Received Date : 04-May-2016

Accepted Date : 10-May-2016

Article type : Original Manuscript

Original article

Assessment of the ability of five culture media for the detection of
Escherichia coli O157

Myriam E Gutierrez¹, Marlene E Janes^{1*}, Damir D Torrico², Kennet M Carabante¹, & Witoon
Prinyawiwatkul¹

¹School of Nutrition and Food Sciences, LSU AgCenter, Baton Rouge, LA 70803, USA

²Current Address: Faculty of Veterinary and Agricultural Sciences, Food and Wine Science, the
University of Melbourne, Parkville 3010 VIC, Australia

Running title: Detection of *E. coli* O157

*Correspondent: Fax: +1-225-342-0027

E-mail: mjanes@agcenter.lsu.edu

This is the author manuscript accepted for publication and has undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the [Version of Record](#). Please cite this article as [doi: 10.1111/ijfs.13164](https://doi.org/10.1111/ijfs.13164)

This article is protected by copyright. All rights reserved

Summary

Cattle are a common reservoir for *Escherichia coli* O157:H7. Prior to confirming its presence in a sample, proper isolation of *E. coli* O157 is necessary. Consequently, this study evaluated the ability of five commercial plating media to isolate *E. coli* O157 from 138 samples of fresh cattle feces, water from water trough and pond, and surfaces of water trough and hay bunk. For the isolation of *E. coli* O157, samples were enriched in TSB, followed by immunoseparation and then plating on SMAC, CT-SMAC, CHROMagar™ O157, Tellurite CHROMagar™ O157, and Vancomycin Cefixime Cefsoludin CHROMagar™ O157. Real-time PCR targeting genes *stx*₁, *stx*₂, and *wzY*_{O157} was used to confirm selected isolates. When analysed together, CT-SMAC and CHROMagar™ O157 were the best combination for isolating *E. coli* O157, giving 79% true positive results and only 0.05% false negative results.

Keywords: *Escherichia coli* O157, *E. coli* O157, SMAC, CHROMagar, cattle, environmental samples.

Introduction

According to CDC (CDC, 2015), *Escherichia coli* is a large and diverse group of bacteria; most of which are harmless, but some can be highly pathogenic. Enterohemorrhagic *Escherichia coli* (EHEC) are considered the leading cause of hemorrhagic colitis (HC). EHEC are identified by the production of Shiga toxins (Stxs), but only isolates that have been clinically associated with HC are designated as EHEC. Hemolytic uremic syndrome (HUS) is caused by Stxs produced in the intestine which act systemically on kidney cells and other organs. Shiga-toxin producing *Escherichia coli* (STEC) is an important group of zoonotic pathogens, and *E. coli* O157:H7 is the most frequent serotype associated with HC and HUS in USA (Hancock *et al.*, 1994; Fratamico & DeRoy, 2010; CDC, 2015).

Proper isolation and identification methods are crucial to diagnose *E. coli* O157. Prior to confirming the presence of *E. coli* O157:H7 in a sample, isolation and identification of *E. coli* O157 in selective and differential culture media are necessary. Currently, selective and differential media for isolation of *E. coli* O157 include Sorbitol MacConkey Agar (SMAC), Cefixime-Tellurite Sorbitol MacConkey Agar (CT-SMAC), CHROMagar™ O157 (CHROMagar), Tellurite CHROMagar™ O157 (T-CHROMagar), and Vancomycin Cefixime Cefsulodin CHROMagar™ O157 (VCC-CHROMagar). Although all media have been claimed by various authors to be superior in isolating and identifying *E. coli* O157, most of those studies were performed in food, inoculated samples, or environmental samples from feedlots (Hancock *et al.*, 1994; Franz *et al.*, 2007; Fratamico & DeRoy, 2010). Pathogens used in challenge studies can adapt to grow in culture media. High availability of nutrients in food samples and high animal density in feedlots (250 ft²/head) provide an optimum environment for pathogens, which makes them more likely to grow in culture media. In contrast, pathogens from small-scale cow/calf farms with low animal density (7,920 ft²/head) are subject to stress due to desiccation and reduced availability of nutrients (Mader & Colgan, 2007; Gutierrez-Barberena, 2016), reducing their ability to grow in culture media. This prompted the focus of this current study.

