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Rapid detection of *Listeria monocytogenes* in ham samples using immunomagnetic separation followed by polymerase chain reaction

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Aims: To develop a 24-h system for the detection of *Listeria monocytogenes* in ham.

Methods and Results: An immunomagnetic separation (IMS) of bacteria directly from ham followed by extraction of DNA and detection using a new multiplex polymerase chain reaction (PCR) was used. The PCR method used one primer pair targeted at the listeriolysin O gene of *L. monocytogenes* and the other pair for a region of the 23S rRNA genes of *Listeria*, giving products of 706 and 239 bp, respectively. The combined IMS/PCR was calculated to be capable of detecting as few as 1·1 *L. monocytogenes* cells g^{-1} in a 25-g ham sample.

Conclusions: The process produced acceptable results, but the IMS step is the main barrier to further improvement of sensitivity. The DNA isolation was the most time-consuming step in the process.

Significance and Impact of the Study: A 24-h test for the presence of *L. monocytogenes* will be useful to the food industry and significantly assist in the timely investigation of outbreaks.

INTRODUCTION

Invasive listeriosis is most often contracted by those who are at the extremes of age and those who are immunocompromised, but when *Listeria monocytogenes* cells are consumed in high numbers they may also cause non-invasive febrile gastroenteritis in otherwise healthy people (Anon. 1997). A range of foods have acted as vehicles in outbreaks of listeriosis, including vegetables, dairy products (Czajka and Batt 1994) and meat products (Salvat *et al*. 1995). Other members of the genus *Listeria* are not considered to be significant human pathogens (Lund 1990).

Conventional detection methods for *L. monocytogenes* include selective enrichment, isolation of colonies on agar and confirmation using haemolysin activity and other biochemical reactions as diagnostic tests. These methods are laborious and take several days to achieve a confirmed identification. A number of alternative detection methods, based on amplification of *L. monocytogenes* DNA using the polymerase chain reaction (PCR), have been reported (Hill 1996). However, most methods of this nature still rely on selective enrichment both to grow sufficient bacteria for detection and to ensure viability.

The isolation of *Listeria* directly from food is an alternative to selective enrichment. This approach presents several challenges, including assessment of the viability of the micro-organisms, physically detaching them from the food and separating them from food materials that may inhibit the PCR. Assessment of viability may be possible via detection of mRNA by reverse transcription PCR (e.g. Stinear *et al*. 1996) while detachment has been explored through the use of physical and chemical techniques (e.g. Rodrigues-Szulc *et al*. 1996). The systems most frequently used for the isolation of pathogens from food homogenates are buoyant density centrifugation (e.g. Lindqvist *et al*. 1997), immunomagnetic separation (IMS) (e.g. Bennett *et al*. 1996) and two phase separation (Lantz *et al*. 1994).

The aim of this study was to develop a rapid method for the detection of *L. monocytogenes* from ham. Ham was chosen as the food matrix for study as it is consumed without further cooking and is thus a potential vehicle for transmission of *L. monocytogenes* and because New Zealand has experienced a number of food recalls of ham and similar products due to the detection of this pathogen. A rapid

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method for the detection of *L. monocytogenes* would therefore be of use to both regulators and the food industry. Pork has also been reported to contain materials which inhibit the operation of the PCR (Lantz *et al*. 1998) and so may represent a 'worst case' food matrix.

To avoid time-consuming enrichment procedures, and to remove PCR inhibitors, bacteria were isolated and concentrated directly from the food using IMS, a technique chosen because of the commercial availability of anti-*Listeria* immunomagnetic beads.

A new PCR detection method was also developed which uses one primer pair specific for *L. monocytogenes* complemented by another primer pair for the genus *Listeria*. The objective was to provide a level of confirmation of the *L. monocytogenes* PCR product, as well as offering the ability to detect other species of the genus *Listeria*. The presence of non-pathogenic *Listeria* species may indicate a critical control point failure that, on another occasion, may have resulted in contamination by *L. monocytogenes*.

MATERIALS AND METHODS

Bacteria used

Table 1 shows the bacteria used to assess the specificity of the PCR primers. Isolates were obtained from the New Zealand Reference Culture Collection Medical Section (ESR, Porirua, New Zealand). Isolate NCTC 7973 was used for experiments on the direct isolation of *Listeria* from ham. Inocula were obtained after incubation of this isolate for 24 h in Brain Heart Infusion (BHI) broth (Oxoid) at 30°C.

