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The optimization of isolation media used in immunomagnetic separation methods for the detection of *Escherichia coli* O157 in foods

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Aims: To compare media used in immunomagnetic separation (IMS) techniques for the isolation of *Escherichia coli* O157 from food.

Methods and Results: Foods, both naturally contaminated and spiked, with low numbers ($< 1\ g^{-1}$) of stressed *E. coli* O157 were enriched in media based on buffered peptone water (BPW), tryptone soya and EC broths incubated at 30, 37, 40 and 42°C. Following immunomagnetic separation, beads were plated on a range of selective agars.

Conclusions: BPW supplemented with vancomycin (8 mg l^{-1}) incubated at 42°C, followed by IMS and subsequent plating of immunobeads onto cefixime tellurite sorbitol MacConkey agar plus either Rainbow™ or CHROMagar™ agars, proved optimum for the recovery of spiked, stressed *E. coli* O157 in minced beef, cheese, apple juice and pepperoni. The same protocol was optimum for recovery from naturally-contaminated minced beef and cheese.

Significance and Impact of the Study: The optimum protocol would increase isolation rates of *E. coli* O157 from foods.

INTRODUCTION

Escherichia coli O157 is a major food-borne pathogen in humans causing increasing concern worldwide as the number of incidences continue to rise (Anon. 1995a). Clinical symptoms include vomiting and diarrhoea, haemorrhagic colitis and haemolytic uraemic syndrome (HUS, a cause of renal failure in children which can lead to long-term complications and death), which is now the single most common cause of acute renal failure in children in North America (Karmali 1989). Pathogenic bacteria causing such severe symptoms require sensitive and precise methods of detection.

A wide variety of foods have been implicated in *E. coli* O157 outbreaks, with many being of cattle and dairy product origin. Post-processing contamination of milk by *E. coli* O157 led to an outbreak resulting in the death of one person (Upton and Coia 1994). Infected cooked meat caused the death of 21 people in the central Scotland outbreak in 1996 (Ahmed and Donaghy 1998). There have been a number of cases in the USA where unfermented apple juice was shown to be the vehicle of infection (Besser *et al.* 1993), and fermented, ready-to-eat meat products have also been associated with *E. coli* O157 (Anon. 1995b).

The infectious dose of *E. coli* O157 is estimated to be very low (Willshaw *et al.* 1994), thus further necessitating the development of sensitive detection techniques. In recent years, immunomagnetic separation (IMS) methods (Chapman *et al.* 1994) have become widespread in food analysis, although initial isolation media were developed for the examination of faecal specimens. Problems were experienced by some workers when these protocols were used in food testing (Bolton *et al.* 1995), and, in particular, for processed foods where bacteria were physiologically damaged due to extremes of heat, desiccation, preservatives, high osmotic pressure or pH changes. Such micro-organisms can be sensitive to antimicrobial additions used to inhibit non-target bacteria and therefore, the choice and concentration of such additions is of vital importance to optimize target recovery.

The routine diagnostic laboratory uses a number of distinct steps in the IMS procedure, culminating in colony confirmation from a selective agar plate. This paper compares chemical composition and incubation tempera-

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tures of the enrichment, and selective agars used in the IMS procedure to isolate both naturally-occurring and artificially-inoculated *E. coli* O157 in foods. To show method sensitivity under extreme circumstances, the spiked studies used low numbers (<1 g^{-1}) of physiologically-stressed target bacteria in the presence of high numbers of healthy background micro-organisms. Foods of known association with *E. coli* O157 were studied, minced (ground) beef was tested initially, with subsequent validations performed on cheese, apple juice and pepperoni.

MATERIALS AND METHODS

Bacterial strains

Three separate cocktails of *E. coli* O157, each containing four or five different strains, were employed to minimize the influence of a typical single strains. The isolates were from food, environmental and clinical sources from the Department's collection. Previous work (data not presented) had shown them to be resistant to a range of antimicrobials (cefixime, cefsulodin and potassium tellurite) used routinely in IMS methods.

Preparation of spiking cocktail

Isolates of *E. coli* were grown individually in nutrient broth at 37°C for 18 h prior to mixing 1·0 ml aliquots in a sterile container to form the cocktail. This was diluted in sterile phosphate-buffered saline (PBS) to a level of approximately 10^2 ml^{-1}.

Preparation and testing artificially-contaminated foods

All foods for spiking studies were initially confirmed as absent from naturally-occurring *E. coli* O157, using buffered peptone water supplemented with vancomycin 8 mg l^{-1} (BPW-V) enrichment at 42°C for 6 h, followed by IMS with beads (0·1 ml) plated equally onto two cefixime tellurite sorbitol MacConkey agar (CTSMAC) plates. After incubation at 37°C for 16–18 h, presumptive *E. coli* O157 colonies (non-sorbitol fermenting) were tested using latex agglutination (Oxoid). Mince samples contained background *E. coli* while cheese and pepperoni contained variable coliform levels. Apple juice contained no background micro-organisms. Minced beef was spiked separately with all cocktails, and other foods were spiked with a single cocktail only.

Aliquots (20 ml) of the diluted cocktail were added to 100 g retail minced beef to give approximate levels of 100 g^{-1}. The mince was then subjected to a number of freeze/thaw cycles (between −20 and + 15°C) in order to reduce numbers and physiologically stress the bacteria. The time periods at + 15°C were short (<4 h) to minimize bacterial growth. The number of survivors were calculated by the Most Probable Number method with each dilution enriched in BPW-V for 6 h at 42°C, followed by IMS and subsequent plating on CTSMAC incubated at 37°C. Four freeze/thaw cycles typically gave a reduction to <1 g^{-1}, but if significantly above this number, the sample was subjected to one more cycle. Prior to testing spiked minced beef, samples were mixed with an equal weight of unfrozen mince previously shown to contain high numbers (approximately 10^7 g^{-1}) of micro-organisms (but containing no *E. coli* O157). Mince samples (200 g) were homogenized for 2 min in a Stomacher (Don Whitley Scientific, Shipley, UK) in 800 ml PBS, and 50 ml volumes of supernatant fluid were added to 50 ml double strength enrichment broth under test. Replicate experiments were performed with all three cocktails.

Pasteurized goats' cheese (60 g) was surface spread with 1·0 ml of cocktail containing approximately 10^6 ml^{-1} and stored at 4°C for 2 days prior to analysis, thus simulating post-processing contamination. Testing of IMS enrichments was performed as for minced beef.