SMAC was first reported for the identification of *E. coli* O111 and O55 that fermented sorbitol slowly or not at all (Rappaport & Henig, 1952). Later it was suggested that the medium could also be effective for the detection of *E. coli* O157:H7 in stool samples from adults and children (Harris *et al.*, 1985; March & Ratnam, 1986). Subsequently, SMAC has been supplemented with Cefixime and Potassium Tellurite for enhanced detection of *E. coli* O157 in samples from feedlots and dairy cattle (Van Donkersgoed *et al.*, 2001; Sargeant *et al.*, 2003). This same medium has also been used to assess fecal shedding of *E. coli* O157:H7 in corn- and barley-fed steers (Bach *et al.*, 2005).

The patented chromogenic chemical substrates for identification of microbial colonies (Rambach, 1979) triggered development of a wide range of media for detection of pathogens. CHROMagar™ O157

medium selectively promotes the growth of *E. coli* O157:H7 and differentiates *E. coli* O157:H7 from non-O157 *E. coli* due to a chromogenic β -galactosidase substrate and a change in pH (Rambach, 2000). CHROMagar™ O157 has been used for the detection of *E. coli* O157 in food as well as environmental samples, and can be more selective with the addition of Potassium Tellurite, or Vancomycin, Cefixime and Cefsulodin (Wallace *et al.*, 1997; Barkocy-Gallagher *et al.*, 2002; Keen *et al.*, 2006; Fratamico & DebRoy, 2010).

The purpose of this study was to evaluate the ability of five commercial media for the detection of *E. coli* O157 in small-scale cow/calf farm environment, to identify which of the five media were the best option for the isolation and presumptive identification of environmental *E. coli* O157 from cattle farms, and to evaluate if the different environmental samples (faecal matter, water and swabs) had a significant effect on the probability of media in detecting *E. coli* O157.

Materials and methods

In this study, 138 samples were collected from six small-scale cow/calf operations in the State of Louisiana during the summer months of June and July. At each operation, 15 samples of fresh undisturbed faecal matter were collected from the ground, 6 samples of water were collected from water troughs and ponds, and 2 swabs, one from the surface of water trough and the other from hay bunk.

Collection of environmental samples

To collect the faecal matter from the ground, gardener sterilized spoons were used, and then the samples were placed in an 18-oz Nasco Whirl-Pak® write-on bags. Water samples were collected from water troughs and ponds using a sterilized plastic ladle, then placed in VWR® 4 oz Polypropylene Specimen containers with Polyethylene lid. For the swabs, an 18-oz Nasco Whirl-Pak® Speci-Sponge® hydrated with Phosphate Buffer Saline (PBS) was used to swab surface of water troughs and hay bunks. All the samples were transported in ice chests to the Food Safety Laboratory, Louisiana State University, Baton Rouge, Louisiana, USA. Samples were placed into enrichment broth the same day of collection.

Isolation of *E. coli* O157

Tryptic Soy Broth (TSB) was used for the enrichment of *E. coli* O157 from all the environmental samples collected for this study. Enrichment was as follows; 5 g of faecal matter samples was added to 50 ml of TSB, 30 ml of environmental water samples was added to 30 ml of double strength TSB, and environmental sponge samples were added to 90 ml of TSB. All the samples were incubated overnight at 35±2°C. After incubation, Immunomagnetic Separation (IMS) was performed using Dynabeads® MAX *E. coli* O157 Kit, following a manual protocol suggested by the manufacturer (Invitrogen Co., Carlsbad, CA). The product from IMS was plated, as suggested by the manufacturer, on SMAC

(Difco™ MacConkey Sorbitol Agar), CT-SMAC (Cefuxune Tellurite Supplement, 77981 Fluka Analytical), CHROMagar (CHROMagar™ O157, CHROMagar), T-CHROMagar (Potassium Tellurite, 02100427 MP Biomedicals, and VCC-CHROMagar (Vancomycin Cefixime Fefsulodin Selective Supplement, 80704 Fluka Analytical). The approximate formula for SMAC and CHROMagar, and concentration of supplements per litre of media are described in Tables 1 and 2, respectively. One to three suspected colonies from selective/differential media were cultured and tested for the presence of *stx*₁, *stx*₂, and *wzy*_{O157} genes by real-time Polymerase Chain Reaction (real-time PCR).

