbreaks have in most cases been contaminated with cattle or other ruminant manure during harvesting and processing.

Conventional methods for the detection of *E. coli* O157 commonly use enrichment, followed by isolation on Sorbitol MacConkey Agar with cefixime and potassium tellurite (CT-SMAC). Most *E. coli* O157 strains do not ferment sorbitol and colonies are colourless on Sorbitol MacConkey Agar, distinguishing them from sorbitol-fermenting non-O157 *E. coli* strains (Fratamico, 2006). However, traditional isolation methods are time-consuming, and competing flora may obscure O157 colonies thereby giving rise to false negative results. Selective capture with *E. coli* O157 specific magnetic beads significantly reduces background flora, and has been incorporated in isolation methods for improved recovery rates and the reduction of false-negatives on selective agars (International Organization for Standardization, 2001; LeJeune, Hancock, & Besser, 2006). Isolation rates may be further improved by the use of chromogenic agars that can detect sorbitol- fermenting *E. coli* O157 strains. In recent years, a number of companies have developed methods for detection that are specific, faster and more sensitive than traditional culture methods. Enzyme-linked immunosorbent fluorescent assays (VIDAS-UP), immunoconcentration (VIDAS-ICE), and real-time PCR (GeneDisc, LightCycler) are examples of rapid methods that are commercially available for the detection of *E. coli* O157.

Investigation of foods implicated in disease outbreaks indicate that the infectious dose for *E. coli* O157:H7 is lower than 50 cells (Meng et al., 2007). Since *E. coli* O157 may be present in food and environmental samples in small numbers (Reinders, Barna, Lipman, & Bijker, 2002) and the infectious dose is low, sensitive and rapid detection methods are required to ensure a safe supply of foods. The objectives of this study were therefore to determine the relative sensitivity and specificity of newly introduced *E. coli* O157 detection methods, and to assess the applicability of each detection method for routine laboratory use based on labour intensiveness, ease of use and interpretation, cost-effectiveness, and length of time required for results.

2 Materials and Methods

2.1 Bacterial strains and culture conditions

A total of 19 sorbitol negative and 23 sorbitol fermenting *E. coli* O157 strains were obtained from the Food Safety Authority (Zutphen, Netherlands) culture stocks. All cultures were maintained in Brain Heart Infusion with 30% glycerol in Microbank vials (Pro-Lab Diagnostics, Netherlands) at -80°C and resuscitated on Tryptone Soya Agar streak plates (Oxoid, Basingstoke, UK) at 37°C for 18-24 hours before use.

2.2 Test samples

Three ground beef samples were obtained from reputable retail outlets in the Zutphen area (Netherlands), and three cattle faeces samples obtained from a dairy farm in the same locality. Absence of *E. coli* O157 in all samples was confirmed by standard ISO 16654:2001(E) culture protocol (International Organization for Standardization, 2001) before subdivision into 25 g portions and storage at -20°C until use.

2.3 Determination of IMS recovery rates in ground beef and cattle faeces on CT-SMAC Agar

Un-inoculated ground beef and cattle faeces samples (25g) in 225 mL Buffered Peptone Water (Oxoid, Basingstoke, UK) or 225 mL modified Tryptone Soya Broth (Oxoid, Basingstoke, UK) with 20 mg L^{-1} novobiocin (Sigma Chemical Co., St. Louis) were incubated for 6 or 18 hours at 41.5°C. Serial dilutions of overnight brain heart infusion broth cultures of seven *E. coli* O157 strains (Table 1) were prepared, and cell counts determined on CT-SMAC agar plates prepared from Sorbitol MacConkey agar (Oxoid, Basingstoke, UK) supplemented with 0.05 mg L^{-1} cefixime and 2.5 mg L^{-1} tellurite (ITK Diagnostics BV, Uithoorn, Netherlands) .
Immunomagnetic separation was carried out in duplicate on 0.1 mL of 10^{-6} dilutions of the overnight cultures mixed with 0.9 mL of 6 h or 18 hour beef or cattle faeces enrichment. For the IMS procedure (Invitrogen-Dynal, 2007), Dynabeads O157 suspensions (20 μL) were dispensed into 1.5 mL microcentrifuge tubes and 1 mL of the test sample added before incubation for 10 minutes in a Dynal MX4 sample mixer (Invitrogen-Dynal, Oslo, Norway). The bead-bacteria complexes were separated in a magnetic separator rack (Dynal MPC-S) for three minutes, washed three times in 1 mL phosphate buffered saline with 0.05% Tween-20 (PBS-T) and re-suspended in 100 μL PBS-T. Re-suspended samples were then spread on CT-SMAC agar plates, and typical colourless colonies after an 18-24 hour incubation at 37°C confirmed as *E. coli* O157 by a latex agglutination test for the O157 antigen (Oxoid, Basingstoke, UK). Recovery rates were calculated as a percentage of the initial inoculum.

