Molecular and Cellular Probes (2001) **15**, 267–274
doi:10.1006/mcpr.2001.0370, available online at http://www.idealibrary.com on IDEAL®

Sample preparation methods for PCR detection of *Escherichia coli* O157:H7, *Salmonella typhimurium,* and *Listeria monocytogenes* on beef chuck shoulder using a single enrichment medium

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(Received 14 March 2001, Accepted 18 May 2001)

To improve the utility of the polymerase chain reaction (PCR) for food samples, methods for preparing template DNA were developed to remove PCR inhibitors. Beef chuck shoulder medallions, artificially contaminated, individually or in combination, with *Escherichia coli* serotype O157:H7 strain FSIS 45753-35, *Salmonella typhimurium* DT104 strain 13HP, or *Listeria monocytogenes* strain Scott A at concentrations of 10, 1 and 0·5 cfu/cm^2 were swabbed with a sponge, and the sponges were enriched for 18 h at 37°C in universal pre-enrichment broth (UPB). Enriched broth cultures (EBC), cell pellets (CP), or phosphate-buffered saline–washed cell pellets (PBSCP) from enriched sponge samples were compared for detection of *E. coli* O157:H7, *S. typhimurium* DT104, or *L. monocytogenes* by the PCR using the BAX™ system. Recovery of the three organisms was effective for detection of each pathogen at initial levels of 10, 1 and 0·5 cfu/cm^2 when inoculated separately, or in combination, onto the beef samples. Use of EBC, CP, or PBSCP of sponge-swabbed samples eliminated problems associated with inhibition of the PCR by food components, time-consuming extraction of DNA, and inhibition due to large amounts of non-target DNA derived from the food. The procedure involving enrichment of sponge-swabbed beef samples in UPB followed by PCR amplification using EBC with the BAX™ system is the most efficient and simple method for detection of *E. coli* O157:H7, *S. typhimurium* DT104, and *L. monocytogenes*.

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KEYWORDS: sponge-swabbing, enrichment, polymerase chain reaction, detection, pathogens, beef.

INTRODUCTION

Contamination of foods with pathogens such as *Escherichia coli* O157:H7, *Salmonella typhimurium*, and *Listeria monocytogenes* is a major public health concern worldwide.[1] There is a need for rapid and reliable methods for detection of these organisms in contaminated food. Contaminated beef has been identified as a source of outbreaks of food-borne diseases caused by *E. coli* O157:H7, *S. typhimurium*, and *L. monocytogenes* infection.[2–4] Detection methods for these pathogens in food are based on enrichment using selective media to increase the concentration of viable bacteria which can be fol-

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0890–8508/01/050267+08 $35.00/0 © 2001 Academic Press

lowed by the polymerase chain reaction (PCR) for identification. There are two major obstacles for simple and rapid detection of these pathogens in food: (a) the lack of one single medium for the simultaneous enrichment of these 3 organisms; and (b) the presence of inhibitors of the PCR in foods such as beef and in synthetic media.[5,6]

Basic differences in the growth characteristics of Gram-negative bacteria such as *E. coli* and *Salmonella*, and a Gram-positive bacterium such as *Listeria* species make the enrichment of these 3 organisms incompatible in a single medium. Most chemical inhibitors or antibiotics which are active against Gram-negative bacteria have little effect on Gram-positive bacteria and vice versa. Furthermore, the presence of selective agents could inhibit the enrichment of injured cells in food. To overcome some of these difficulties, the universal pre-enrichment broth (UPB) was developed and used for simultaneous recovery of injured *Salmonella* and *Listeria* in food.[7]

Efficient sample treatment methods are needed to fully exploit the potential of the PCR for detection of foodborne pathogens. Consequently, several methods to concentrate bacteria and remove PCR inhibitors from food and growth media have been suggested, including the use of lysozyme, proteinase K, detergents, centrifugation and filtration, magnetic beads coated with antibodies, aqueous two phase systems, and extraction of DNA directly from food.[8–13] In addition to the presence of inhibitors in food and growth media, DNA-extraction solutions may influence the effectiveness of the PCR by reducing assay sensitivity due to loss of target DNA.[5,6] The array of methods developed for preparing PCR samples reflects the need for techniques that are tailored to each type of food. To overcome these limitations, we have developed a sampling method by sponge-swabbing food samples to recover the bacteria followed by enrichment of the culture and then PCR amplification of a cell pellet-derived lysate for detection of plasmid-bearing virulent serotypes of *Yersinia enterocolitica*.[14] The detection of other foodborne pathogens by this technique is also feasible if a suitable enrichment medium specific for the target pathogen(s) is used.