Polymerase chain reaction primer design

The listeriolysin O gene (*hly*) was chosen as a target for the *L. monocytogenes*-specific primers and the 23S rRNA gene for the *Listeria*-specific primers (Table 2). DNA sequences obtained from GenBank (http://ncbi.nlm.nih.gov/genbank) were aligned using the Wisconsin Package (GCG, Madison, WI, USA) and primers designed to identify isolates at the required taxonomic rank. Primers were supplied by Life Technologies (Rockville, MD, USA). The *L. monocytogenes*-specific primers produce a 706-bp product and the *Listeria*-specific primers one of 239 bp.

Nucleic acid extraction

Pure cultures and immunomagnetic beads. Cell pellets or trapped immunomagnetic beads were resuspended in 300 μl extraction buffer (0·025 mol l^{-1} TRIZMA base (Sigma, St. Louis, MO, USA), 0·010 mol l^{-1} EDTA and 0·050 mol l^{-1} glucose). Cell lysis was performed by the addition of 20 μl 50 mg ml^{-1} lysozyme for 5 min at room temperature. Sodium dodecyl sulphate (12 μl, 20%) and 4 μl 10 mg ml^{-1} proteinase K were added and incubation continued at 37°C for 30 min.

The lysed cells were extracted twice with an equal volume of phenol : chloroform : isoamyl alcohol (25 : 24 : 1; Sigma) by gently mixing for 10 min and then centrifuging for 15 min at 13 800 *g*. The organic layers were discarded and the remaining aqueous layer further extracted in an equal volume of chloroform : isoamyl alcohol (24 : 1). After gentle mixing, centrifugation and removal of the organic layer, a one-tenth volume of 3 mol l^{-1} sodium acetate (pH 5·2) and two volumes of absolute ethanol were added. DNA was precipitated by storage at –20°C overnight or for at least 1 h at –70°C and then recovered by centrifugation. The DNA was washed in 600 μl ethanol : water (70 : 30), the supernatant fluid removed and any remaining ethanol evaporated by gentle heating. Purified DNA was dissolved in milli Q (Millipore, Bedford, MA, USA) H_2O and stored at –20°C. For cells recovered by IMS the DNA was dissolved in 20 μl water.

The quantity of DNA and purity of the extracted sample were estimated by u.v. spectrometry and a 100 ng ml^{-1} working solution prepared.

Food enrichments. Enrichments were mixed briefly and transferred to sterile centrifuge tubes. Cells were harvested at 1500 *g* for 20 min at room temperature. Pellets were washed twice in 5 ml cold phosphate-buffered saline (PBS), recentrifuged and the pellet resuspended in 100 μl cold PBS. A volume (10 μl) of the cell suspension was added to 90 μl MilliQ water and then heated at 96°C for 12 min. Cell debris was removed by centrifugation and 10 μl of the supernatant fluid used in the PCR.

Amplification and polymerase chain reaction product detection

The PCR amplification was performed in a total reaction volume of 50 μl containing 0·050 mol l^{-1} KCl, 0·010 mol l^{-1} Tris HCl (pH 8·4), 0·025 mol l^{-1} $MgCl_2$, 5 μl 2 mg ml^{-1} bovine serum albumin, 5 pmol each primer, 200 μmol l^{-1} each dNTP, 2·5 U *Taq* DNA polymerase, 1 μl purified DNA (2 ng ml^{-1} final concentration), 10 μl heat-extracted DNA or the entire 20 μl from cells recovered by IMS. The PCR was conducted using a programmable DNA thermal cycler (Hybaid Omnigene; Hybaid, Ashford, UK) and subjected to 40 cycles of amplification.

The PCR was conducted as follows: 95°C denaturing for 1 min, 62°C annealing for 1 min and 72°C extension for 1 min over 40 cycles followed by a final 8-min extension step at 72°C.