2.4 Detection limits of *E. coli* O157 isolation kits

For each of the three ground beef and three cattle faeces samples, 25g were transferred to 225mL modified Tryptone Soya Broth (mTSB+n) or Buffered Peptone Water (BPW) and inoculated with 0.1 mL of either 10^{-8}, 10^{-7} or 10^{-6} dilution of an overnight brain heart infusion broth culture of *E. coli* O157 (strain NCTC 12079) to give initial cellular counts of 1, 10 and 100 cells, respectively. Inoculated samples were incubated at 41.5°C for 6h or the ISO recommended 18h, and the level and presence of *E. coli* O157 determined using immunomagnetic separation (Dynabeads anti-*E. coli* O157), VIDAS-ICE , VIDAS-UP , GeneDisc O157, and the LightCycler. Uninoculated samples served as negative controls for all detection methods.

Immunomagnetic separation

Dynabeads O157 suspensions (20 μL) were dispensed into 1.5 mL microcentrifuge tubes and 1 mL of 6 hour and 18 hour enrichment test samples added prior to immunomagentic separation as described in 2.3. Re-suspended samples (100μL) were divided into two equal portions and spread on CT-SMAC plates and on CT- CHROM agar O157 (Oxoid, Basingstoke, UK) supplemented with 0.025 mg L^{-1} cefixime and 1.25 mg L^{-1} tellurite. Typical colourless colonies on CT-SMAC, and pink colonies on ½ CT-CHROM after an 18-24 hour incubation at 37°C were confirmed as *E. coli* O157 by a latex agglutination test for the O157 antigen (Oxoid, Basingstoke, U.K).

VIDAS - ICE

VIDAS immuno-concentration *E. coli* O157 (ICE) is a fully automated method for the selective concentration of *E. coli* O157 from enrichment broth, and consists of two components:

Table 1: Recovery rates for *E. coli* O157 with IMS in beef and cattle faeces enrichments.

Strain	Mean % recovery ± sd*							
	Beef				Cattle faeces			
	mTSB+n		BPW		mTSB+n		BPW	
	6h	18h	6h	18h	6h	18h	6h	18h
Eco081	99.1 ± 0.0	65.4 ± 3.2	67.6 ± 6.4	31.5 ± 19.1	54.5 ± 0.0	45.5 ± 0.0	45.5 ± 0.0	5 ± 2.3
EA57	98.0 ± 0.0	63.7 ± 6.9	85.8 ± 17.3	15.7 ± 5.5	34.5 ± 0.9	34.5 ± 6.1	35.1 ± 8.1	8.1 ± 3.7
EI57	67.0 ± 31.6	44.7 ±12.6	46.9 ± 3.2	22.4 ± 6.3	70 ± 2.5	44.1 ± 3.2	29.5 ± 11.4	6.4 ± 2.8
EJ45	57.2 ± 13.5	52.4 ± 6.7	28.6 ± 0.0	8.6 ± 1.3	70 ± 4.6	66.7 ± 0.0	54.7 ± 1.9	6.0 ± 3.3
EB31	91.7 ± 0.0	20.9 ± 5.9	50.0 ± 11.7	8.4 ± 5.9	65.4 ± 4.0	46.6 ± 1.1	25.1 ± 13.7	6.9 ± 4.5
EB29	64.1 ± 6.1	40.6 ± 2.9	51.3 ± 24.1	14.1 ± 4.2	84.2 ± 14.9	54.5 ± 5.0	71.8 ± 7.4	14.9 ± 4.9
NCTC 12900	80.0 ± 0.0	36.0 ± 5.6	52.0 ± 5.6	24.0 ± 5.6	37.4 ± 2.8	33.5 ± 2.0	12.2 ± 0.4	8.7 ± 0.8
Grand Mean	79.6 ± 19.1	46.2 ± 16.0	54.6 ± 19.5	17.8 ± 10.3	59.4 ± 17.9	46.5 ± 11.1	39.1 ± 20.1	8.0 ± 4.5