Therefore, the first objective of the present study was to evaluate the UPB developed by Cox and Bailey[7] for enrichment of sponge-swabs of beef samples artificially contaminated with *E. coli* O157:H7, *S. typhimurium* DT104, and *L. monocytogenes*, individually or in a combination, with subsequent application of the PCR for detection of each specific pathogen. The second objective was to adapt the PCR sample preparation method which was previously developed in our laboratory for pathogenic *Y. enterocolitica* for detection of *E. coli* O157:H7, *S. typhimurium* DT104, and *L. monocytogenes* inoculated onto beef.

MATERIALS AND METHODS

Bacteria and media

E. coli O157:H7 strain FSIS 45753-35 isolated from bovine kidney, *S. typhimurium* DT104 strain 13HP (US) isolated from swine, and *L. monocytogenes* strain Scott A a clinical isolate, were used in this study. All strains were obtained from the culture collection of the Microbial Food Safety Research Unit of the USDA Eastern Regional Research Center, Wyndmoor, PA, USA. A detailed description of the strains and sources is given elsewhere.[15,16] Peptone, universal pre-enrichment broth (UPB), brain heart infusion (BHI) broth, and brain heart infusion agar (BHIA) (Difco Laboratories, Detroit, MI, USA) were prepared as described by the manufacturer.

Preparation of food samples

Beef chuck shoulder was purchased from a local market. Beef chuck shoulder was then placed in sterile Whirl Pak™ bag (Nasco, Ft. Atkinson, WI, USA), transported to the laboratory on ice, and processed within 1 h of collection. Medallions from beef chuck shoulder were prepared using a 10 cm^2 coring tool. Unsterilized medallions were artificially contaminated with the three pathogens, alone or in combination, and subjected to enrichment and then the PCR assay. Other medallions were double-sealed in vacuum packaging bags and irradiated at −30°C with a 42 kGy dose in a Lockheed Georgia Company self-contained caesium[137] irradiator using the facilities of Food Safety Research Unit of the USDA Eastern Regional Research Center, Wyndmoor, PA, USA. This treatment provides sterile samples based on a 12D reduction of *Clostridium botulinum*.[17] The sterile medallions were used to determine the efficiency of sponge-swabbing and the post enrichment bacterial counts (see below), because the selective media currently available for *E. coli* O157:H7, *S. typhimurium* DT104, and *L. monocytogenes* identify these 3 organisms only as presumptive positives and further tests are needed for definitive identification.

Inoculation of samples

Bacterial cultures were grown in BHI for 18 h at 37°C with shaking to a population density of approximately

10^9 cfu/ml, and then were directly diluted in 0·1% peptone water. Dilutions (100 µl) of *E. coli* O157:H7, *S. typhimurium* DT104, and *L. monocytogenes* in 0·1% peptone water containing about 1000, 100, and 50 cfu/ml of each organism, individually or in combination with the other 2 pathogens, were pipetted over the surface of a 10 cm^2 unsterilized beef medallion to obtain final surface concentrations of about 10, 1, and 0·5 cfu/cm^2. Unspiked and unsterilized beef medallions were used as negative controls. To simulate natural surface contamination, the samples were allowed to stand for 15 min at room temperature prior to sponge-swabbing.