The PCR products were analysed by gel electrophoresis using 2% agarose gels in TBE buffer (0·09 mol l^{-1}

Table 1 Bacteria used for primer verification

Bacterium	Source	PCR products	
		Genus	Species
Listeria monocytogenes serotype 1a	NZRM 2591, ATCC 19111	+	+
Listeria monocytogenes serotype 1a	NZRM 44, NCTC 7973	+	+
Listeria monocytogenes serotype 2	NZRM 2592, ATCC 19112, NCTC 5348	+	+
Listeria monocytogenes serotype 3a	NZRM 2594, ATCC 19113, NCTC 5105	+	+
Listeria monocytogenes serotype 4a	NZRM 2595, ATCC 19114, NCTC 5214	+	+
Listeria monocytogenes serotype 4c	NZRM 2596, ATCC 19116	+	+
Listeria monocytogenes serotype 4d	NZRM 2597, ATCC 19117	+	+
Listeria monocytogenes	NZRM 3312, ATCC 7646	+	+
Listeria monocytogenes	NZRM 3370, ATCC 49594	+	+
Listeria monocytogenes	NZRM 3384	+	+
Listeria monocytogenes	NZRM 3387	+	+
Listeria monocytogenes serotype 1/2a	NZRM 3449	+	+
Listeria monocytogenes serotype 1/2a	NZRM 3450	+	+
Listeria grayii	NZRM 1088^{T}	+	–
Listeria innocua	NZRM 3024, ATCC 33090	+	–
Listeria ivanovii	NZRM 797	+	–
Listeria seeligeri	NZRM 3287, ATCC 35967	+	–
Listeria welshimeri	NZRM 3286, NCTC 11857^{T}	+	–
Aerococcus urinae	NZRM 3583, ATCC 51268, NCTC 12142	–	–
Aerococcus viridans	NZRM 3204, ATCC 11563	–	–
Aeromonas hydrophila	ATCC 7966, NCTC 8049, NCIB 9240	–	–
Bacillus cereus	NZRM 5, ATCC 10702, NCTC 8035, NCIB 8122	–	–
Bacillus subtilis	NZRM 134, ATCC 6051, NCTC 3610, NCIB 3610	–	–
Bacillus thuringiensis	NZRM 2981	–	–
Brochothrix thermosphacta	NZRM 3320, ATCC 11509	–	–
Brochothrix campestris	NZRM 3569, ATCC 43754	–	–
Campylobacter jejuni	ATCC 33560	NT	–
Carnobacterium divergens	NZRM 3572, ATCC 35677, NCIMB 11952	–	–
Carnobacterium gallinarum	NZRM 3575, ATCC 49517	–	–
Carnobacterium mobile	NZRM 3576, ATCC 49516, DSM 4848, NCIMB 12847	–	–
Carnobacterium piscicola	NZRM 3571, ATCC 35586, NCIMB 2264	–	–
Enterobacter aerogenes	ATCC 13048, NCTC 10006	–	–
Enterococcus colombae	NZRM 3574, NCIMB 13013	–	–
Enterococcus faecalis	ATCC 19433, NCTC 775, NCDO 581	–	–
Enterococcus sulphureus	NZRM 3570, ATCC 49903, NCIMB 13117	–	–
Escherichia coli	ATCC 25922	–	–
Morganella morganii	ATCC 25380, NCTC 235	–	–
Rhodococcus coprophilus	ATCC 29080, NCTC 10994, DSM 43347^{T}	–	–
Pseudomonas aeruginosa	ATCC 25668, NCTC 10662	–	–
Salmonella menston	NRZM 383	NT	–
Shigella flexneri	NZRM 972	NT	–
Shigella sonnei	NZRM 86	–	–
Staphylococcus aureus	NZRM 917, ATCC 25923	–	–
Staphylococcus epidermidis	ATCC 12228	–	–
Vagococcus fluvialis	NZRM 3573, ATCC 49515, NCIMB 13038	–	–
Vagococcus salmonarium	NZRM 3577, ATCC 51200, NCIMB 13133	–	–
Yersinia enterocolitica	ATCC 9610	NT	–

ATCC, American Type Culture Collection; NCTC, National Collection of Type Cultures; NCIMB, National Collection of Industrial and Marine Bacteria; DSM, Deutsche Sammlung von Mikroorganismen; NZRM, New Zealand Reference Culture Collection, Medical Section;+, polymerase chain reaction (PCR) product detected; –, PCR product not detected; NT, not tested.