Apple juice (containing no preservative) was tested with both stressed and unstressed *E. coli* O157 to simulate recoveries from pre- and post-processing contamination. To stress the cells, the cocktail (10^9 ml^{-1}) was inoculated into 200 ml apple juice (pH 3·5) and stored at 4°C for 10 days, after which time the count had fallen to approximately 10^6 ml^{-1}. The survivors were further diluted and inoculated into fresh apple juice (0·02 ml into 200 ml) to a final concentration of 10^2 ml^{-1}. Unstressed cells of the same cocktail at similar numbers in apple juice were tested separately. Volumes (25 ml) of spiked apple juice were added to 225 ml enrichment broths followed by IMS.

Pepperoni (pre-sliced, 100 g) was spiked separately with stressed and unstressed *E. coli* O157 to simulate recoveries from pre- and post-processing contamination. Stressed cells were produced by inoculating (1 ml) of the cocktail (10^9 ml^{-1}) into 100 ml high salt (NaCl, 13·5% w/v), low pH (4·9), low temperature (5°C) broth (McCarthy *et al.* 1998). Counts fell by 3 log numbers over a 10-day period. The pepperoni slices (300 g) were dipped (20 s) into 300 ml cocktail (10^2 ml^{-1}), giving a final concentration of <10^1 g^{-1}, and stored at 4°C for 18 h prior to testing as for minced beef. Numbers of unstressed *E. coli* O157 in apple juice and pepperoni were adjusted to approximately the same as in stressed *E. coli* O157 assays.

Comparison of enrichment broths and incubation temperatures

Enrichment broths tested were based on modifications of BPW (Oxoid) with additions of vancomycin (8 mg l^{-1}, David Bull Laboratories,), cefixime (0·05 mg l^{-1}, Wyeth-

Ayerst Research) and cefsulodin (10 mg l^{-1}, Sigma) (BPW–VCC). BPW with vancomycin alone (BPW-V), and with cefixime and cefsulodin concentrations reduced to one quarter strength (BPW-V + ¼ C + C), were also tested. Modifications of tryptone soya broth (Oxoid) and EC medium (Difco) were investigated (Table 1). Incubation temperatures tested included 30, 37, 40 and 42°C, and incubation times remained constant at 6 h. All broths were equilibrated at 20°C prior to food addition and incubation.

Immunomagnetic separation technique

Enrichment volumes (1 ml) were transferred to sterile Eppendorf tubes containing 0·02 ml Captivate™ O157 immunomagnetic beads (International Diagnostics Group, Bury, UK). The Eppendorf tubes were placed in a rack, mixed for 30 min, before discarding the supernatant fluid and re-suspending the beads (without the magnet) in wash buffer (PBS + 0·05% Tween 20). Two further washing stages were performed prior to transferring the beads (in 0·1 ml buffer) equally onto two selective agar plates incubated at 37°C for 16–18 h.

Selective agars

Those under test (Table 2) included sorbitol MacConkey agar (SMAC, Oxoid), SMAC with additions (Mast Diagnostics) of cefixime (C, 0·05 mg l^{-1}) and potassium tellurite (T, 2·5 mg l^{-1}) (CTSMAC), SMAC with one third strength CT additions and SMAC with two third strength CT additions. The performance of three chromogenic agars was investigated, including CHROMagar$_{TM}$ O157 (M-Tech Diagnostics Ltd), Rainbow™ Agar O157 (Don Whitley Scientific) and SD-39 (QA Life Sciences). All were made according to the manufacturer's instructions and stored at chill temperatures for <7 days prior to use. All agars were incubated at 37°C, except SD-39 which was tested at 42°C, and incubation times were approximately 16–18 h at which time presumptive target colonies could be distinguished from background flora (according to manufacturer's instructions) and tested by latex agglutination. Confirmed target colony numbers were recorded.

Table 1 Recovery of spiked *Escherichia coli* O157 from minced beef by different IMS enrichments at different temperatures

Enrichment medium	37 °C	40 °C	42 °C
BPW-VCC*	+–	+	+ +
BPW-V pH 7·0†	+	+ + + +	+ + + + +
BPW-V pH 6·0‡	+	NT	+ + +
BPW-V + 1/4; C + C§	+	+ + + +	+ + + + +
MTSB + N¶	+	+ + +	+ + + +
EC medium + N**	+–	NT	+ + +

*BPW + vancomycin (8 mg l^{-1}) + cefixime (0·05 mg l^{-1}) + cefsulodin (10 mg l^{-1}).
†Buffered peptone water (BPW) + vancomycin (8 mg l^{-1}) pH 7·0.
‡BPW + vancomycin (8 mg l^{-1}) pH 6·0.
§BPW + vancomycin (8 mg l^{-1}) + cefixime (0·0125 mg l^{-1}) + cefsulodin (2·5 mg l^{-1}).
¶ Tryptone soya broth + bile salts (1·5 g l^{-1}) + novobiocin (20 mg l^{-1}).
**EC medium + novobiocin (20 mg l^{-1}).
NT, not tested; ±, < 10 or zero recovery; +, 10–25; + +, 26–50; + + +, 51–100; + + + +, 101–250; + + + + +, > 250 target colonies recovered. Colony numbers are representative of several replicate experiments.

Testing of naturally-contaminated foods

Samples of cheese and minced beef naturally contaminated with *E. coli* O157 were included in this study. The cheese was responsible for a small, localized outbreak in north-east Scotland and the minced beef was obtained from routine surveillance. Both strains of *E. coli* O157 were VT1 negative, VT2 positive and were phage type 21/28. Samples were homogenized in PBS; the supernatant fluid was subdivided and added to equal volumes of double strength BPW-V and mTSB + N incubated at 42°C, and BPW-VCC incubated at 37°C, for 16–18 h. Immunobeads were divided equally and plated onto CTSMAC and CHROMagar$_{TM}$ O157 agars.

Table 2 Comparison of *Escherichia coli* O157 enrichment media and incubation temperatures from different foods

		Apple juice		Pepperoni	
Enrichment medium	Cheese	Stressed	Unstressed	Stressed	Unstressed
BPW-VCC 37 °C*	–	+ + +	+ + +	–	+ + +
mTSB + N 42 °C†	+ + + +	+ + + +	+ + + + +	+ + + +	+ + + + +
BPW-V 42 °C‡	+ + + +	+ + + + +	+ + + + +	+ + + + +	+ + + + +

*BPW + vancomycin (8 mg l^{-1}) + cefixime (0·05 mg l^{-1}) + cefsulodin (10 mg l^{-1}).
† Tryptone soya broth + bile salts (1·5 g l^{-1}) + novobiocin (20 mg l^{-1}).
‡ Buffered peptone water (BPW) + vancomycin (8 mg l^{-1}) pH 7·0.
–, Indicates zero recovery; + + +, 51–100; + + + +, 101–250; + + + + +, >250 target colonies recovered.