*duplicate samples on CT-SMAC

a solid phase receptacle whose interior is coated with anti- *E. coli* O157 antibodies, and a reagent strip which contains all the wash and release solutions (BioMerieux, Inc. 2008). The enrichment broth (0.5 mL) was dispensed into the reagent strip and the sample cycled in and out of the solid phase receptacle in the VIDAS Immunoassay Analyzer (BioMerieux, Marcy-l'Etoile, France) in a 40 minute procedure. *E. coli* O157 cells present in the broth were bound to the anti- *E. coli* O157 antibodies coating the interior of the solid phase receptacle, and released in a final enzymatic step into one of the strip wells. The immunoconcentrated samples were then streaked on selective agar plates using sterile cotton swabs, before inspection for typical colourless colonies on CT-SMAC agar and pink colonies on ½ CT-CHROM agar after an 18-24 hour incubation period at 37°C. Typical colonies were confirmed as *E. coli* O157 by a latex agglutination test for the O157 antigen.

VIDAS-UP O157

Enriched samples (1 mL) were transferred to 1.5 mL micro-centrifuge tubes and heated for 5 minutes at 95-96°C. Cooled samples (0.5 mL) were then dispensed into VIDAS-UP reagent strips and the presence and level of *E. coli* O157 determined by automated assay in the VIDAS Immunoassay Analyzer (BioMerieux, Marcy-l'Etoile, France). VIDAS-UP, unlike the VIDAS-ICE system, does not require plating on selective agar for the screening of *E. coli* O157, and is based on an enzyme-linked fluorescent assay. The interior of the solid phase receptacle is coated with recombinant phage tail fibre protein for the capture of *E. coli* O157, and the fluorescence of the final product measured at 450 nm at the end of the 50 minute assay (BioMerieux, Inc. 2009).

GeneDisc *E. coli* O157

A GeneSystems (Bruz, France) Food Extraction Pack was used for extracting DNA from beef and

cattle faeces *E. coli* O157 enrichments. Enrichment broth samples (1 mL) were centrifuged at room temperature for 5 minutes at 500g to remove any cell fragments, before transferring 900 μL of supernatant to lysis tubes for a further 5 minute centrifugation at 10 000 g. Bacterial pellets were then re-suspended in 200 μL of dilution buffer supplied in the extraction pack and lysed by incubation for 10 minutes at 100°C. Faecal sample extracts were further diluted ten-fold in sterile deionized water before analysis, to reduce PCR inhibition caused by bilirubin and bile salts in faeces (Wilson, 2000). For the PCR analysis, 37 μL of a Master Mix supplied with the GeneDisc pack was mixed with 37 μL of DNA sample in 200 μL micro-centrifuge tubes, and 72 μL transferred to each reservoir in the GeneDisc before analysis in the GeneDisc Cycler for presence of *ehx*, *Stx*1, *Stx*2, O157 and *eae* genes in a 55 minute assay (GeneSystems, 2009b, 2009a).

LightCycler

The LightCycler protocol was designed for a 20 μL standard reaction (Roche Diagnostics, 2006). Volumes in the PCR mix for one reaction were: 10.5 μL PCR-grade water, 1 μL O157 forward primer, 1 μL O157 reverse primer, 1 μL O157 probe, 4 μL of Mastermix (Roche Diagnostics, Mannheim, Germany), and 2.5 μL of DNA sample extracted as outlined in Section 2.4.4. Each reaction mix was transferred to a capillary tube, loaded in a rotor, and the PCR analysis carried out in a Roche LightCycler 2.0 system in a 60 minute assay.

Specificity of VIDAS-UP O157 and GeneDisc O157

The specificity of VIDAS-UP O157 and the GeneDisc O157 was determined by analysis of identification results for 17 non-sorbitol-fermenting *E. coli* O157 strains, 22 sorbitol-fermenting *E. coli* O157 strains and 9 non-O157 strains. Overnight Brain Heart Infusion cultures (1 mL) were transferred to 1.5 mL micro-centrifuge tubes and the presence of *E. coli* O157 determined by VIDAS-UP analysis (Section 2.4.3) and GeneDisc analysis (Section 2.4.4). Strains were confirmed as *E. coli* O157 by latex agglutination tests for the O157 antigen.