Sampling of medallions, enrichment of sponge-swabbed samples, and plating of enrichment broths

The beef medallions were sampled by surface sponge-swabbing using sterile 5 × 5 × 1·2 cm cellulose sponges (Nasco) moistened in 10 ml of UPB as described previously.[14,18] Each sponge was placed in a sterile Whirl Pak™ bag (Nasco) containing 90 ml of UPB and pummelled in a Stomacher (Model Lab Blender 400, A. J. Seward, London, UK) for 2 min, and then incubated at 37°C for 18 h in a shaking incubator (100 rpm). Each enrichment bag was pummelled for 2 min immediately prior to removing samples for testing by the PCR. For control samples, the UPB was removed by squeezing the liquid from the sponges directly into a sterile Whirl Pak™ bag to determine the number of bacteria removed from the surface of the medallions by sponge-swabbing. The full volume of this liquid was plated on a series of BHIA plates using an Autoplate 4000 Spiral Plater (Spiral Biotech Inc., Bethesda, MD, USA). Plates were incubated at 37°C for 18 h and colonies were counted using an automated colony counter (Model 500A, Spiral System, Inc., Bethesda, MD, USA). For determination of the post enrichment bacterial counts, enriched cultures from spiked and unspiked sterile beef medallions were assayed to determine bacterial concentrations. Each enrichment was serially diluted in 0·1% peptone water and then plated in triplicate on BHIA, incubated at 37°C for 18 h, and colonies were counted as described above.

Sample preparation for PCR assay

The concentration of the cells in the enriched culture was determined by optical density at 600 nm (A_{600}) after 18 h incubation at 37°C. The 100 ml of each enriched culture was divided into three aliquots of 33 ml each. The first 33 ml sample (10^9 cfu/ml as determined by A_{600}) had no additional post-enrichment processing and was identified as a enriched broth culture (EBC). For the second 33 ml sample, the cells were harvested by centrifugation (7000 × ***g*** for 10 min at 4°C) and the resulting cell pellets were designated as CP. For the third 33 ml sample, cells were harvested by centrifugation (7000 × ***g*** for 10 min at 4°C) and the pellets were washed twice with phosphate-buffered saline (PBS) without Mg^{2+} and Ca^{2+} as described previously.[19] These samples were identified as PBS-washed cell pellets (PBSCP). The CP and PBSCP samples were suspended in 33 ml of sterile distilled water to give a cell concentration (ca. 10^9 cfu/ml) equivalent to that of the EBC samples. The EBC, CP, and PBSCP were also prepared from the enriched culture of unspiked beef medallions and were used as negative controls. These sample groups were analyzed by the PCR using the BAX™ systems for *E. coli* O157:H7, *S. typhimurium* DT104, and *L. monocytogenes* according to manufacturer's instruction (Qualicon™, Wilmington, DE, USA).

PCR screening of samples for *E. coli* O157:H7, *S. typhimurium* DT104, and *L. monocytogenes* using the BAX™ systems

A 3 µl portion for *E. coli* O157:H7 or a 5 µl portion for *L. monocytogenes* and *S. typhimurium* DT104 from the EBC, CP, and PBSCP samples prepared from enrichments of each pathogen inoculated individually or in combination with the other 2 bacteria was lysed in 200 µl of lysis reagent according to the protocol provided in the BAX™ PCR amplification kits (Qualicon™). The *E. coli* O157:H7 and *S. typhimurium* DT104 samples were incubated at 37°C for 20 min and the *L. monocytogenes* samples were incubated at 55°C for 60 min. All samples were then heated at 95°C for 10 min. Fifty microliter samples from each lysate were evaluated as template DNA for PCR analyses. Fifty microliters of UPB only was added to PCR tubes as a negative control. Lysates (50 µl) derived from pure cultures of each organism grown in UPB were added to tubes as positive controls. Fifty microliters of lysate from EBC, CP, and PBSCP from unspiked beef samples were also added to tubes as negative controls for each organism. A DNA template from the PCR amplification kit was used as an internal positive control for the PCR reaction. Amplification was performed in a programmable heating block incubator (GeneAmp PCR system 9600, Perkin-Elmer Corporation, Norwalk, CT, USA) using parameters specified by the manufacturer for each BAX™ kit. The PCR products were separated electrophoretically on