RESULTS

Three criteria were used when comparing results in this study: the presence/absence of target pathogen, the volume of background flora and the numbers of recovered *E. coli* O157.

Enrichment studies

An initial comparison of enrichment incubation temperatures at 30, 37 and 42°C indicated that 30°C gave significantly poorer target recoveries after overnight incubation (data not presented). Incubation at 30°C was therefore omitted from subsequent investigations. A comparison of *E. coli* O157 recoveries from minced beef by six different enrichment broths incubated at 37, 40 and 42°C is presented in Table 1. The results are representative of approximately 40 replicate experiments from a number of minced beef samples spiked separately with the three cocktails. Cocktail composition had no effect on the ratings shown in Table 1. Throughout the tests, BPW supplemented with vancomycin at 8 mg l^{-1}, with or without one quarter normal strength cefixime and cefsulodin, gave optimum recovery. BPW-V at pH 7·0 was superior to BPW-V at pH 6·0. While BPW with the full supplement of vancomycin, cefixime and cefsulodin showed minimal background flora, it consistently gave a poor or zero recovery of *E. coli* O157. Modified TSB proved to be slightly inferior to BPW-V pH 7·0 at 42°C, but superior to EC broth + N. There was a marked difference between incubation temperatures with 42°C giving best, and 37°C giving poorest recovery.

Validation studies of IMS enrichment media in foods other than minced beef compared BPW-VCC (conventional IMS broth), mTSB + N (proposed ISO broth) and BPW-V (optimum broth as shown in Table 1). The results (Table 2) clearly indicate the poor performance of BPW-VCC, which failed to isolate *E. coli* O157 from cheese and the stressed *E. coli* O157 from pepperoni. The recoveries of unstressed cells in pepperoni, and stressed and unstressed *E. coli* O157 from apple juice, were low in number from BPW-VCC. No differences were observed in the performance of the other two enrichments except in the numbers of stressed cells recovered, which were slightly less from mTSB+N. Replicate testing of cheese and pepperoni showed natural levels of associated microflora, varying between 10^3 g^{-1} and 10^7 g^{-1}, while apple juice contained $<10^2$ ml^{-1}.

Selective agars

Selective agars based on either SMAC or different chromogenic reactions were tested and are listed in Table 3. The food source did not have an effect on agar ratings. The agars with the best rating had high numbers of easily observed target bacteria, while those rated poorly were often completely overgrown with background flora, making individual isolate recognition difficult. Of the media based on MacConkey agar, CTSMAC proved superior, with minimal non-target growth. Two chromogens showed good recovery of *E. coli* O157 with low background microflora. Rainbow™ was slightly superior to CHROMagar$_{TM}$, having an almost complete absence of background flora. In the majority of cases, SD-39 failed to isolate the target which was totally obscured by a lawn of competing enteric bacteria. Extended incubation periods (>18 h) often resulted in overgrowth by background flora.

Table 3 Comparison of *Escherichia coli* O157 selective agars for immunomagnetic separation

Selective agar	Rating
CTSMAC*	+ + + +
SMAC†	+ +
SMAC + 1/3 CT‡	+ +
SMAC + 2/3 CT§	+ +
CHROMagar$_{TM}$¶	+ + + +
SD-39**	–
Rainbow™††	+ + + + +

*Cefixime tellurite sorbitol MacConkey, cefixime 0·05 mg l^{-1}, potassium tellurite 2·5 mg l^{-1}.
†Sorbitol MacConkey.
‡Sorbitol MacConkey + cefixime and tellurite at one third normal strength.
§Sorbitol MacConkey + cefixime and tellurite at two thirds normal strength.
¶CHROMagar$_{TM}$ O157 isolation medium.
**Quality Life Sciences *E. coli* O157 isolation medium.
††Biolog Rainbow™ *E. coli* O157 isolation medium.
–,Indicates zero recovery, + + , 26–50; + + + + , 101–250; + + + + + , >250 target colonies recovered.

Naturally-contaminated foods

Foods previously found to contain *E. coli* O157 were tested by three IMS enrichments. The number of replicate tests reflects the small sample size available for testing. The results are presented in Table 4, which indicates the number of samples giving a presence or absence of the target pathogen. BPW-V (42°C) gave the greatest number of positives and BPW-VCC failed to isolate *E. coli* O157 from all samples. Modified TSB + N (42°C) gave one positive from a cheese sample and zero from the minced beef samples.

DISCUSSION

When IMS first became commercially available, routine food testing laboratories regularly employed a sample enrichment in BPW-VCC at 37°C, followed by bead

Table 4 Testing naturally-contaminated foods for *Escherichia coli* O157 by three enrichment procedures

	Cheese	Mince
Enrichment conditions	No. positive	No. positive
BPW-VCC 37 °C*	0/6	0/2
mTSB + N 42 °C†	1/6	0/2
BPW-V 42 °C‡	2/6	1/2;

*BPW + vancomycin (8 mg l^{-1}) + cefixime (0·05 mg l^{-1}) + cefsulodin (10 mg l^{-1}).
†Tryptone soya broth + bile salts (1·5 g l^{-1}) + novobiocin (20 mg l^{-1}).
‡Buffered peptone water (BPW) + vancomycin (8 mg l^{-1}) pH 7·0.

incubation on CTSMAC (Bell and Kyriakides 1998), as indicated by the immunobead manufacturers. However, some strains of *E. coli* O157 were shown to be sensitive to many of the antimicrobials used in this particular method (MacRae *et al.* 1997), and a wide range of enrichment conditions (media and temperature) were tested by a number of laboratories in order to improve recovery. Several enrichments were based on BPW, which is used widely to aid enrichment of physiologically-stressed micro-organisms such as *Salmonella* spp. (Edel and Kampelmacher 1973). Bolton *et al.* (1995) recognized that the cefixime and cefsulodin supplements in the enrichment step were potentially inhibitory to some *E. coli* O157 and tested a range of reduced concentrations, resulting in an optimum BPW enrichment containing one quarter of the normal antimicrobial strength.

