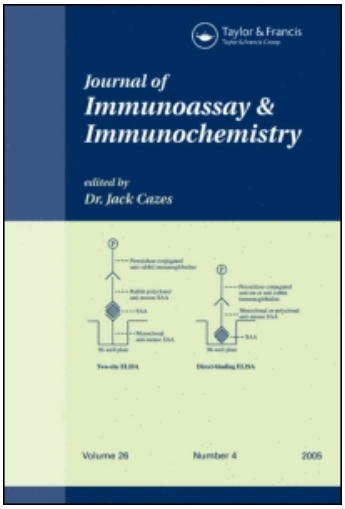

Journal of Immunoassay and Immunochemistry

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597271

Detection of *Salmonella* Typhimurium in Raw Meats using In-House Prepared Monoclonal Antibody Coated Magnetic Beads and PCR Assay of the *fimA* Gene

Ângela N. Moreira[ab]; Fabricio R. Conceição[ac]; Rita de Cássia S. Conceição[bc]; Roberta J. Ramos[ab]; José B. Carvalhal[b]; Odir A. Dellagostin[a]; José A. G. Aleixo[ab]

[a] Centro de Biotecnologia, Universidade Federal de Pelotas, Pelotas, RS, Brazil [b] Faculdade de Nutrição, Universidade Federal de Pelotas, Pelotas, RS, Brazil [c] Faculdade de Veterinária, Universidade Federal de Pelotas, Pelotas, RS, Brazil

To cite this Article Moreira, Ângela N. , Conceição, Fabricio R. , Conceição, Rita de Cássia S. , Ramos, Roberta J. , Carvalhal, José B. , Dellagostin, Odir A. and Aleixo, José A. G.(2008) 'Detection of *Salmonella* Typhimurium in Raw Meats using In-House Prepared Monoclonal Antibody Coated Magnetic Beads and PCR Assay of the *fimA* Gene', Journal of Immunoassay and Immunochemistry, 29: 1, 58 — 69

To link to this Article: DOI: 10.1080/15321810701735096

URL: http://dx.doi.org/10.1080/15321810701735096

Journal of Immunoassay & Immunochemistry, 29: 58–69, 2008

ISSN 1532-1819 print/1532-4230 online
DOI: 10.1080/15321810701735096

Detection of *Salmonella* Typhimurium in Raw Meats using In-House Prepared Monoclonal Antibody Coated Magnetic Beads and PCR Assay of the *fimA* Gene

Ângela N. Moreira,[1,2] Fabricio R. Conceição,[1,3] Rita de Cássia S. Conceição,[2,3] Roberta J. Ramos,[1,2] José B. Carvalhal,[2] Odir A. Dellagostin,[1] and José A. G. Aleixo[1,2]

[1]Centro de Biotecnologia, Universidade Federal de Pelotas, Pelotas, RS, Brazil
[2]Faculdade de Nutrição, Universidade Federal de Pelotas, Pelotas, RS, Brazil
[3]Faculdade de Veterinária, Universidade Federal de Pelotas, Pelotas, RS, Brazil

Abstract: A method for detection of *Salmonella* Typhimurium in meat samples that uses in-house monoclonal antibody (MAb) coated magnetic beads for immunomagnetic separation (IMS) associated with PCR amplification of the gene *fimA* was developed. An internal amplification control (IAC) of the PCR reaction was constructed. The *fimA* PCR has shown 100% sensitivity and specificity when tested with various bacteria. The detection limit of the IMS-PCR method, using a post-enrichment in BHI broth for 6 h between IMS and PCR, was 1–10 CFU/mL. The method proved to be rapid (27 hrs), highly sensitive (1–10 CFU/25 g), and specific for detection of *S*. Typhimurium from experimentally contaminated pork and chicken meat samples.

Keywords: *Salmonella*, Foods, Rapid methods, PCR, IMS, IAC

Correspondence: Dr. José A. G. Aleixo, Centro de Biotecnologia, Universidade Federal de Pelotas, Campus Universitário, s/nº, Pelotas, RS, CEP 96010-900, Brazil. E-mail: biotjaga@ufpel.edu.br

INTRODUCTION

The conventional culture method for detection of *Salmonella* in foods is laborious and requires at least 4 days to obtain negative results, and 6 to 7 days to identify and confirm *Salmonella*-positive samples.[1] This long time of analysis is due to the requirement of a pre-enrichment, selective enrichment, selective plating, and confirmation of presumptive *Salmonella* colonies by biochemical and serological tests. Many novel rapid methods have been developed in an effort to replace traditional techniques by combining diverse capture and/or detection technologies to diminish total assay time.