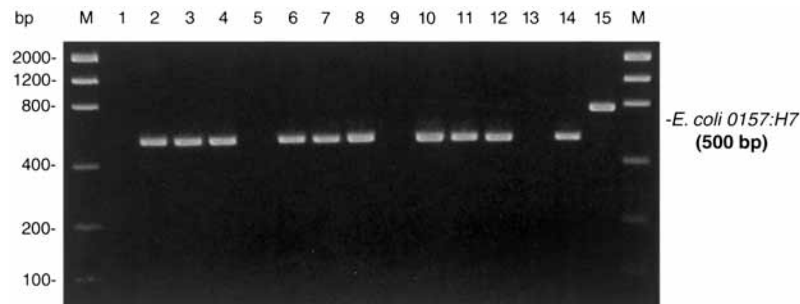

Fig. 1. Detection of *E. coli* O157:H7 strain FSIS 45753-35 on artificially contaminated beef by PCR amplification of a 500 bp sequence. Lanes; M, 100–2000 bp ladder marker; lane 1, UPB as a negative control; lanes 5, 9, and 13 negative controls for EBC, CP, and PBSCP from unspiked beef, respectively. *E. coli* O157:H7 showing the presence of the 500 bp product from spiked beef: EBC (lanes 2–4); CP (lanes 6–8); PBSCP (lanes 10–12). The panel shown is representative of the amplification results after beef was spiked with 10, 1 and 0·5 cfu/cm^2 concentrations of *E. coli* O157:H7, from left to right, respectively. Lane 14; positive control, pure culture lysate of *E. coli* O157:H7 showing the presence of the 500 bp product. Lane 15; positive control for PCR assay, control DNA template showing 800 bp product.

a 2·0% agarose gel and detected in UV light after ethidium bromide staining.[20] A positive result for samples containing *E. coli* O157:H7, *S. typhimurium* DT104, and *L. monocytogenes* was indicated by the presence of a band corresponding to the 500 bp, 750 bp, and 410 bp respectively.

RESULTS AND DISCUSSION

The goal of this study was to evaluate sample preparation methods for detection of *E. coli* O157:H7, *S. typhimurium* DT104, and *L. monocytogenes* on beef using a PCR-based assay. In this work, a previously described[14,18] sponge-swabbing technique was applied to the recovery of *E. coli* O157:H7, *S. typhimurium* DT104, and *L. monocytogenes* followed by the PCR for detection on unsterilized beef. The sensitivity of sponge-swabbing in combination with EBC, CP and PBSCP sample preparation methods for PCR was compared using the BAX™ system. The use of the BAX™ system simplified the PCR analyses through the use of prepared lysis reagents and tableted PCR reagents from the manufacturer. Pipetting time was reduced and potential cross-contamination was decreased.

The *E. coli* O157:H7, *S. typhimurium* DT104, and *L. monocytogenes* could be detected using the BAX™ system from enriched sponge-swabbed samples of beef medallions inoculated with each organism separately or in combination after 18 h of enrichment at 37°C. As expected, the primers amplified a 500 bp product from *E. coli* O157:H7, (Fig. 1 EBC lanes 2–4, CP lanes 6–8, PBSCP lanes 10–12), a 750 bp product from *S. typhimurium* DT104 (Fig. 2 EBC lanes 2–4, CP lanes 6–8, PBSCP lanes 10–12), and a 410 bp product from *L. monocytogenes* (Fig. 3 EBC lanes 2–3, CP lanes 6–7, PBSCP lanes 10–11). Bands corresponding to each inoculum level (10, 1, and 0·5 cfu/cm^2) for the EBC, CP and PBSCP sample preparations were visible for *E. coli* O157:H7 and *S. typhimurium* DT104. The 410 bp product from *L. monocytogenes* was detectable in the EBC samples, however, band intensity was greater with the CP and PBSCP sample preparations for the two highest inoculum levels (10 and 1 cfu/cm^2). These results demonstrate that *E. coli* O157:H7 and *S. typhimurium* DT104 can be directly detected in EBC, CP, and PBSCP sample preparations while the CP and PBSCP sample preparations from *L. monocytogenes* amplified the target DNA with high efficiency as it was evidenced from the intensity of the amplified band on the gel. This may be due to the presence of PCR inhibitors in the EBC samples which are removed by the washing steps involved in the CP and PBSCP samples and could account for the differences in sample preparation. This observation about the EBC samples is more apparent with the *L. monocytogenes* samples versus preparation of the *E. coli* O157:H7 and *S. typhimurium* DT04 because of the overall reduced cell lysis efficiency for Gram-positive characteristics such as the composition and thickness of the cell wall of *L. monocytogenes* resulting in the non-optimal concentration of template DNA.