Table 2 Primer sequences used in the polymerase chain reaction

Primer	Specificity	Sequence (5′–3′)	bp	T_m (°C)	% GC
L318F	*Listeria*	GGGGAACCCACTATCTTTAGTC	22	67	50
L559R	*Listeria*	GGGCCTTTCCAGACCGCTTCA	21	70	61
310F	*L. monocytogenes*	GCCTGCAAGTCCTAAGACGCCAATC	25	71	56
1016R	*L. monocytogenes*	CTTGCAACTGCTCTTTAGTAACAGC	25	67	44

T_m, Melting temperature calculated by manufacturer; bp, base pairs.

TRIZMA, 0·09 mol l^{-1} boric acid, 0·002 mol l^{-1} EDTA, pH 8·0). Gels were run for 75 min at 100 V in TBE buffer containing 0·5 μg ml^{-1} ethidium bromide to enable visualization of the PCR products by u.v. transillumination. Molecular weight markers were included on each gel (123- or 100-bp DNA ladders; Life Technologies).

Determination of polymerase chain reaction sensitivity

Four foods (surimi crabmeat, marinated mussels, ham and farmhouse pâté) were purchased, blended, autoclaved at 121°C for 20 min and refrigerated overnight. Portions (25 g) of the foods were aseptically weighed into Whirl Pak bags (NASCO, Fort Atkinson, WI, USA). *Listeria monocytogenes* ATCC 19111 cells (1 ml) from 10^{-7} and 10^{-8} dilutions of an overnight broth culture were added to two separate portions of each food. Other portions served as uninoculated controls. Samples were homogenized by stomaching for 1–2 min in a Colworth 400 stomacher (A.J. Seward, London, UK). A volume (225 ml) of *Listeria* Enrichment Broth (Oxoid) was added to each bag and the contents again homogenized. The number of bacteria in the overnight culture used to prepare the dilution series was estimated by serial dilution and plating on Columbia Blood Agar containing 5% (v/v) sheep blood, followed by incubation at 37°C for 24 h and counting.

The food enrichments were incubated at 30°C for 4 h after which filter-sterilized stock solutions of nalidixic acid, acriflavine and cycloheximide were added to achieve final concentrations of 40, 10 and 50 mg l^{-1}, respectively. Incubation was continued for a further 44 h at 30°C. A 0·1 ml volume was then subcultured to 10 ml Oxoid UVMII (McClain and Lee 1988) and this broth incubated for 24 h at 35°C prior to PCR analysis.

Direct isolation of *Listeria monocytogenes* from ham

Anti-*Listeria* antibody-coated beads and a Magnetic Particle Concentrator (MPC®-M) were purchased from Dynal (Oslo, Norway). Prior to using the beads a 1-ml volume was washed twice in 1 ml PBS (pH 7·3) containing 0·5% (w/v) bovine serum albumin (BSA). The beads were resuspended in 1 ml PBS/BSA and stored at 4°C.

Ham was purchased from local supermarkets and cut into slices of approximately 5 g each. The slices were autoclaved and five slices aseptically transferred to Whirl Pak stomacher bags incorporating a filter barrier film to give a sample size of approximately 25 g. The bags were stored at –20°C until required and defrosted prior to use.

Strain NCTC 7973 was grown overnight in BHI broth at 30°C, harvested by centrifugation, resuspended in PBS twice and a tenfold dilution series prepared. Cell counts were performed by spread plating the dilution series onto PALCAM agar (Difco, Detroit, MI, USA) (Van Netten *et al.* 1989) followed by incubation at 37°C for 48 h and counting.

The 25 g packs of ham slices were inoculated from the same dilution series by distributing 0·5 ml of the suspension over the surface of the ham slices. The inoculated samples were incubated for 24 h at 14·6°C to allow the bacteria to attach and grow by approximately one log cycle, as predicted by the USDA/ARS Pathogen Modelling Program, Version 5.1 (www.arsercc.gov/mfs/pathogen.htm).