The polymerase chain reaction (PCR) which is used for DNA amplification and detection is a rapid technique whose specificity and sensitivity depend on the DNA sequence selected. Sequences present only in the target microorganism must be chosen for amplification. Although different PCR assays for *Salmonella* detection have been developed, most of them do not contain an internal amplification control (IAC), a prerequisite in PCR standardization since it allows identification of false-negative results caused by PCR inhibitors,[2] and have limitations on sensitivity or specificity.[3–5] PCR sensitivity may be reduced dramatically when it is applied to complex biological samples such as food. This occurs due to the presence of a number of components commonly found in foods, such as lipids, salts, proteins, DNA, and cells other than the target organism, which inhibit or reduce the amplification. Therefore, the removal of these inhibitory components in the preparation of samples by immunomagnetic separation (IMS) and the use of an IAC in order to prevent false negative results is important.

IMS is a technique of separation and concentration of specific microorganisms that has been used for improving sensitivity of detection methods of pathogenic bacteria in foods and reducing the total analysis time. This technique increases the sensitivity of the PCR because it concentrates the target cells and separates them from food samples containing inhibitory components and competitive microbiota.[6]

Products of animal origin, such as chicken and pork meats, are among the foods most commonly implicated in food borne infections caused by *Salmonella enterica*, and *S. enterica* serovar Typhimurium is one of the serovars more frequently involved in these infections[7] and in outbreaks caused by multi-drug resistant salmonellae.[8]

In this study, we report on the use of in-house prepared MAb coated magnetic beads and primers to the *fimA* gene from *Salmonella* to develop a method that combines IMS and PCR for detection of *S.* Typhimurium in meat samples. To prevent false-negative results, an IAC was constructed. The result was a detection method which is highly sensitive and specific, which allows a significant reduction in the length of time normally required for detection of this microorganism.

EXPERIMENTAL

Bacterial Growth Conditions and DNA Extraction

Salmonella strains of 23 different serovars (Paratyphi, Agona, Derby, Typhimurium, Heidelberg, Saint Paul, Mbandaka, Oranienberg, Choleraesuis, Infantis, Hadar, Emek, Albany, Enteritidis, Gallinarum, Dublin, Panama, Anatum, Newington, Senftenberg, Rubislaw, Worthington and Florida), and 9 other bacteria (*Proteus vulgaris*, *Enterobacter aerogenes*, *Klebsiella pneumoniae*, *Serratia marcescens*, *Citrobacter freundii*, *Escherichia coli*, *Shigella sonnei*, *Edwardsiella tarda*, and *Pseudomonas aeruginosa*) were obtained from the culture collection of Fundação Oswaldo Cruz, Rio de Janeiro, RJ, Brazil and EMBRAPA–CNPSA, Concórdia, SC, Brazil. For the experiments, bacteria were grown on brain heart infusion (BHI) broth at 37°C with constant shaking (150 rpm) and the cellular densities were adjusted to 0.9 at 600 nm. The actual number of cells used in the experiments was determined by viable cell plate counts.

Genomic DNA was extracted from BHI cultures according to instructions of the PureGene® DNA purification kit (Gentra Systems, USA). For extraction of bacterial DNA from enrichment broth of meat samples, aliquots of 1 mL were centrifuged at 15,000 × g for 8 min, the cells were washed with sterile 0.01 M phosphate-buffered saline (PBS, pH 7.2), and the DNA was extracted by heating at 95°C for 15 min in 20 μL of lysis solution (1:1 of 0.125% SDS and 0.05 M NaOH). In the experiments where lysis followed IMS, 20 μL of the lysis solution was directly added to beads in microtubes.

Selection of the Oligonucleotide Primers

The *Salmonella*-specific sequence of nucleotides chosen for amplification by PCR was the *fimA* gene of *S.* Enteritidis (GenBank accession number S76043, 566 bp). This sequence was selected after an extensive bibliographic revision and comparison to sequences of salmonellae and other bacteria available in DNA databanks (GenBank, EMBL) using the software BLAST N (National Center for Biotechnology Information-NCBI). Primers (forward 5′ GGGGGATCCATGAAACATAAATTAATGACCT 3′ and reverse 5′ CCGAATTCTCACATGATAAAGGTGG-CG 3′) were designed with the aid of Vector NTI (Invitrogen®, USA) and synthesized by MWG-Biotech (Los Angeles, CA, USA).