Identification of the strain

All cultures from suspected colonies were analysed by real-time PCR. FDA Bacteriological Analytical Manual (BAM) protocol was used for the DNA extraction (Feng *et al.*, 2011). SmartCycler II platform was used to perform real-time PCR, targeting *stx*₁, *stx*₂, and *wzy*_{O157} genes with product sizes of 200, 140, and 112 bp, respectively (Table 3); The PCR was performed using 6.2 µl of OmniMix HS for Cepheid SmartCycler® Systems (TaKaRa Bio Inc.), which was prepared as recommended by the manufacturer. The concentration of primers and probes used for the assay were 0.25 µM and 0.125 µM, respectively, and the volume of sample was 2.5µl. The protocol used for the PCR was 2 min at 94°C, followed by 40 cycles at 94°C for 20 s, 60°C for 30 s, and 72°C for 50 s (Fratamico and DebRoy, 2010). The threshold between positive and negative was set at 30 fluorescence units.

Statistical analysis

Contingency tables were used to analyse the sensitivity and specificity of each media individually. To analyse the results of the combination of two media, Logistic Regression was applied, modelling the presence/absence of *E. coli* O157 as a dependent variable, and the culture media (SMAC, CT-SMAC, CHROMagar, T-CHROMagar, and VCC-CHROMagar) as regressor variables of the model. Moreover, the effects of different environmental cattle farm samples on the detection of *E. coli* O157 were evaluated using logistic regression models. Statistical Analysis Software® (SAS 2012) at $\alpha = 0.05$ was used for all data analysis.

Results

Ability of each media to detect *E. coli* O157 in environmental samples from small-scale cow/calf operations in Louisiana is displayed in Fig.1, where SMAC showed the highest sensitivity having 109 *E. coli* O157 positive samples from 138 samples, followed by CT-SMAC with 53 *E. coli* O157 positive samples, and CHROMagar with 14 *E. coli* O157 positive samples. Furthermore, T-CHROMagar and

VCC-CHROMagar were unable to detect *E. coli* O157 naturally found in any of the environmental samples tested in the present study (Fig. 1).

The interaction between each culture media and real-time PCR was analysed to establish the ability of each media to detect *E. coli* O157 in environmental samples from cattle farms in Louisiana. To achieve this, contingency tables were used, where the positive and negative results from each media, and the positive and negative results from real-time PCR were crossed to obtain positive, negative, false positive and false negative as marginal totals (Table 4). The false negative results represented the inability of a medium to detect *E. coli* O157, compared to *E. coli* O157 isolated in other media and confirmed as positive by real-time PCR. False positive results were colonies isolated from the medium but not confirmed as positive by real-time PCR. The total of *E. coli* O157 positive results detected using real-time PCR as a validation method was 27 from 138 environmental samples from cattle farms. When the real-time PCR results were compared with each media individually, it was found that T-CHROMagar and VCC-CHROMagar had the highest number of *E. coli* O157 false negative results with 27 for both of them, followed by CHROMagar with 23, CT-SMAC with 13, and SMAC with 7 false negative results (Table 4). The logistic regression model indicated that media had a significant effect on the predicted probability of correct detection of *E. coli* O157 in environmental samples from cattle farms in the present study (media $P < 0.0001$). Additionally, the probability of detecting *E. coli* O157 in environmental samples using CHROMagar or CT-SMAC was significantly higher than using SMAC, and CHROMagar (odds ratio over SMAC = 7.296) more effective than CT-SMAC (odds ratio over SMAC = 3.789) (Table 5).