The PCR assay confirmed the presence of each pathogen when inoculated in combination with the other two pathogens, amplifying a 500 bp sequence for *E. coli* O157:H7, a 750 bp sequence for *S. typhi-*

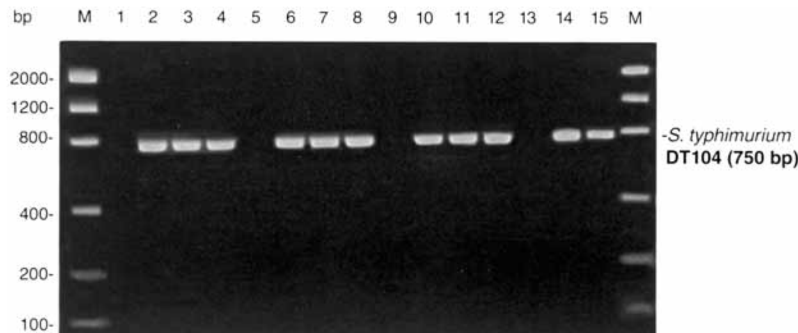

Fig. 2. Detection of *S. typhimurium* DT104 strain 13HP on artificially contaminated beef by PCR amplification of a 750 bp sequence. Lanes; M, 100–2000 bp ladder markers; lane 1, UPB as a negative control; 5, 9 and 13 negative controls for EBC, CP, and PBSCP from unspiked beef, respectively. *S. typhimurium* DT104 showing the presence of the 750 bp product from spiked beef: EBC (lanes 2–4); CP (lanes 6–8); PBSCP (lanes 10–12). The panel shown is representative of the amplification results after beef was spiked with 10, 1 and 0·5 cfu/cm^2 concentrations of *S. typhimurium* DT104 from left to right, respectively. Lane 14; positive control, pure culture lysate of *S. typhimurium* DT104 showing the presence of the 750 bp. Lane 15; positive control for PCR assay, control DNA template showing 800 bp product.

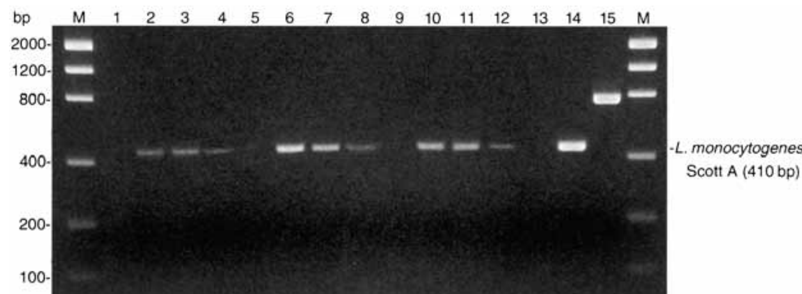

Fig. 3. Detection of *L. monocytogenes* strain Scott A on artificially contaminated beef by PCR amplification of a 410 bp sequence. Lanes; M, 100–2000 bp ladder markers; lane 1, UPB as a negative control; 5, 9, and 13 negative controls for EBC, CP, and PBSCP from unspiked beef, respectively. *L. monocytogenes* showing the presence of the 410 bp product from spiked beef: EBC (lanes 2–4); CP (lanes 6–8); PBSCP (lanes 10–12). The panel shown is representative of the amplification results after beef was spiked with 10, 1 and 0·5 cfu/cm^2 concentrations of *L. monocytogenes* from left to right, respectively. Lane 14; positive control, pure culture lysate of *L. monocytogenes* showing the presence of the 410 bp product. Lane 15; positive control for PCR assay, control DNA template showing 800 bp product.