Development of IAC

An IAC was developed according to the strategy described by Sachadyn and Kur[9] to amplify a fragment of 850 bp in the same reaction of the *fimA* gene. The IAC DNA consisted of a fragment of 810 bp of the pGAPZαA vector

(Invitrogen®, USA) flanked by target sequences of the FimA primers. Chimera primers (forward 5′ ATGAAACATAAATTAATGACC*GCAATAA-TAGCGGGCGGAC* 3′ and reverse 5′ TTTCATGATAAAGGTGGCG*CAAA CCCCTA-CCACAAGATAT* 3′), which possess 5′ overhanging ends with identical sequences to the FimA primer sequences (underlined sequence) and 3′ ends complementary to a DNA sequence of pGAPZαA vector (Invitrogen, USA) (italic sequence), were designed and used to amplify the DNA from this vector. The PCR product was purified according to the instructions of the GFX™ PCR DNA and gel band purification kit (Amersham Biosciences®, UK) and used as template in a second PCR using the FimA primers. This second PCR product was purified and cloned into PCR®2.1-TOPO® plasmid (Invitrogen®, USA) to create the PCR®2.1-TOPO®/IAC plasmid. Plasmid DNA was used to transform electrocompetent *Escherichia coli* TOP10 cells, extracted according to instructions of GFX™ Micro plasmid prep kit (A mersham Biosciences®, UK) and used as IAC in the *fimA* PCR assay.

The concentration of IAC (PCR®2.1-TOPO®/IAC) was estimated spectrophotometrically at 260 nm and the optimal concentration for use in the *fimA* PCR was determined by titration studies. First, the lowest reproducible concentration was determined using decimal dilutions of IAC (50 to 0.05 pg) as template DNA in a PCR with FimA primers. Then, different concentrations of IAC (3, 2, 1, 0.5, and 0.1 pg), as defined by previous PCR, were amplified in the presence of 100 pg of DNA from *S.* Typhimurium.

PCR

One microliter of bacterial DNA was added to 24 μL of the amplification mixture containing 1.5 mM $MgCl_2$, 25 pmol of each primer, 200 μM of each dNTP, 1U of *Taq* DNA polymerase, 1 × PCR buffer and 0.5 pg of IAC. Reactions with and without DNA from *S*. Typhimurium were used as positive and negative controls, respectively. Amplification was carried out in a thermocycler Mastercycler Gradient (Eppendorf, Germany) using a temperature program consisting of initial denaturation for 5 min at 95°C; 35 cycles of denaturation for 30 s at 95°C; annealing of primers for 30 s at 60°C; primer extension for 30 s at 72°C; and a final extension for 7 min at 72°C. Amplified products were detected by gel electrophoresis on 0.8% agarose gels containing ethidium bromide. Results were considered positive when bands of 566 and 850 bp, or only the first one, were visualized under UV light, and negative when only the latter was seen. When neither the IAC nor the target DNA was amplified, it was assumed that inhibition of the PCR has occurred.

PCR Detection Limits, Sensitivity, and Specificity

First, genomic DNA from *S*. Typhimurium was diluted with sterile 10 mM Tris-1 mM EDTA (TE, pH 8.0) to concentrations ranging from 30 to

0.75 pg/μL and used to assess the lowest concentration of DNA required to detect the *fimA* gene by PCR. Then, bacterial DNA from 1 mL of decimal dilutions (10^0 to 10^9 CFU/mL) from a 6 h culture of S. Typhimurium was used to assess the detection limit of the PCR assay with *Salmonella* cells. Briefly, cells from 1 mL of each dilution were spun down (15,000 × g for 8 min), washed with PBS, and suspended in 20 μL of lyses solution for DNA extraction. These experiments were repeated three times. Finally, genomic DNA from salmonellae of different serogroups and of other bacteria were used as templates to assess the sensitivity and the specificity of the PCR.

IMS

A volume of 20 μL of in-house prepared *S.* Typhimurium-specific MAb-coated magnetic beads[10] was added to microtubes containing 1 mL aliquots from a *S*. Typhimurium culture or from meat samples enriched in buffered peptone water (BPW). The microtubes were incubated at room temperature for 15 min with continuous shaking. The beads-antigen complex was separated from the liquid phase, concentrated on the tube wall during 3 minutes using a magnetic concentrator (MPC-S, Dynal, Norway), and washed three times with 1 mL of PBS with 0.05% of Tween 20 (PBST, pH 7.4) to remove food debris and other microorganisms. The *S*. Typhimurium cells attached to beads were either resuspended in 20 μL of lysis solution to extract DNA for PCR or post-enriched in BHI broth.

Detection Limit of the IMS-PCR Method

To determine the lowest number of *Salmonella* required for detection of the *fimA* gene by PCR after IMS, beads were added to two aliquots of 1 mL of decimal dilutions (10^0 to 10^9 CFU/mL) from a 6 h culture of *S.* Typhimurium, in triplicate, and the IMS was performed as described above. The beads from the first aliquot were suspended in 20 μL of lysis solution for DNA extraction and PCR, and those from the second aliquot were suspended in 1 mL of BHI broth and submitted to a post-enrichment for 6 h at 37°C before PCR. This experiment was repeated three times.