In this study, BPW-V pH 7·0 and BPW-V + ¼ C + C were the optimum enrichment media for the recovery of stressed *E. coli* O157 in minced beef. It is probable that cefixime and cefsulodin at this reduced level are below the concentrations that have an effect on target recovery. Doyle and Schoeni (1987) proposed the use of modified TSB + N at 37°C, which in our study compared favourably with other media tested, although incubation at 42°C improved recovery. However, it proved slightly inferior to BPW-V pH 7·0 and BPW-V + ¼ C + C. Szabo *et al.* (1990) tested TSB at 35 and 43°C, and found the addition of bile salts and novobiocin at the higher temperature improved recovery of *E. coli* O157 in foods containing competing micro-organisms. Bolton *et al.* (1995) tested mTSB at 37 and 42°C alongside BPW with different additions and found mTSB at 42°C to be optimum. This medium, incubated at 41·5°C, was proposed by Scotter *et al.* (2000) and has been accepted as a draft International Standard by the International Organization for Standardization; it is widely used by a number of laboratories in the UK. The recommended medium of the US Department of Agriculture is a modified version of EC medium with novobiocin (Okrend and Rose 1989) but in our study, it proved inferior to BPW-V pH 7·0. Originally designed to enumerate coliforms in water and shellfish, EC media contain bile salts and are therefore more selective than BPW-V, and possibly, more inhibitory to sub-lethally damaged micro-organisms.

This study demonstrates the importance of enrichment incubation temperature. Temperature elevation above 37°C increased the number of samples testing positive for *E. coli* O157 and also increased the number of target bacteria recovered (Table 1). This was independent of enrichment composition and was possibly due to the higher temperatures being inhibitory to some of the natural microbial flora in the foods tested. In the majority of cases, there were fewer non-target bacteria on plates from 42°C enrichments.

Further experiments were performed in other foods associated with *E. coli* O157 contamination (Table 2). A zero recovery from cheese by BPW-VCC (37°C) was somewhat surprising as good recovery was observed from the other two enrichments. The numbers of *E. coli* O157 spiked into cheese were slightly lower than in pepperoni, but the degree of physiological stress was assumed to be minimal (2 days of storage at 4°C). The use of BPW-VCC also failed to recover stressed *E. coli* O157 from pepperoni, and it is assumed that cells were damaged to the extent that the high antimicrobial concentration did not allow sufficient recovery in the 6 h enrichment time. BPW-VCC did recover unstressed target bacteria from pepperoni, supporting this theory. BPW-VCC was found to recover both stressed and unstressed *E. coli* O157 from apple juice, and perhaps the degree of stress with this product was less damaging than other methods applied in this work. The difference between BPW-V and mTSB + N lay in their ability to recover damaged bacteria in apple juice and pepperoni; fewer *E. coli* O157 were recovered from the mTSB + N enrichment. This difference may have been due to partial inhibition of damaged organisms by the presence of bile salts in TSB, or to the superior recovery of such cells in the less nutritious peptone water (Edel and Kampelmacher 1973). All results indicate that optimum enrichment is achieved with minimum antimicrobial addition, specifically from those with known activity against Gram-negative micro-organisms such as cefixime and cefsulodin (Lambert and O'Grady 1992).

When Zadik *et al.* (1993) introduced cefixime and tellurite additions to improve *E. coli* O157 selectivity, the majority of isolation procedures in the UK used SMAC. The introduction of cefixime and tellurite into SMAC has made the observation of target pathogen clearer by reducing background flora. However, MacRae *et al.* (1997) showed that a small but significant number of *E. coli* O157 isolates exhibited partial inhibition in the presence of such antimicrobials, and suggested that the use of such additions may contribute to foods being incorrectly labelled as free from *E. coli* O157. It must be stressed that all strains of

E. coli O157 used in this study were not sensitive to cefixime and tellurite and therefore, CTSMAC gave excellent results when compared with SMAC, with or without reduced concentrations of the two antimicrobials (Table 3). CTSMAC alone will not detect the presence of sorbitol-positive strains of *E. coli* O157. These are currently isolated only rarely in the UK but it would be unwise to assume that they do not pose a significant threat to human health in the future. Other European countries, such as Germany (Beutin 1999), isolate sorbitol-positive strains on a regular basis, which necessitates the use of an alternative selective plating medium. A number of selective agars based on alternatives to sorbitol MacConkey are available commercially, and most are based on chromogenic reactions to distinguish the target colony from background flora. Taormina *et al.* (1998) concluded that Rainbow™ and a modified eosin methylene blue agar gave best recovery from heat-stressed *E. coli* O157 in ground beef. The present study found Rainbow™ to be superior to two alternative chromogenic agars (having less background flora) and indeed, showed better recovery of target cells compared with CTSMAC. CHROMagar$_{TM}$ O157 was equal in performance to CTSMAC. In addition to the discrimination of *E. coli* O157 from other enterics and food-associated microflora, CHROMagar$_{TM}$ O157 can be used to identify serogroups O111 and O103 (Bettelheim 1998a), while Rainbow™ can distinguish serotypes O111:H8 and O26:H11 from *E. coli* O157 (Bettelheim 1998b). Quality Life Sciences *E. coli* O157 medium (SD-39) gave poor results in this study (incubated at 42°C), with large numbers of competing microflora preventing correct identification of *E. coli* O157. However, it must be stressed that the manufacturers recommend incubation at 44°C, a temperature unavailable during the course of this work. Some workers (Doyle and Schoeni 1984) have reported partial or complete inhibition of *E. coli* O157 at this elevated temperature. Based on the results of this study, the present method for culturing *E. coli* O157 in our laboratory is to distribute IMS beads equally between CTSMAC and a chromogenic agar. The current economical choice is CHROMagar$_{TM}$ O157. Throughout this study using spiked cockails, it was not possible to identify the individual component strains recovered after enrichment and plating, and the possibility exists that the media may not be optimal for all strains used.

Two foods naturally contaminated with *E. coli* O157 were available in sufficient quantities for replicate testing during the course of this work. The levels of *E. coli* O157 in the two foods (as enumerated by MPN) were found to be low at $<10\ g^{-1}$, but their physiological state was unknown. Although both minced beef and cheese were supplied by the retailer as fresh produce, their history was unclear. For example, the beef may have been frozen prior to sale and the cheese could have been stored for an extensive period (several months) before purchase, both of which could result in sub-lethally-damaging target bacteria. Results showing such differences between enrichments suggest that stressed *E. coli* O157 could have been present in all samples.

The use of optimum IMS enrichment media as described has enabled this laboratory to isolate and confirm the presence of low numbers of sub-lethally-damaged *E. coli* O157 from a number of foods. In the majority of tests, there was a high background level of micro-organisms which did not interfere with recognition of the target bacteria. A protocol is proposed of enriching in BPW-V at 42°C for 6 h, and plating immunobeads on CTSMAC and CHROMagar$_{TM}$ O157, to improve *E. coli* O157 isolation from foods.