After incubation, 50 ml sterile 0·1% peptone water were added to each ham sample and the bag stomached for 2 min. The liquid portion was decanted (using the barrier film to retain large pieces of ham) into a sterile 50 ml centrifuge tube. Remaining particulate material was removed by centrifugation for 2 min at 1560 *g*. The supernatant fluid was recovered and decanted into another 50 ml tube and the cells recovered by centrifugation at 1560 *g* for 20 min. The pellet was resuspended in 700 μl sterile PBS/0·1% Tween 20 and this suspension transferred to a sterile 1·5 ml centrifuge tube to which 20 μl anti-*Listeria*-coated beads were added. The tube was gently rocked back and forth (approximately 10 cycles min^{-1}) on a platform for 10 min, the tube being turned once through 180° to prevent settling of the beads. Beads were trapped with the magnet and as much supernatant fluid as possible removed. Occasionally it was necessary to split the beads into two tubes and to add further PBS/Tween to allow the beads to separate from liquid which contained fine particulate matter. The beads were resuspended in 300 μl extraction buffer prior to phenol : chloroform extraction and PCR as described above.

The numbers of *L. monocytogenes* in supernatant fluids sampled before and after the 2 min centrifugation (to remove large particulate matter) and the 20 min centrifugation (to pellet the cells) were assessed by plate counts on PALCAM agar.

The above protocol was the end result of a number of experiments to assess the effect on recovery of cells from cell suspensions in PBS after different agitation methods (rocking platform *vs* 360° rotation on a turntable at 33 rev min^{-1}) and different numbers of washes after trapping (i.e. repeated magnetic trapping, aspiration and resuspension). After each trial the recovery was assessed by spread plating onto PALCAM agar followed by incubation at 37°C for 48 h and counting.

Initial experiments included a step where the stomached ham samples were treated with 50 ml of a chemical/enzyme mix and shaken (100 rev min^{-1}) for 20 min. The chemical/enzyme mixture included 0·1 mol l^{-1} NaCl, 0·02 mol l^{-1} $CaCl_2$, 0·1% Tween, 0·05 mg ml^{-1} collagenase, 0·05% (w/v) trypsin, 25 mg ml^{-1} glucose and 25 mg ml^{-1} mannose in 0·1% (w/v) peptone (Rodrigues-Szulc *et al.* 1996). After this treatment the samples were centrifuged and subjected to IMS as described above.

Uninoculated broth and uninoculated ham controls were carried through the entire process. At the DNA extraction step a reagent blank was included as well as a blank of unused antibody-coated beads. At the PCR amplification stage positive and negative controls were included.

RESULTS

Polymerase chain reaction primer specificity and sensitivity

The primers and PCR conditions described correctly identified 13 *L. monocytogenes* isolates and five other *Listeria* species tested. None of the other 29 species of bacteria tested (Table 1) contained DNA that yielded either PCR product under the conditions described. The multiplex PCR allowed the identification of genus and target species in one amplification.

Enrichment followed by PCR consistently detected *L. monocytogenes* when inoculated at 0·4 cfu g^{-1} or more into ham, pâté and surimi prior to enrichment. With marinated mussels detection could also be achieved at this level, but not consistently.

Development of the immunomagnetic separation protocol

Concentration of bacteria prior to immunomagnetic separation. Enumeration of *L. monocytogenes* in supernatant fluids sampled before and after the 2 min centrifugation (to remove large particulate matter) and the 20 min centrifugation (to pellet the cells) confirmed that no significant losses of cells were occurring in these two steps. The mean recovery after both centrifugations was 83% over five experiments, which was considered acceptable.

The use of barrier bags to retain large particulate material after stomaching was useful. They did not eliminate the need for the initial 2 min centrifugation, but did mean that the volume of suspension available for further processing was increased.

In our hands, treatment of inoculated ham samples with chemicals and enzymes developed for physicochemical separation of bacteria from meat surfaces (Rodrigues-Szulc *et al.* 1996) did not markedly increase the recovery of organisms beyond that which was achieved simply by stomaching with peptone water as a diluent (data not shown). In order to minimize the analysis time this treatment was not used.

Optimizing immunomagnetic separation. The number of beads in the 20 μl added, as indicated by the manufacturer, was $1{\cdot}4 \times 10^7$ which would have been in excess of the number of cells in any of the experiments. It was found that very gentle mixing (on a rocking platform) during binding gave better recoveries than more vigorous agitation (data not shown). Initial trials used three repeated washings in order to remove possible PCR inhibitors derived from the ham. However, it was found that this number of washing steps resulted in an unacceptable rate of recovery. Typically, recoveries were reduced by approximately 50% for each washing of the beads meaning that, after three washes, the recovery was only of the order of 1%. A single aspiration after trapping, followed by immediate resuspension in PCR extraction buffer was adopted to minimize these losses, but still only gave a recovery of the order of 10–20%. This compromise between removal of potential PCR inhibitors and retention of cells produced material that could be successfully amplified.