After evaluating each media individually it was decided to assess results of a combination of two media simultaneously to determine if the detection level of *E. coli* O157 could be improved and the false negative results could be reduced. Logistic regression was used to analyse the probability of two media in detecting *E. coli* O157 in environmental samples in this study simultaneously, where the presence and absence of *E. coli* O157 was used as a dependent variable, and each media as regressors. The odds ratio obtained from the logistic regression were converted into probability (%) for correctly detecting *E. coli* O157 in the samples. Due to the high selectivity of T-CHROMagar and VCC-CHROMagar, and none detection of any *E. coli* O157 in the samples analysed in the present study, these two media were excluded from the logistic regression analysis. From all the samples that were positive based on real-time PCR results; SMAC, CT-SMAC and CHROMagar were analysed individually, then results from different combinations of two media were analysed simultaneously. The calculated probability of correctly detecting *E. coli* O157 was 18% with SMAC, 26% with CT-SMAC and 29% with CHROMagar; all these compared with samples tested positive by real-time PCR. This percentage increased when a combination of two media was assessed simultaneously; 52% for SMAC and

CHROMagar, followed by 57% for SMAC and CT-SMAC, and 79% for CT-SMAC and CHROMagar (Table 6).

Another important aspect in this study was the effect of different environmental samples on the detection level of *E. coli* O157 using each different media; this was also analysed using logistic regression, where the presence and absence of *E. coli* O157 was used as a dependent variable, and fecal matter, water, swabs, SMAC, CT-SMAC, and CHROMagar as regressors. The logistic regression model indicated that different environmental samples did not have significant effect on the predicted probability of correct detection of *E. coli* O157 in environmental samples from cattle farms in the present study (type of sample, $P = 0.7420$).

Discussions

In this present study, while SMAC, CT-SMAC, and CHROMagar allowed the growth of *E. coli* O157 in environmental samples from small-scale cow/calf operations (Fig. 1), results from CT-SMAC and CHROMagar analysed simultaneously appeared to be the best option for the detection of *E. coli* O157 (Table 6). Furthermore, T-CHROMagar and VCC-CHROMagar were unable to detect *E. coli* O157 in the environmental samples that were tested in this study (Fig. 1). Although SMAC appeared to be most sensitive medium, it showed a high number of false positive results for environmental samples. However, when supplemented with Cefixime and Potassium Tellurite as recommended for the analysis of environmental samples, it became more selective (Chapman *et al.*, 1994; Hancock *et al.*, 1994; Van Donkersgoed *et al.*, 2001; Zimbrow *et al.*, 2009; Fedio *et al.*, 2011).

CHROMagar had a good sensitivity and specificity in the detection of *E. coli* O157 in the environmental samples analysed in this study. CHROMagar has been used as a selective medium for the isolation, differentiation and presumptive identification of *E. coli* O157 from stool specimens, foods, veterinary and environmental samples (Bettelheim, 1998; Barkocy-Gallagher *et al.*, 2002; Keen *et al.*, 2006; Fratamico & DebRoy, 2010). Due to the chromogenic substances in the medium, colonies of *E. coli* O157 will produce mauve color, thus allowing presumptive identification. The addition of Potassium Tellurite (2.5mg/L), or Vancomycin, Cefixime and Cefsulodin (4.0, 0.025, and 5.0 mg/L, respectively) as recommended by several authors (Invitrogen, 2008; Zimbrow *et al.*, 2009; Fratamico & DebRoy, 2010) resulted in very selective media, not allowing the growth of *E. coli* O157 naturally found in environmental samples, specifically from small-scale cow/calf operations investigated in this study. Even though Potassium Tellurite has been used as supplement in SMAC agar for the isolation of *E. coli* O157 (Chapman *et al.*, 1994; Hancock *et al.*, 1994; Fedio *et al.*, 2011), when it was used with CHROMagar, T-CHROMagar inhibited the growth of *E. coli* O157 in environmental samples evaluated in this study (Table 4). While the matrix of each environmental sample analysed in the present study

was different, it was found that there was no significant effect of the type of samples (fecal matter, water and swabs) on the probability of each media to correctly detect the presence of *E. coli* O157 in environmental samples.