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The optimization of isolation media used in immunomagnetic separation methods for the detection of *Escherichia coli* O157 in foods

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Aims: To compare media used in immunomagnetic separation (IMS) techniques for the isolation of *Escherichia coli* O157 from food.

Methods and Results: Foods, both naturally contaminated and spiked, with low numbers (< 1 g^{-1}) of stressed *E. coli* O157 were enriched in media based on buffered peptone water (BPW), tryptone soya and EC broths incubated at 30, 37, 40 and 42°C. Following immunomagnetic separation, beads were plated on a range of selective agars.

Conclusions: BPW supplemented with vancomycin (8 mg l^{-1}) incubated at 42°C, followed by IMS and subsequent plating of immunobeads onto cefixime tellurite sorbitol MacConkey agar plus either Rainbow™ or CHROMagar™ agars, proved optimum for the recovery of spiked, stressed *E. coli* O157 in minced beef, cheese, apple juice and pepperoni. The same protocol was optimum for recovery from naturally-contaminated minced beef and cheese.

Significance and Impact of the Study: The optimum protocol would increase isolation rates of *E. coli* O157 from foods.

INTRODUCTION

Escherichia coli O157 is a major food-borne pathogen in humans causing increasing concern worldwide as the number of incidences continue to rise (Anon. 1995a). Clinical symptoms include vomiting and diarrhoea, haemorrhagic colitis and haemolytic uraemic syndrome (HUS, a cause of renal failure in children which can lead to long-term complications and death), which is now the single most common cause of acute renal failure in children in North America (Karmali 1989). Pathogenic bacteria causing such severe symptoms require sensitive and precise methods of detection.

A wide variety of foods have been implicated in *E. coli* O157 outbreaks, with many being of cattle and dairy product origin. Post-processing contamination of milk by *E. coli* O157 led to an outbreak resulting in the death of one person (Upton and Coia 1994). Infected cooked meat caused the death of 21 people in the central Scotland outbreak in 1996 (Ahmed and Donaghy 1998). There have been a number of cases in the USA where unfermented apple juice was shown to be the vehicle of infection (Besser *et al.* 1993), and fermented, ready-to-eat meat products have also been associated with *E. coli* O157 (Anon. 1995b).

The infectious dose of *E. coli* O157 is estimated to be very low (Willshaw *et al.* 1994), thus further necessitating the development of sensitive detection techniques. In recent years, immunomagnetic separation (IMS) methods (Chapman *et al.* 1994) have become widespread in food analysis, although initial isolation media were developed for the examination of faecal specimens. Problems were experienced by some workers when these protocols were used in food testing (Bolton *et al.* 1995), and, in particular, for processed foods where bacteria were physiologically damaged due to extremes of heat, desiccation, preservatives, high osmotic pressure or pH changes. Such micro-organisms can be sensitive to antimicrobial additions used to inhibit non-target bacteria and therefore, the choice and concentration of such additions is of vital importance to optimize target recovery.

The routine diagnostic laboratory uses a number of distinct steps in the IMS procedure, culminating in colony confirmation from a selective agar plate. This paper compares chemical composition and incubation tempera-

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tures of the enrichment, and selective agars used in the IMS procedure to isolate both naturally-occurring and artificially-inoculated *E. coli* O157 in foods. To show method sensitivity under extreme circumstances, the spiked studies used low numbers (<1 g^{-1}) of physiologically-stressed target bacteria in the presence of high numbers of healthy background micro-organisms. Foods of known association with *E. coli* O157 were studied, minced (ground) beef was tested initially, with subsequent validations performed on cheese, apple juice and pepperoni.

MATERIALS AND METHODS

Bacterial strains

Three separate cocktails of *E. coli* O157, each containing four or five different strains, were employed to minimize the influence of a typical single strains. The isolates were from food, environmental and clinical sources from the Department's collection. Previous work (data not presented) had shown them to be resistant to a range of antimicrobials (cefixime, cefsulodin and potassium tellurite) used routinely in IMS methods.

Preparation of spiking cocktail

Isolates of *E. coli* were grown individually in nutrient broth at 37°C for 18 h prior to mixing 1·0 ml aliquots in a sterile container to form the cocktail. This was diluted in sterile phosphate-buffered saline (PBS) to a level of approximately 10^2 ml^{-1}.

Preparation and testing artificially-contaminated foods

All foods for spiking studies were initially confirmed as absent from naturally-occurring *E. coli* O157, using buffered peptone water supplemented with vancomycin 8 mg l^{-1} (BPW-V) enrichment at 42°C for 6 h, followed by IMS with beads (0·1 ml) plated equally onto two cefixime tellurite sorbitol MacConkey agar (CTSMAC) plates. After incubation at 37°C for 16–18 h, presumptive *E. coli* O157 colonies (non-sorbitol fermenting) were tested using latex agglutination (Oxoid). Mince samples contained background *E. coli* while cheese and pepperoni contained variable coliform levels. Apple juice contained no background micro-organisms. Minced beef was spiked separately with all cocktails, and other foods were spiked with a single cocktail only.

Aliquots (20 ml) of the diluted cocktail were added to 100 g retail minced beef to give approximate levels of 100 g^{-1}. The mince was then subjected to a number of freeze/thaw cycles (between –20 and + 15°C) in order to reduce numbers and physiologically stress the bacteria. The time periods at + 15°C were short (<4 h) to minimize bacterial growth. The number of survivors were calculated by the Most Probable Number method with each dilution enriched in BPW-V for 6 h at 42°C, followed by IMS and subsequent plating on CTSMAC incubated at 37°C. Four freeze/thaw cycles typically gave a reduction to <1 g^{-1}, but if significantly above this number, the sample was subjected to one more cycle. Prior to testing spiked minced beef, samples were mixed with an equal weight of unfrozen mince previously shown to contain high numbers (approximately 10^7 g^{-1}) of micro-organisms (but containing no *E. coli* O157). Mince samples (200 g) were homogenized for 2 min in a Stomacher (Don Whitley Scientific, Shipley, UK) in 800 ml PBS, and 50 ml volumes of supernatant fluid were added to 50 ml double strength enrichment broth under test. Replicate experiments were performed with all three cocktails.

Pasteurized goats' cheese (60 g) was surface spread with 1·0 ml of cocktail containing approximately 10^0 ml^{-1} and stored at 4°C for 2 days prior to analysis, thus simulating post-processing contamination. Testing of IMS enrichments was performed as for minced beef.