Experiments without the use of bead trapping and washing of cells were unsuccessful (Fig. 1). In these experiments the inoculated ham slices were simply homogenized and centrifuged twice as described above and the DNA extracted from the second pellet. Amplification failure was probably due to the presence of PCR inhibitors from the ham. Consequently, although the recovery of cells by IMS was not optimal, inclusion of IMS was essential to provide DNA suitable for amplification.

Characteristics of the combined polymerase chain reaction/immunomagnetic separation method

Following refinement of the sample treatment, DNA extraction and PCR amplification protocols it was possible to observe consistently distinct PCR products from *L. monocyto-*

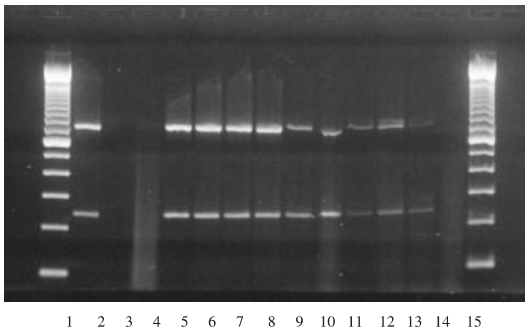

Fig. 1 Polymerase chain reaction amplicons generated from serial dilutions of *Listeria monocytogenes* onto ham samples. Lane(s): 1, size ladder (100 bp); 2, positive control; 3, negative control; 4, pellet DNA amplified without immunomagnetic separation; 5 and 6, duplicate samples 1000 cfu g^{-1}; 7 and 8, duplicate samples 100 cfu g^{-1}; 9 and 10, duplicate samples 10 cfu g^{-1}; 11 and 12, duplicate samples 1 cfu g^{-1}; 13 and 14, duplicate samples 0·1 cfu g^{-1}; 15, size ladder (100 bp)

genes in 25-g samples containing as few as 1–2 cfu g^{-1} although, as these counts were made at the start of the isolation process (i.e. prior to the centrifugation steps), it is possible that some growth of cells could have occurred during the sample processing prior to IMS and DNA isolation.

The experiment was repeated on three occasions. In the first two trials calculated recoveries were 1·8 and 1·1 cfu g^{-1} *L. monocytogenes* detected in both of the duplicate samples. In the latter, one of the two duplicates containing 0·1 cfu g^{-1} was also positive. In the third trial 57 cfu g^{-1} were detected in both samples and 5·7 cfu g^{-1} in only one.

The final protocol developed from these experiments is shown schematically in Fig. 2. A typical gel from a dilution series of cells is shown in Fig. 1. The time sequence for this analysis included stomaching, centrifugation, IMS, DNA extraction and precipitation on the first day. The PCR amplification was performed overnight and gel electrophoresis the next morning. Overall the time for the analysis was approximately 24 h.

DISCUSSION

A number of methods have been published for the rapid detection of *L. monocytogenes* (e.g. Carroll *et al*. 2000; Entis and Lerner 2000; Firstenberg-Eden and Shelef 2000), but the requirement to confirm positive results adds to the time taken. A 5–6-h method using PCR followed by ELISA detection has been described, but was applied only to broth cultures, and did not address isolation or enrichment of cells from food samples (Scheu *et al*. 1999). Methods not using enrichment tend to require more cells to be present before detection is achieved, for example Li *et al*. (2000) reported a limit of detection of 40 cfu ml^{-1}.