murium DT104 and a 410 bp sequence for *L. monocytogenes* (data not shown). The sensitivity of the PCR assays for all 3 organisms in EBC, CP, and PBSCP sample preparations at all levels tested (10, 1, and 0·5 cfu/cm^2) was similar as described above when beef medallions were inoculated with each organism alone. The protocol using the EBC-derived lysate substantially simplified sample handling and reduced the time required for sample preparation compared to our previously described method by eliminating the need for centrifugation and washing of cells.[14]

Sponge-swabbing offers several advantages over other sampling techniques for the PCR analysis. Removing the food matrix from the enrichment medium eliminates the presence of many food components and non-specific food-derived DNA which may inhibit the PCR. The sponge-swabbing technique is applicable for foods such as fruits, vegetables, and sliced cheese where surfaces are the primary site of bacterial contamination and a surface sampling procedure is applicable.[14,18] It is not applicable for liquid, semisolid, or ground foods.

Since UPB is highly buffered by the presence of mono- and dibasic-phosphates and is low in carbohydrates (0·05% dextrose), it prevents a rapid drop in pH with the growth of extraneous micro-organisms found in foods.[7] Thus, this single medium was suitable for simultaneous recovery and detection of *E. coli* O157:H7, *S. typhimurium* DT104, and *L. monocytogenes* in food samples. This medium allowed as few as 0·5 cfu/cm^2 target organisms to multiply in sufficient numbers following a 24 h enrichment at 37°C. Inhibition of the PCR by components of enrichment media has been reported.[5,6,21] However, enrichment in UPB did not affect the effectiveness of the PCR in the present study. Enrichment in UPB also eliminates the use of a "second-stage" enrichment of these organisms as reported previously,[7] although further

Table 1. Efficiency of sponge-swabbing for recovery of bacteria from artificially contaminated sterile beef medallions

Organism	Number of bacteria on 10 cm^2 medallion	Number[a] (%) of bacteria recovered from medallion
E. coli O157:H7	100	72·3 ± 2·5 (72·3 ± 2·5)
strain FSIS 45753-35	10	8·0 ± 1·0 (80·0 ± 10·0)
	5	2·3 ± 0·6 (46·7 ± 11·5)
	0	0
S. typhimurium DT104	100	42·0 ± 2·0 (42·0 ± 2·0)
strain 13HP	10	2·7 ± 0·6 (26·7 ± 5·8)
	5	1·3 ± 0·6 (26·7 ± 11·5)
	0	0
L. monocytogenes	100	38·7 ± 8·1 (38·7 ± 8·1)
strain Scott A	10	4·0 ± 2·0 (40·0 ± 20·0)
	5	2·3 ± 1·5 (46·7 ± 30·6)
	0	0

[a] M ± S where M is the mean of three experiments and S is the standard deviation.

Table 2. Bacterial levels obtained after enrichment of sponges in universal pre-enrichment broth and corresponding initial inoculum levels on sterile beef medallions

Organism	Initial concentration on beef medallion cfu/cm^2	Final enrichment level log_{10} cfu/ml[a]
E. coli O157:H7	10	9·34 ± 0·13
strain FSIS 45753-35	1	9·36 ± 0·05
	0·5	9·25 ± 0·09
	0	no growth
S. typhimurium DT104	10	9·16 ± 0·13
strain 13HP	1	9·16 ± 0·04
	0·5	9·19 ± 0·02
	0	no growth
L. monocytogenes	10	8·89 ± 0·33
strain Scott A	1	8·83 ± 0·28
	0·5	8·91 ± 0·28
	0	no growth

[a] M ± S where M is the mean of three experiments and S is the standard deviation.

studies are needed to determine if the use of a second-stage enrichment would increase sensitivity.