3 Results and Discussion

3.1 IMS recovery rates in beef and cattle faeces enrichments

In six hour beef enrichments, mean recovery rates of *E. coli* O157 with Dyna beads anti-*E. coli* O157 were 79.6 ± 19.1% in mTSB+n and 54.6 ± 19.5% in BPW (Table 1) compared to mean recovery rates in 18h beef enrichments of 46.2 ± 16.0, and 17.8 ± 10.3%, in mTSB+n and BPW, respectively. Similar trends were noted in cattle faeces enrichments, where recovery rates were 59.4 ± 17.9, 39.1 ± 20.1, 46.5 ± 11.1, and 8.0 ± 4.5% in 6h mTSB+n, 6h BPW, 18h mTSB+n and 18 h BPW enrichments, respectively. Recovery rates were significantly lower in BPW than mTSB+n in 18h enriched beef and cattle faeces samples, and also lower for 18h enrichments compared to 6 h enrichments due to overgrowth by background flora on the CT-SMAC plates. It was also noted that recovery rates of inoculum in cattle faeces were significantly lower than in beef samples, presumably due to the higher level of background flora in faecal samples.

3.2 Relative sensitivity of *E. coli* O157 detection methods

Dynabeads and the GeneDisc were the most sensitive detection method for *E. coli* O157 and could detect an initial 1 CFU in 25 g beef samples after a 6 hour enrichment in both modified Tryptone Soya Broth (mTSB+n) and Buffered Peptone Water (Table 2). VIDAS-UP could detect an initial 10 CFU, while VIDAS-ICE and the LightCycler were less sensitive and could only detect an initial 100 CFU in 6 hour enrichments. In 6 hour cattle faeces enrichments, Dynabeads were the most sensitive isolation method and could detect an initial 1 CFU in two of the three mTSBn samples while VIDAS-ICE and VIDAS UP could detect an initial 10 CFU, and the GeneDisc and LightCycler could only detect an initial 100 CFU (Table 3). Detection thresholds were higher in buffered peptone water, where an

Table 2: Relative sensitivity of *E. coli* O157 detection methods in spiked beef samples.

		No. of positives in three trials						
Enrichment	Inoculum Level* (CFU/25g)	VIDAS UP	VIDAS-ICE CT-SMAC	VIDAS-ICE ½ CT-CHROM	Dynabeads (IMS) CT-SMAC	Dynabeads (IMS) ½ CT-CHROM	Gene Disc	Light Cycler
6h mTS+Bn	100 CFU	3	2	2	3	3	3	2
	10 CFU	2	0	0	3	3	3	0
	1 CFU	0	0	0	3	3	3	0
	0 CFU	0	0	0	0	0	0	0
6h BPW	100 CFU	3	3	2	3	3	3	1
	10 CFU	0	0	0	3	3	3	0
	1 CFU	0	0	0	3	3	3	0
	0 CFU	0	0	0	0	0	0	0
18h mTSB+n	100 CFU	3	3	3	3	3	3	3
	10 CFU	3	3	3	3	3	3	3
	1 CFU	3	3	3	3	3	3	3
	0 CFU	0	0	0	0	0	0	0
18h BPW	100 CFU	3	3	3	3	3	3	3
	10 CFU	3	3	3	3	3	3	3
	1 CFU	3	3	3	2	3	3	3
	0 CFU	0	0	0	0	0	0	0

*Strain NCTC 12079

Table 3: Relative sensitivity of *E. coli* O157 detection methods in cattle faeces enrichments.

		No. of positives in three trials						
Enrichment	Inoculum Level* (CFU/25g)	VIDAS UP	VIDAS-ICE CT-SMAC	VIDAS-ICE ½ CT-CHROM	Dynabeads (IMS) CT-SMAC	Dynabeads (IMS) ½ CT-CHROM	Gene Disc	Light Cycler
6h mTS+Bn	100 CFU	2	3	3	3	3	3	1
	10 CFU	1	2	2	3	3	0	0
	1 CFU	0	0	0	2	2	0	0
	0 CFU	0	0	0	0	0	0	0
6h BPW	100 CFU	1	2	1	3	3	1	0
	10 CFU	0	0	0	2	2	0	0
	1 CFU	0	0	0	0	2	0	0
	0 CFU	0	0	0	0	0	0	0
18h mTSB+n	100 CFU	3	3	3	3	3	3	2
	10 CFU	2	2	3	3	3	2	2
	1 CFU	2	2	2	2	2	2	2
	0 CFU	0	0	0	0	0	0	0
18h BPW	100 CFU	1	2	1	1	2	1	1
	10 CFU	1	1	0	1	2	1	1
	1 CFU	0	0	0	1	2	0	0
	0 CFU	0	0	0	0	0	0	0