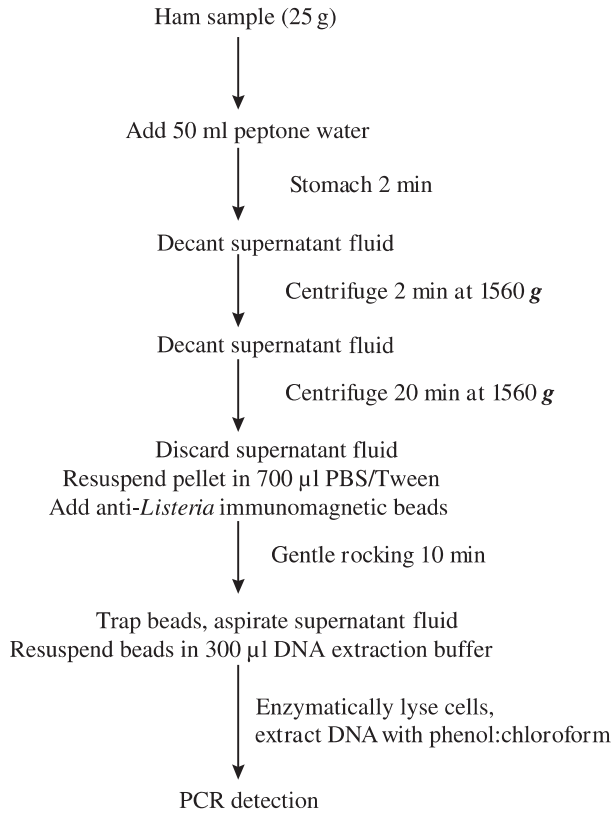

Fig. 2 Schematic representation of immunomagnetic separation/polymerase chain reaction (PCR) protocol for the detection of *Listeria monocytogenes* in ham. PBS, Phosphate-buffered saline

None of these methods have yet achieved detection and identification of *L. monocytogenes* present at 1 cell g^{-1} within 24 h and this was the aim of the work presented here.

In the development of our IMS/PCR method the intention was to mimic real samples as closely as possible. It is possible that autoclaving the ham prior to incubation altered the matrix, but this was essential to eliminate any autochthonous *Listeria* present. The 24-h preisolation incubation, which resulted in a 2–11 fold increase in cell numbers, should have been sufficient to allow the inoculum to adhere to the matrix and more closely represent a 'naturally' contaminated food. The kinetics of adherence of *L. monocytogenes* to food contact surfaces such as stainless steel or rubber (Smoot and Pierson 1998a, b) indicate that adherence reaches a maximum after about 2 h. There are few data on the kinetics of attachment of *L. monocytogenes* to food surfaces, although one study did indicate that actively growing cells appear to adhere more readily than those in stationary phase (Dickson 1991). Sub-optimal growth at 14·6°C also allowed flagellum formation which is required for antibody binding.

Although the chemical/enzyme treatment did not improve recovery from the ham, this may not be the case for other food matrices.

A recovery of 1–2 cfu g^{-1}, as achieved in two complete experiments, is comparable to that achieved by Uyttendaele *et al.* (2000) using a similar protocol for the recovery of *L. monocytogenes* from cheese. The lower sensitivity in the third complete run might be explained by the low inoculum levels being used allowing for some samples to remain uninoculated. For example, a calculated 0·4 cfu was added to a pair of samples of which only one yielded a PCR product.

It must be acknowledged that the method as described could possibly detect non-viable intact *Listeria* cells. A study of the use of IMS to isolate *Escherichia coli* O157:H7 (Uyttendaele *et al.* 1999) indicated that two washing steps circumvented the detection of heat-killed cells, presumably because the heat-killed cells were no longer captured by the immunomagnetic beads. For dead cells to be detected, DNA will have to be retained within the cell, which will also need to retain functional antigens. Our method uses only a single wash as otherwise recovery was poor, and this highlights the need to improve the recovery of cells in the IMS step. Additional washing steps would also function to increase the removal of potential PCR inhibitors in residual food material. Work to investigate the ability of IMS to discriminate between dead and viable *Listeria* cells is planned.

These experiments focused on the use of the method to detect *L. monocytogenes* in ham. Since the immunomagnetic beads are claimed by the manufacturer to capture all *Listeria* serotypes and we have used a PCR which includes a primer pair to detect *Listeria* spp., it seems likely that the combined IMS/PCR approach would be able to detect all *Listeria* species even if it is only able to identify one species within the genus.

Improvements in detection time could be achieved through the use of rapid DNA isolation and purification protocols, rather than the lengthy phenol : chloroform procedure. The use of real-time PCR, where amplification and detection occur simultaneously, could further decrease analysis time. A 24-h real-time PCR test for *L. monocytogenes* in dairy samples has been described (Cox *et al.* 1998). As this protocol included 20 h for enrichment, an IMS/real-time PCR analysis that takes one working day seems a realistic aspiration. Options to reduce the time taken to perform the assay are under investigation in our laboratory, as is the extension of the method to other food matrices.

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