Conclusions

Culture media analysed individually are less likely to correctly detect environmental *E.coli* O157, compared to when results of two different media were analysed simultaneously. In this study, the best combination of two media used for detection of *E.coli* O157 was CT-SMAC and CHROMagar. Furthermore, the findings suggested that T-CHROMagar and VCC-CHROMagar were very selective and did not allow growth of *E.coli* O157 from environmental samples from small-scale cow/calf operations tested in Louisiana, USA. Collectively, it is recommended to use CHROMagar and CT-SMAC simultaneously as a tool for the detection of STEC O157 naturally found in environmental samples from cattle farms. Future study with samples collected from small-scale cow/calf operations in other locations is needed to confirm the findings from this study.

Acknowledgment

This material was based on research work that was supported by the National Institute of Food and Agriculture, U.S. Department of Agriculture, under award number 2010-85212-20578. The Authors thank farmers in the state of Louisiana for allowing access to their operations and the collection of samples used for this study.

References

- Bach, S.J., Stanford, K. & McAllister, T.A. (2005). Survival of *Escherichia coli* O157:H7 in feces from corn- and barley-fed steers. *FEMS Microbiology Letters*, **252**, 25–33.
- Barkocy-Gallagher, G.A., Berry, E.D., Rivera-Betancourt, M., Arthur, T.M., Nou, X. & Koohmaraie, M. (2002). Development of methods for the recovery of *Escherichia coli* O157:H7 and *Salmonella* from beef carcass sponge samples and bovine fecal and hide samples. *Journal of food protection*, **65**, 1527–1534.
- Bettelheim, K.A. (1998). Reliability of CHROMagarR O157 for the detection of enterohaemorrhagic *Escherichia coli* (EHEC) O157 but not EHEC belonging to other serogroups. *Journal of Applied Microbiology*, **85**, 425–428.
- CDC. (2015). *E. coli* (*Escherichia coli*) [Internet document] URL <http://www.cdc.gov/ecoli/>. Accessed 31/05/2015.
- Chapman, P.A., Wright, D.J., Siddons, C.A., Road, H. & Sheffield, S. (1994). A comparison of immunomagnetic separation and direct culture for the isolation of verocytotoxin-producing *Escherichia coli* O 157 from bovine faeces. *Journal of Medical Microbiology*, **40**, 424–427.
- CHROMagar. (2013). CHROMagar™ O157.
- Donkersgoed, J. Van, Berg, J., Potter, A., Hancock, D., Besser, T., Rice, D., LeJeune, J. & Klashinsky, S. (2001). Environmental sources and transmission of *Escherichia coli* O157 in feedlot cattle. *The Canadian veterinary journal. La revue vétérinaire canadienne*, **42**, 714–20.
- Fedio, W.M., Jinneman, K.C., Yoshitomi, K.J., Zapata, R., Wendakoon, C.N., Browning, P. & Weagant, S.D. (2011). Detection of *E. coli* O157:H7 in raw ground beef by Pathatrix™ immunomagnetic-separation, real-time PCR and cultural methods. *International journal of food microbiology*, **148**, 87–92.
- Feng, P., Weagant, S.D. & Jinneman, K. (2011). Laboratory Methods BAM Diarrheagenic *Escherichia coli* [Internet document] . *U.S. Food and Drug Administration* URL <http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm070080.htm>. Accessed 25/07/2011.
- Franz, E., Klerks, M.M., Vos, O.J. De, Termorshuizen, A.J. & Bruggen, A.H.C. van. (2007). Prevalence of Shiga toxin-producing *Escherichia coli* stx1, stx2, eaeA, and rfbE genes and survival of *E. coli* O157:H7 in manure from organic and low-input conventional dairy farms. *Applied and environmental microbiology*, **73**, 2180–90.