*Strain NCTC 12079

initial 100 CFU for VIDAS-ICE, VIDAS-UP and the GeneDisc, and>100 CFU for the LightCycler were required for *E. coli* O157 detection. Increasing the incubation time to 18 hours improved the sensitivity of all isolation methods. An initial 1 CFU in mTSB+n enrichments could be detected with all five methods, while the threshold for detection in BPW enrichments was 1 CFU for the Dynabeads and 10 CFU for VIDAS-ICE, VIDAS-UP, GeneDisc, and the LightCycler.

In similar studies, (Vernozy-Rozand et al., 1998) consistently detected *E. coli* O157 contamination from food samples containing 8 CFU/25 g with VIDAS-ICE after a 24 hr incubation, whilst Wright, Chapman, and Siddons (1994) could detect an initial inocula of 200 organisms/g from beef samples in overnight BPW enrichments by direct subculture on CT-SMAC and 2 organ-

isms/g by Immunomagnetic separation. Additionaly, Islam, Heuvelink, Talukder, and de Boer (2006) reported a relative sensitivity, specificity, and accuracy of the VIDAS ICE method in comparison with Dynabeads anti-*E. coli* O157 of 63.0%, 99.3% and 96.7% respectively, for faecal samples, and 55.6%, 99.1%, and 98.1% for beef samples.
Ohtsuka, Tanaka, Ohtsuka, Takatori, and Hara-Kudo (2010) compared the sensitivity of the direct plating method, plating after immunomagnetic separation (IMS), and loop-mediated isothermal amplification (LAMP) for the Shiga toxin (Stx) gene and O157 antigen gene. Fifty percent and 45% of samples were positive by Stx-LAMP assay and O157 antigen LAMP assay, respectively, compared to 35% and 10% by the IMS and direct plating methods, respectively. The sensitivity of real-time PCR detection for *E. coli* O157 in the presence of high concentrations of non-O157 *E. coli* has also been demonstrated by Heijnen and Medema (2006).

3.3 Specificity of VIDAS-UP *E. coli* O157 and GeneDisc O157

Both VIDAS-UP *E. coli* O157 and the Gene Disc O157 correctly identified 17 non-sorbitol-fermenting and 22 sorbitol- fermenting *E. coli* O157 strains with a 0% false negative rate, and also correctly identified 9 non-*E. coli* strains. VIDAS-UP and the GeneDisc O157 are therefore highly specific for *E. coli* O157 and can be used as rapid and reliable pre-screening tests for the presence of *E. coli* O157.

3.4 Relative cost and applicability of *E. coli* O157 detection methods

Immunomagnetic separation was the least expensive of the five detection methods due to the lower cost of test equipment. However, IMS and the LightCycler methods were the most labour intensive, while the GeneDisc, VIDAS-UP, and VIDAS-ICE were automated and therefore less laborious and required less manipulation. In addition, the Gene Disc, VIDAS-UP and LightCycler were the most rapid, requiring only between 2-5 hours for preliminary test results after a 6 hour pre-enrichment, while IMS and VIDAS ICE required at least 26 hours before presumptive colony identification. It should however be noted that although VIDAS –UP and GeneDisc methods are rapid and convenient methods for *E. coli* O157 detection, the ISO procedure requires confirmation of presumptive positive samples by identification of typical colonies on selective agar. Thus, VIDAS –UP, GeneDisc, and LightCycler methods may serve only as rapid screening methods for the absence or presence of *E. coli* O157.

4 Conclusions

The GeneDisc and VIDAS-UP methods have a high sensitivity and may be used as convenient, reliable and rapid screening methods for *E. coli* O157 presence. VIDAS-ICE in addition to convenience, has the added advantage of colony isolation on selective agar for confirmatory tests, as required by ISO test protocols. Although CT-SMAC Agar is the ISO (International Organization for Standardization) recommended isolation medium, ½ CT-CHROM agar is more selective, resulting in a significant reduction of background flora, and may be used as a second isolation medium in combination with a mTSB+n enrichment to improve *E. coli* detection rates.

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