Apple juice (containing no preservative) was tested with both stressed and unstressed *E. coli* O157 to simulate recoveries from pre- and post-processing contamination. To stress the cells, the cocktail (10^9 ml^{-1}) was inoculated into 200 ml apple juice (pH 3·5) and stored at 4°C for 10 days, after which time the count had fallen to approximately 10^6 ml^{-1}. The survivors were further diluted and inoculated into fresh apple juice (0·02 ml into 200 ml) to a final concentration of 10^2 ml^{-1}. Unstressed cells of the same cocktail at similar numbers in apple juice were tested separately. Volumes (25 ml) of spiked apple juice were added to 225 ml enrichment broths followed by IMS.

Pepperoni (pre-sliced, 100 g) was spiked separately with stressed and unstressed *E. coli* O157 to simulate recoveries from pre- and post-processing contamination. Stressed cells were produced by inoculating (1 ml) of the cocktail (10^9 ml^{-1}) into 100 ml high salt (NaCl, 13·5% w/v), low pH (4·9), low temperature (5°C) broth (McCarthy *et al.* 1998). Counts fell by 3 log numbers over a 10-day period. The pepperoni slices (300 g) were dipped (20 s) into 300 ml cocktail (10^2 ml^{-1}), giving a final concentration of <10^1 g^{-1}, and stored at 4°C for 18 h prior to testing as for minced beef. Numbers of unstressed *E. coli* O157 in apple juice and pepperoni were adjusted to approximately the same as in stressed *E. coli* O157 assays.

Comparison of enrichment broths and incubation temperatures

Enrichment broths tested were based on modifications of BPW (Oxoid) with additions of vancomycin (8 mg l^{-1}, David Bull Laboratories,), cefixime (0·05 mg l^{-1}, Wyeth-

Ayerst Research) and cefsulodin (10 mg l^{-1}, Sigma) (BPW–VCC). BPW with vancomycin alone (BPW-V), and with cefixime and cefsulodin concentrations reduced to one quarter strength (BPW-V + ¼ C + C), were also tested. Modifications of tryptone soya broth (Oxoid) and EC medium (Difco) were investigated (Table 1). Incubation temperatures tested included 30, 37, 40 and 42°C, and incubation times remained constant at 6 h. All broths were equilibrated at 20°C prior to food addition and incubation.

Immunomagnetic separation technique

Enrichment volumes (1 ml) were transferred to sterile Eppendorf tubes containing 0·02 ml Captivate™ O157 immunomagnetic beads (International Diagnostics Group, Bury, UK). The Eppendorf tubes were placed in a rack, mixed for 30 min, before discarding the supernatant fluid and re-suspending the beads (without the magnet) in wash buffer (PBS + 0·05% Tween 20). Two further washing stages were performed prior to transferring the beads (in 0·1 ml buffer) equally onto two selective agar plates incubated at 37°C for 16–18 h.

Selective agars

Those under test (Table 2) included sorbitol MacConkey agar (SMAC, Oxoid), SMAC with additions (Mast Diagnostics) of cefixime (C, 0·05 mg l^{-1}) and potassium tellurite (T, 2·5 mg l^{-1}) (CTSMAC), SMAC with one third strength CT additions and SMAC with two third strength CT additions. The performance of three chromogenic agars was investigated, including CHROMagar$_{TM}$ O157 (M-Tech Diagnostics Ltd), Rainbow™ Agar O157 (Don Whitley Scientific) and SD-39 (QA Life Sciences). All were made according to the manufacturer's instructions and stored at chill temperatures for <7 days prior to use. All agars were incubated at 37°C, except SD-39 which was tested at 42°C, and incubation times were approximately 16–18 h at which time presumptive target colonies could be distinguished from background flora (according to manufacturer's instructions) and tested by latex agglutination. Confirmed target colony numbers were recorded.

Table 1 Recovery of spiked *Escherichia coli* O157 from minced beef by different IMS enrichments at different temperatures

Enrichment medium	37 °C	40 °C	42 °C
BPW-VCC*	+–	+	+ +
BPW-V pH 7·0†	+	+ + + +	+ + + + +
BPW-V pH 6·0‡	+	NT	+ + +
BPW-V + 1/4; C + C§	+	+ + + +	+ + + + +
MTSB + N¶	+	+ + +	+ + + +
EC medium + N**	+–	NT	+ + +

*BPW + vancomycin (8 mg l^{-1}) + cefixime (0·05 mg l^{-1}) + cefsulodin (10 mg l^{-1}).
†Buffered peptone water (BPW) + vancomycin (8 mg l^{-1}) pH 7·0.
‡BPW + vancomycin (8 mg l^{-1}) pH 6·0.
§BPW + vancomycin (8 mg l^{-1}) + cefixime (0·0125 mg l^{-1}) + cefsulodin (2·5 mg l^{-1}).
¶ Tryptone soya broth + bile salts (1·5 g l^{-1}) + novobiocin (20 mg l^{-1}).
**EC medium + novobiocin (20 mg l^{-1}).
NT, not tested; ± , < 10 or zero recovery; + , 10–25; + + , 26–50; + + + , 51–100; + + + + , 101–250; + + + + + , > 250 target colonies recovered. Colony numbers are representative of several replicate experiments.

Testing of naturally-contaminated foods

Samples of cheese and minced beef naturally contaminated with *E. coli* O157 were included in this study. The cheese was responsible for a small, localized outbreak in north-east Scotland and the minced beef was obtained from routine surveillance. Both strains of *E. coli* O157 were VT1 negative, VT2 positive and were phage type 21/28. Samples were homogenized in PBS; the supernatant fluid was subdivided and added to equal volumes of double strength BPW-V and mTSB + N incubated at 42°C, and BPW-VCC incubated at 37°C, for 16–18 h. Immunobeads were divided equally and plated onto CTSMAC and CHROMagar$_{TM}$ O157 agars.

Table 2 Comparison of *Escherichia coli* O157 enrichment media and incubation temperatures from different foods

		Apple juice		Pepperoni	
Enrichment medium	Cheese	Stressed	Unstressed	Stressed	Unstressed
BPW-VCC 37 °C*	–	+ + +	+ + +	–	+ + +
mTSB + N 42 °C†	+ + + +	+ + + +	+ + + + +	+ + + +	+ + + + +
BPW-V 42 °C‡	+ + + +	+ + + + +	+ + + + +	+ + + + +	+ + + + +

*BPW + vancomycin (8 mg l^{-1}) + cefixime (0·05 mg l^{-1}) + cefsulodin (10 mg l^{-1}).
† Tryptone soya broth + bile salts (1·5 g l^{-1}) + novobiocin (20 mg l^{-1}).
‡ Buffered peptone water (BPW) + vancomycin (8 mg l^{-1}) pH 7·0.
–, Indicates zero recovery; + + + , 51–100; + + + + , 101–250; + + + + + , >250 target colonies recovered.