- Fratamico, P.M. & DebRoy, C. (2010). Detection of Escherichia coli O157:H7 in Food Using Real-Time Multiplex PCR Assays Targeting the stx1, stx2, wzyO157, and the fliCh7 or eae Genes. *Food Analytical Methods*, **3**, 330–337.
- Gutierrez-Barberena, M.E. (2016). *Isolation and identification of foodborne pathogens and spoilage bacteria in environmental and food samples*.
- Hancock, D.D., Besser, T.E., Kilsel, M.L., Tarr, P.I., Rice, D.H. & Paros, M.G. (1994). The Prevalence of Escherichia coli O157 . H7 in Dairy and Beef Cattle in Washington State. *Epidemiology and Infection*, **113**, 199–207.
- Harris, A.A., Kaplan, R.L., Goodman, L.J., Doyle, M., Landau, W., Segreti, J., Mayer, K. & Levin, S. (1985). Results of a screening method used in a 12-month stool survey for Escherichia coli O157:H7. *The Journal of infectious diseases*, **152**, 775–777.
- Invitrogen. (2008). Dynabeads ® MAX E. coli O157 Kit [Internet document] URL http://tools.lifetechnologies.com/content/sfs/manuals/dynabeadsMAX_ecoli_man.pdf. Accessed 16/08/2011.
- Keen, J.E., Wittum, T.E., Dunn, J.R., Bono, J.L. & Durso, L.M. (2006). Shiga-toxigenic Escherichia coli O157 in agricultural fair livestock, United States. *Emerging Infectious Diseases*, **12**, 780–786.
- Mader, T.L. & Colgan, S.L. (2007). *Pen Density and Straw Bedding During Feedlot Finishing Pen Density and Straw Bedding During Feedlot Finishing. Nebraska Beef Cattle Reports*.
- March, S.B. & Ratnam, S. (1986). Sorbitol-MacConkey medium for detection of Escherichia coli O157:H7 associated with hemorrhagic colitis. *Journal of Clinical Microbiology*, **23**, 869–872.
- Rambach, A. (1979). Chromogenic chemical sustrate for identification of microbial colonies.
- Rambach, A. (2000). Culture medium fro revealing enterohemorrhagic e coli bacteria and procedure therefore US006165743A.
- Rappaport, F. & Henig, E. (1952). Media for the isolation and differentiation of pathogenic Esch. coli (serotypes O111 and O55). *Journal of Clinical Pathology*, **5**, 361.
- Sargeant, J.M., Sanderson, M.W., Smith, R.A. & Griffin, D.D. (2003). Escherichia coli O157 in feedlot cattle feces and water in four major feeder-cattle states in the USA. *Preventive Veterinary Medicine*, **61**, 127–135.
- Wallace, J.S., Cheasty, T. & Jones, K. (1997). Isolation of vero cytotoxin-producing Escherichia coli O157 from wild birds. *Journal of applied microbiology*, **82**, 399–404.
- Zimbro, M.J., Power, D.A., Miller, S.M., Wilson, G.E. & Johnson, J.A. (Eds.). (2009). *Difco & BBL*

299 *Manual of Microbiological Culture Media*. Second Edi. Sparks, MD: BD Diagnostics -
300 Diagnostic Systems.
301

Author Manuscript

Table 1 SMAC and CHROMagar™ O157 approximate formula per litre

SMAC ^a	Amount (g)	CHROMagar™ O157 ^b	Amount (g)
Peptone	15.5	Agar	15.0
Proteose Peptone	3.0	Peptone and Yeast extract	13.0
D-Sorbitol	10.0	Chromogenic mix	1.2
Bile Salts	1.5		
Sodium Chloride	5.0		
Agar	15.0		
Neutral Red	0.03		
Crystal Violet	0.001		

^aSource: (Zimbro *et al.*, 2009)

^bSource:(CHROMagar, 2013)

306 **Table 2** Supplement concentration per litre of selective/differential media

	Cefixime	Potassium Tellurite	Cefsoludin	Vancomycin
CT SMAC	0.050 mg	2.5 mg	n/a	n/a
T CHROMagar O157	n/a	2.5 mg	n/a	n/a
VCC CHROMagar™ O157	0.025 mg	n/a	5.0 mg	4.0 mg

307 n/a: not applicable

308 **Table 3** Oligonucleotide primers and probes used in the real-time Multiplex PCR assay for detection of *E. coli* O157