To determine the efficiency of sponge-swabbing for removal of bacteria from the food surface, sterile 10 cm^2 beef medallions were artificially contaminated with a total of 100, 10, and 5 cfu/cm^2 of *E. coli* O157:H7, *S. typhimurium* DT104, and *L. monocytogenes* (Table 1). The sterile samples were used to accurately enumerate the *E. coli* O157:H7, *S. typhimurium* DT104, and *L. monocytogenes* removed by sponge-swabbing without interference of the background beef microflora. Results of the experiments showed that the recovery of bacteria was 46·7–80% for *E. coli* O157:H7, 26·7–42% for *S. typhimurium* DT104, and 38·7–46·7% for *L. monocytogenes* (Table 1). The different levels of recovery for each pathogen can not be explained from this study. No bacteria were recovered from uncontaminated samples (Table 1). These data established that as low as 5 cfu/10 cm^2 could be removed by sponge-swabbing beef, enriching in UPB, detecting by the PCR in the presence of competitive microflora.

Bacterial levels after an 18 h enrichment at 37°C in UPB were determined for *E. coli* O157:H7, *S. typhimurium* DT104, and *L. monocytogenes* inoculated at levels of 10, 1, and 0·5 cfu/cm^2 onto sterile beef medallions. Enrichments yielded approximately $10^{9\cdot2}$ cfu/ml for *E. coli* O157:H7 and *S. typhimurium* DT104 and approximately $10^{8\cdot9}$ CFU/ml for *L. monocytogenes* at every inoculum level (Table 2). There was no growth in the enrichments of uncontaminated sponge-swabbed samples (Table 2). It is likely that these levels of *E. coli* O157:H7, *S. typhimurium* DT104, and *L. monocytogenes* obtained from inoculation of sterile medallions are higher than would be obtained in enrichments in which background

microflora is present because the extraneous micro-organisms normally present in beef might affect the levels of enrichment of *E. coli* O157:H7, *S. typhimurium* DT104, and *L. monocytogenes*. However, the results showed that sufficient enrichment was achieved for the 3 pathogens at all initial inoculum levels tested for detection by the PCR.

This work indicates that sponge-swabbing of beef samples, when used in combination with PCR amplification of an enriched broth culture-derived lysate, is an effective method for the detection of *E. coli* O157:H7, *S. typhimurium* DT104, and *L. monocytogenes*. Furthermore, it is a convenient sample preparation method for processing large numbers of samples. We demonstrate for the first time that the UPB is effective for simultaneous enrichment of *E. coli* O157:H7, *S. typhimurium* DT104, and *L. monocytogenes*, and that the enrichment can be directly employed for detection of the organisms by the PCR. Because of the unique properties of UPB, further studies are in progress to determine the applicability of this medium to resuscitate and allow multiplication to sufficiently high numbers of stressed/injured *E. coli* O157:H7, *S. typhimurium* DT104, and *L. monocytogenes* for detection by the PCR in different foods.

ACKNOWLEDGEMENTS

We thank Dr Donald W. Thayer, Research Leader of the Food Safety Research Unit at the USDA, Eastern Regional Research Center, Wyndmoor, PA, USA for irradiating the beef samples used in this study. The authors also thank Dr John Phillips, Statistician, at the USDA/ARS/NAA who performed the statistical calculations and provided consultation on the statistical analyses.