RESULTS

Three criteria were used when comparing results in this study: the presence/absence of target pathogen, the volume of background flora and the numbers of recovered *E. coli* O157.

Enrichment studies

An initial comparison of enrichment incubation temperatures at 30, 37 and 42°C indicated that 30°C gave significantly poorer target recoveries after overnight incubation (data not presented). Incubation at 30°C was therefore omitted from subsequent investigations. A comparison of *E. coli* O157 recoveries from minced beef by six different enrichment broths incubated at 37, 40 and 42°C is presented in Table 1. The results are representative of approximately 40 replicate experiments from a number of minced beef samples spiked separately with the three cocktails. Cocktail composition had no effect on the ratings shown in Table 1. Throughout the tests, BPW supplemented with vancomycin at 8 mg l^{-1}, with or without one quarter normal strength cefixime and cefsulodin, gave optimum recovery. BPW-V at pH 7·0 was superior to BPW-V at pH 6·0. While BPW with the full supplement of vancomycin, cefixime and cefsulodin showed minimal background flora, it consistently gave a poor or zero recovery of *E. coli* O157. Modified TSB proved to be slightly inferior to BPW-V pH 7·0 at 42°C, but superior to EC broth + N. There was a marked difference between incubation temperatures with 42°C giving best, and 37°C giving poorest recovery.

Validation studies of IMS enrichment media in foods other than minced beef compared BPW-VCC (conventional IMS broth), mTSB + N (proposed ISO broth) and BPW-V (optimum broth as shown in Table 1). The results (Table 2) clearly indicate the poor performance of BPW-VCC, which failed to isolate *E. coli* O157 from cheese and the stressed *E. coli* O157 from pepperoni. The recoveries of unstressed cells in pepperoni, and stressed and unstressed *E. coli* O157 from apple juice, were low in number from BPW-VCC. No differences were observed in the performance of the other two enrichments except in the numbers of stressed cells recovered, which were slightly less from mTSB+N. Replicate testing of cheese and pepperoni showed natural levels of associated microflora, varying between 10^3 g^{-1} and 10^7 g^{-1}, while apple juice contained $<10^2$ ml^{-1}.

Selective agars

Selective agars based on either SMAC or different chromogenic reactions were tested and are listed in Table 3. The food source did not have an effect on agar ratings. The agars with the best rating had high numbers of easily observed target bacteria, while those rated poorly were often completely overgrown with background flora, making individual isolate recognition difficult. Of the media based on MacConkey agar, CTSMAC proved superior, with minimal non-target growth. Two chromogens showed good recovery of *E. coli* O157 with low background microflora. Rainbow™ was slightly superior to CHROMagar$_{TM}$, having an almost complete absence of background flora. In the majority of cases, SD-39 failed to isolate the target which was totally obscured by a lawn of competing enteric bacteria. Extended incubation periods (>18 h) often resulted in overgrowth by background flora.

Table 3 Comparison of *Escherichia coli* O157 selective agars for immunomagnetic separation

Selective agar	Rating
CTSMAC*	+ + + +
SMAC†	+ +
SMAC + 1/3 CT‡	+ +
SMAC + 2/3 CT§	+ +
CHROMagar$_{TM}$¶	+ + + +
SD-39**	–
Rainbow™††	+ + + + +

*Cefixime tellurite sorbitol MacConkey, cefixime 0·05 mg l^{-1}, potassium tellurite 2·5 mg l^{-1}.
†Sorbitol MacConkey.
‡Sorbitol MacConkey + cefixime and tellurite at one third normal strength.
§Sorbitol MacConkey + cefixime and tellurite at two thirds normal strength.
¶CHROMagar$_{TM}$ O157 isolation medium.
**Quality Life Sciences *E. coli* O157 isolation medium.
††Biolog Rainbow™ *E. coli* O157 isolation medium.
–,Indicates zero recovery, + + , 26–50; + + + + , 101–250; + + + + + , >250 target colonies recovered.

Naturally-contaminated foods

Foods previously found to contain *E. coli* O157 were tested by three IMS enrichments. The number of replicate tests reflects the small sample size available for testing. The results are presented in Table 4, which indicates the number of samples giving a presence or absence of the target pathogen. BPW-V (42°C) gave the greatest number of positives and BPW-VCC failed to isolate *E. coli* O157 from all samples. Modified TSB + N (42°C) gave one positive from a cheese sample and zero from the minced beef samples.

DISCUSSION

When IMS first became commercially available, routine food testing laboratories regularly employed a sample enrichment in BPW-VCC at 37°C, followed by bead

Table 4 Testing naturally-contaminated foods for *Escherichia coli* O157 by three enrichment procedures

	Cheese	Mince
Enrichment conditions	No. positive	No. positive
BPW-VCC 37 °C*	0/6	0/2
mTSB + N 42 °C†	1/6	0/2
BPW-V 42 °C‡	2/6	1/2;

*BPW + vancomycin (8 mg l^{-1}) + cefixime (0·05 mg l^{-1}) + cefsulodin (10 mg l^{-1}).
†Tryptone soya broth + bile salts (1·5 g l^{-1}) + novobiocin (20 mg l^{-1}).
‡Buffered peptone water (BPW) + vancomycin (8 mg l^{-1}) pH 7·0.

incubation on CTSMAC (Bell and Kyriakides 1998), as indicated by the immunobead manufacturers. However, some strains of *E. coli* O157 were shown to be sensitive to many of the antimicrobials used in this particular method (MacRae *et al.* 1997), and a wide range of enrichment conditions (media and temperature) were tested by a number of laboratories in order to improve recovery. Several enrichments were based on BPW, which is used widely to aid enrichment of physiologically-stressed micro-organisms such as *Salmonella* spp. (Edel and Kampelmacher 1973). Bolton *et al.* (1995) recognized that the cefixime and cefsulodin supplements in the enrichment step were potentially inhibitory to some *E. coli* O157 and tested a range of reduced concentrations, resulting in an optimum BPW enrichment containing one quarter of the normal antimicrobial strength.