<i>stx</i> ₁ , <i>stx</i> ₂ , and <i>wzy</i> _{O157}	Primer sequences 5' to 3'	PCR product size	GenBank accession no.
Primer sets			
stx1-418-F	CTCGACTGCAAAGACGTATG	200	U47614
stx1-617-R	TTCGTTCAACAATAAGCCGTAGATT		
stx2-314-F	ACGATAGACTTTTCGACCCAACAA	140	M16626
stx2-453-R	AAATAACTGCCCCGGTGGGGT		
wzyO157-528-F	CCTGTCAAAGGATAACCGTAATCC	112	X07865
wzyO157-639-R	TTGTTCTCCGTCTTGTCTAACT		
Probes			
stx1-P	5'-Texas Red-CGCTGAATGTCATTCGCTCTGCA-BHQ2		
stx2-P	5'-Texas Red-AACAGACACCGATGTGGTCCCCTGAGAT-BHQ2		
wzyO157-P	5'-FAM-AAAACAACGAGCATACAACCCCTACCAAT-BHQ1		

Source (Fratamico & DebRoy, 2010)

309

310 **Table 4** Detection of *E. coli* O157 in environmental samples from small-scale cow/calf farms; comparing results from SMAC, CT-SMAC,
 311 CHROMagar, T- CHROMagar, VCC- CHROMagar with real-time PCR.

	SMAC	CT-SMAC	CHROMagar	T CHROMagar	VCC CHROMagar	real-time PCR
Negative	29	85	124	138	138	111
Positive	109	53	14	0	0	27
False negative^{1*}	7	13	23	27	27	n/a**
False positive^{2*}	89	39	10	0	0	n/a**
Concur with real-time PCR	42	86	105	111	111	n/a**

312 ¹ represented the inability of a medium to detect *E. coli* O157, compared to *E. coli* O157 isolated in other media and confirmed as positive by real-
 313 time PCR.

314 ² colonies isolated from the medium but not confirmed as positive by real-time PCR.

315 *based on contingency tables comparing results from each media with real-time PCR results.

316 **non applicable.

317 **Table 5** Logistic Regression analysis on selected media for detection of *E. coli* O157 in environmental samples

Media	$P>\chi^2$	Odds ratio	C.I.L (95%)	C.L.U (95%)
CHROMagar vs SMAC	<0.0001	7.296	4.277	12.444
CT-SMAC vs SMAC	<0.0001	3.789	2.297	6.250

318 C.I.L. = Lower confidence interval

319 C.I.U. = Upper confidence interval

320 **Table 6** Probability* of detecting correctly *E. coli* O157 in environmental samples, either analysing
 321 results from each media individually or two media simultaneously.

Media	Probability of detection
SMAC	18%
CT-SMAC	26%
CHRomagar	29%
SMAC & CHRomagar	52%
SMAC & CT-SMAC	57%
CT-SMAC & CHRomagar	79%

322 *based on logistic regression analysis

Figure 1 Detection of *E. coli* O157 in environmental samples from small-scale cow/calf farms; comparing results from SMAC, CT-SMAC, CHROMagar, T- CHROMagar, VCC-CHROMagar with real-time PCR. SMAC had higher sensitivity (109/138) followed by CT-SMAC (53/138), CHROMagar (14/138), and T- CHROMagar and VCC- CHROMagar (0/138).

Minerva Access is the Institutional Repository of The University of Melbourne

Author/s:

Gutierrez, ME;Janes, ME;Torrico, DD;Carabante, KM;Prinyawiwatkul, W

Title:

Assessment of the ability of five culture media for the detection of Escherichia coli O157

Date:

2016-08-01

Citation:

Gutierrez, M. E., Janes, M. E., Torrico, D. D., Carabante, K. M. & Prinyawiwatkul, W. (2016). Assessment of the ability of five culture media for the detection of Escherichia coli O157. INTERNATIONAL JOURNAL OF FOOD SCIENCE AND TECHNOLOGY, 51 (8), pp.1910-1915. <https://doi.org/10.1111/ijfs.13164>.

Persistent Link:

<http://hdl.handle.net/11343/291441>