REFERENCES

1. Mead, P. S., Slutsker, L., Dietz, V., McCaig, L. F., Bresee, J. S., Shapiro, C., Griffin, P. M. & Tauxe, R. V. (1999). Food-related illness and death in the United States. *Emerging Infectious Disease* **5**, 607–25.
2. Doyle, M. P., Zhao, T., Meng, J. & Zhao, S. (1997). *Escherichia coli* O157:H7. In *Food Microbiology, Fundamentals and Frontiers* (Doyle, M. P., Beuchat, L. R., & Montville, T. J., eds), Pp. 171–91. Washington DC: ASM Press.
3. Centers for Disease Control and Prevention. (1995). Outbreak of *Salmonella* serotype Typhimurium infection associated with eating raw ground beef—Wisconsin, 1994. *MMWR* **4**, 905–9.
4. Farber, J. M. & Peterkin, P. I. (1992). Incidence and behavior of *Listeria monocytogenes* in meat products. In *Listeria, Listeriosis and Food Safety* (Ryser, E. T. & Marth, E. H., eds) 2nd edn, Pp. 505–64. New York: Marcel Dekker, Inc.
5. Rossen, L., Nørskov, P., Holmstrøm, K. & Rasmussen, O. F. (1992). Inhibition of PCR by components of food samples, microbial diagnostic assays and DNA-extraction solutions. *International Journal of Food Microbiology* **17**, 37–45.
6. Lantz, P.-G., Hahn-Hägerdal, B. & Rådström, P. (1994). Sample preparation methods in PCR-based detection of food pathogens. *Trends in Food Science & Technology* **5**, 384–9.
7. Cox, N. A. & Bailey, J. T. (1992). Universal preenrichment broth for simultaneous detection of *Salmonella* and *Listeria* in foods. *Journal of Food Protection* **55**, 256–9.
8. Lindqvist, R., Norling, B. & Thisted-Lambertz, S. T. (1997). A rapid sample preparation method for PCR detection of food pathogens based on buoyant density centrifugation. *Letters in Applied Microbiology* **24**, 306–10.
9. Rychlík, I., van Kesteren, L., Cardová, L., Švestkoá, A., Martínková, R. & Šíšák, F. (1999). Rapid detection of *Salmonella* in field samples by nested polymerase chain reaction. *Letters in Applied Microbiology* **29**, 269–72.
10. Soumet, C., Ermel, G., Fach, P. & Colin, P. (1994). Evaluation of different DNA extraction procedures for the detection of *Salmonella* from chicken products by polymerase chain reaction. *Letters in Applied Microbiology* **19**, 294–8.
11. Lantz, P.-G., Tjerneld, F., Borch, E., Hahn-Hägerdal, B. & Rådström, P. (1994). Enhanced sensitivity in PCR detection of *Listeria monocytogenes* in soft cheese through use of an aqueous two-phase system as a sample preparation method. *Applied and Environmental Microbiology* **60**, 3416–8.
12. Wang, R.-F., Cao, W.-W., Franklin, W., Campbell, W. & Cerniglia, C. E. (1994). A 16S rDNA-based PCR method for rapid and specific detection of *Clostridium perfringens* in food. *Molecular and Cellular Probes* **8**, 131–8.
13. Lindqvist, R. (1997). Preparation of PCR samples from food by a rapid and simple centrifugation technique evaluated by detection of *Escherichia coli* O157:H7. *International Journal of Food Microbiology* **37**, 73–82.
14. Bhaduri, S. & Cottrell, B. (1998). A simplified sample preparation from various foods for PCR detection of pathogenic *Yersinia enterocolitica*: a possible model for other food pathogens. *Molecular and Cellular Probes* **12**, 79–83.
15. Fratamico, P. M. & Strobaugh, T. P. (1998). Simultaneous detection of *Salmonella* spp. and *Escherichia coli* O157:H7 by multiplex PCR. *Journal of Industrial Microbiology & Biotechnology* **21**, 92–8.
16. Klein, P. G. & Juneja, V. K. (1997). Sensitive detection of viable *Listeria monocytogenes* by reverse transcription-PCR. *Applied and Environmental Microbiology* **63**, 4441–8.
17. Thayer, D. W. & Boyd, G. (1995). Radiation sensitivity of *Listeria monocytogenes* on beef as affected by temperature. *Journal of Food Science* **60**, 237–40.
18. Bhaduri, S. & Cottrell, B. (1997). Direct detection and isolation of plasmid-bearing virulent serotypes of *Yersinia enterocolitica* from various foods. *Applied and Environmental Microbiology* **63**, 4952–5.
19. Bhaduri, S. & Demchick, P. H. (1983). Simple and rapid method for disruption of bacteria for protein studies. *Applied and Environmental Microbiology* **46**, 941–3.

20. Bhaduri, S., Kasai, T., Schlessinger, D. & Raskas, H. J. (1980). pMB9 plasmids bearing the *Salmonella typhimurium his* operon and *gnd* gene. *Gene* **8**, 239–53.
21. Baez, L. A. & Juneja, V. K. (1995). Detection of enterotoxigenic *Clostridium perfringens* in raw beef by polymerase chain reaction. *Journal of Food Protection* **58**, 154–9.