In this study, BPW-V pH 7·0 and BPW-V + ¼ C + C were the optimum enrichment media for the recovery of stressed *E. coli* O157 in minced beef. It is probable that cefixime and cefsulodin at this reduced level are below the concentrations that have an effect on target recovery. Doyle and Schoeni (1987) proposed the use of modified TSB + N at 37°C, which in our study compared favourably with other media tested, although incubation at 42°C improved recovery. However, it proved slightly inferior to BPW-V pH 7·0 and BPW-V + ¼ C + C. Szabo *et al.* (1990) tested TSB at 35 and 43°C, and found the addition of bile salts and novobiocin at the higher temperature improved recovery of *E. coli* O157 in foods containing competing micro-organisms. Bolton *et al.* (1995) tested mTSB at 37 and 42°C alongside BPW with different additions and found mTSB at 42°C to be optimum. This medium, incubated at 41·5°C, was proposed by Scotter *et al.* (2000) and has been accepted as a draft International Standard by the International Organization for Standardization; it is widely used by a number of laboratories in the UK. The recommended medium of the US Department of Agriculture is a modified version of EC medium with novobiocin (Okrend and Rose 1989) but in our study, it proved inferior to BPW-V pH 7·0. Originally designed to enumerate coliforms in water and shellfish, EC media contain bile salts and are therefore more selective than BPW-V, and possibly, more inhibitory to sub-lethally damaged micro-organisms.

This study demonstrates the importance of enrichment incubation temperature. Temperature elevation above 37°C increased the number of samples testing positive for *E. coli* O157 and also increased the number of target bacteria recovered (Table 1). This was independent of enrichment composition and was possibly due to the higher temperatures being inhibitory to some of the natural microbial flora in the foods tested. In the majority of cases, there were fewer non-target bacteria on plates from 42°C enrichments.

Further experiments were performed in other foods associated with *E. coli* O157 contamination (Table 2). A zero recovery from cheese by BPW-VCC (37°C) was somewhat surprising as good recovery was observed from the other two enrichments. The numbers of *E. coli* O157 spiked into cheese were slightly lower than in pepperoni, but the degree of physiological stress was assumed to be minimal (2 days of storage at 4°C). The use of BPW-VCC also failed to recover stressed *E. coli* O157 from pepperoni, and it is assumed that cells were damaged to the extent that the high antimicrobial concentration did not allow sufficient recovery in the 6 h enrichment time. BPW-VCC did recover unstressed target bacteria from pepperoni, supporting this theory. BPW-VCC was found to recover both stressed and unstressed *E. coli* O157 from apple juice, and perhaps the degree of stress with this product was less damaging than other methods applied in this work. The difference between BPW-V and mTSB + N lay in their ability to recover damaged bacteria in apple juice and pepperoni; fewer *E. coli* O157 were recovered from the mTSB + N enrichment. This difference may have been due to partial inhibition of damaged organisms by the presence of bile salts in TSB, or to the superior recovery of such cells in the less nutritious peptone water (Edel and Kampelmacher 1973). All results indicate that optimum enrichment is achieved with minimum antimicrobial addition, specifically from those with known activity against Gram-negative micro-organisms such as cefixime and cefsulodin (Lambert and O'Grady 1992).

When Zadik *et al.* (1993) introduced cefixime and tellurite additions to improve *E. coli* O157 selectivity, the majority of isolation procedures in the UK used SMAC. The introduction of cefixime and tellurite into SMAC has made the observation of target pathogen clearer by reducing background flora. However, MacRae *et al.* (1997) showed that a small but significant number of *E. coli* O157 isolates exhibited partial inhibition in the presence of such antimicrobials, and suggested that the use of such additions may contribute to foods being incorrectly labelled as free from *E. coli* O157. It must be stressed that all strains of

E. coli O157 used in this study were not sensitive to cefixime and tellurite and therefore, CTSMAC gave excellent results when compared with SMAC, with or without reduced concentrations of the two antimicrobials (Table 3). CTSMAC alone will not detect the presence of sorbitol-positive strains of *E. coli* O157. These are currently isolated only rarely in the UK but it would be unwise to assume that they do not pose a significant threat to human health in the future. Other European countries, such as Germany (Beutin 1999), isolate sorbitol-positive strains on a regular basis, which necessitates the use of an alternative selective plating medium. A number of selective agars based on alternatives to sorbitol MacConkey are available commercially, and most are based on chromogenic reactions to distinguish the target colony from background flora. Taormina *et al.* (1998) concluded that Rainbow™ and a modified eosin methylene blue agar gave best recovery from heat-stressed *E. coli* O157 in ground beef. The present study found Rainbow™ to be superior to two alternative chromogenic agars (having less background flora) and indeed, showed better recovery of target cells compared with CTSMAC. CHROMagar$_{TM}$ O157 was equal in performance to CTSMAC. In addition to the discrimination of *E. coli* O157 from other enterics and food-associated microflora, CHROMagar$_{TM}$ O157 can be used to identify serogroups O111 and O103 (Bettelheim 1998a), while Rainbow™ can distinguish serotypes O111:H8 and O26:H11 from *E. coli* O157 (Bettelheim 1998b). Quality Life Sciences *E. coli* O157 medium (SD-39) gave poor results in this study (incubated at 42°C), with large numbers of competing microflora preventing correct identification of *E. coli* O157. However, it must be stressed that the manufacturers recommend incubation at 44°C, a temperature unavailable during the course of this work. Some workers (Doyle and Schoeni 1984) have reported partial or complete inhibition of *E. coli* O157 at this elevated temperature. Based on the results of this study, the present method for culturing *E. coli* O157 in our laboratory is to distribute IMS beads equally between CTSMAC and a chromogenic agar. The current economical choice is CHROMagar$_{TM}$ O157. Throughout this study using spiked cockails, it was not possible to identify the individual component strains recovered after enrichment and plating, and the possibility exists that the media may not be optimal for all strains used.

Two foods naturally contaminated with *E. coli* O157 were available in sufficient quantities for replicate testing during the course of this work. The levels of *E. coli* O157 in the two foods (as enumerated by MPN) were found to be low at $<10\ g^{-1}$, but their physiological state was unknown. Although both minced beef and cheese were supplied by the retailer as fresh produce, their history was unclear. For example, the beef may have been frozen prior to sale and the cheese could have been stored for an extensive period (several months) before purchase, both of which could result in sub-lethally-damaging target bacteria. Results showing such differences between enrichments suggest that stressed *E. coli* O157 could have been present in all samples.

The use of optimum IMS enrichment media as described has enabled this laboratory to isolate and confirm the presence of low numbers of sub-lethally-damaged *E. coli* O157 from a number of foods. In the majority of tests, there was a high background level of micro-organisms which did not interfere with recognition of the target bacteria. A protocol is proposed of enriching in BPW-V at 42°C for 6 h, and plating immunobeads on CTSMAC and CHROMagar$_{TM}$ O157, to improve *E. coli* O157 isolation from foods.

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