IMS-PCR with Experimentally Contaminated Meat Samples

To verify how the natural flora of meats affects the performance of the IMS-PCR method, samples of pork and chicken meats obtained from local retail suppliers and confirmed to be negative for salmonellae by conventional

detection methodology[1] were experimentally contaminated with 1–10, 10–100 and 100–1000 CFU of *S.* Typhimurium and subjected to three different enrichment protocols before IMS and PCR (protocols 1, 2, and 3, Table 1). Fifteen milliliters of BPW from each protocol were centrifuged at 2,000 × g for 2 min to remove food debris and the supernatant was further centrifuged at 10,000 × g for 8 min to harvest cells. The cell pellets were suspended in 1 mL of PBS, 20 μL of MAb-coated beads was added, and the IMS was performed as described above. Beads from protocols numbers 2 and 3 were then suspended in 1 mL of BHI broth for a post-enrichment step. At the end of this step, BHI broth was centrifuged at 15,000 × g for 10 min and the pellets obtained, as well as the beads from protocol number 1, were suspended in 20 μL of lysis solution for DNA extraction. To confirm the need for the IMS step, the three enrichment protocols were also carried without IMS before PCR (protocols 4, 5, and 6, Table 1). Twenty-five grams of meat, at each level of contamination, was also analyzed by conventional methodology. This experiment was repeated three times.

Table 1. Enrichment protocols for detection of *S.* Typhimurium in samples of Experimentally contaminated meats by IMS-PCR

Protocol number	Pre-enrichment[a] (h)	IMS[b]	Post-enrichment[c] (h)	Total time of analysis (h)
1	16–18	+	−	19–21
2	16–18	+	6	25–27
3	8	+	16–18	27–29
4	16–18	−	−	19–21
5	16–18	−	6	25–27
6	8	−	16–18	27–29

[a]Buffered peptone water (BPW) at 37°C.
[b]Immunomagnetic separation: + yes, − no.
[c]BHI broth at 37°C.

RESULTS

Development of IAC

The optimal concentration of the PCR®2.1-TOPO®/IAC for use in the PCR was 0.5 pg per 25 μL of reaction (Fig. 1). This concentration of IAC did not affect the detection limit of DNA by PCR since the intensity of the PCR products amplified from different concentrations of *S.* Typhimurium DNA was the same either in the absence or presence of IAC (Fig. 2).

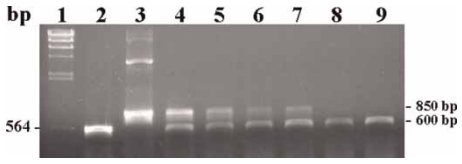

Figure 1. Determination of the optimal IAC concentration for PCR. Agarose gel electrophoresis of the PCR products amplified with DNA from *S.* Typhimurium (ST, 100 pg) and different concentrations of IAC. Lanes: 1-λ*Hind*III; 2-Positive control; 3-IAC only; 4-IAC 3 pg; 5-IAC 2 pg; 6-IAC 1 pg; 7-IAC 0.5 pg; 8-IAC 0.1 pg; 9-ST DNA.

PCR Detection Limits, Sensitivity, and Specificity

The detection limit of the PCR using pure DNA was 1.5 pg per 25 μL of reaction mixture and was not affected by presence of 0.5 pg of DNA from IAC (Fig. 2). The detection limit of the PCR with *S.* Typhimurium cells was 10^4 CFU/mL or 500 CFU in the reaction mixture (data not shown). A fragment of approximately 566 bp was amplified from DNA of all salmonellae tested. On the other hand, when DNA from other bacteria was used as template, there was amplification of the IAC only (data not shown).

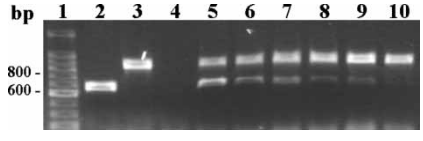

Figure 2. Detection limit of DNA by PCR. Agarose gel electrophoresis of the PCR products amplified with different concentrations of DNA from *S.* Typhimurium in the presence of IAC DNA (0.5 pg). Lanes: 1-100 pb DNA Ladder; 2-Positive control; 3-IAC only; 4-Negative control; 5-ST DNA 30 pg; 6-ST DNA 15 pg; 7-ST DNA 7.5 pg; 8-ST DNA 3 pg; 9-ST DNA 1.5 pg.

Detection Limit of the IMS–PCR Method

The detection limit of the IMS associated to PCR was 10^4 CFU/mL. This limit was reduced to 1–10 CFU/mL when a 6 h post-enrichment in BHI broth was introduced between IMS and PCR (data not shown).

IMS-PCR with Experimentally Contaminated Meat Samples

Protocols that included IMS were more sensitive than those that did not include IMS (Fig. 3). The time length of the protocols 1, 2, and 3, which

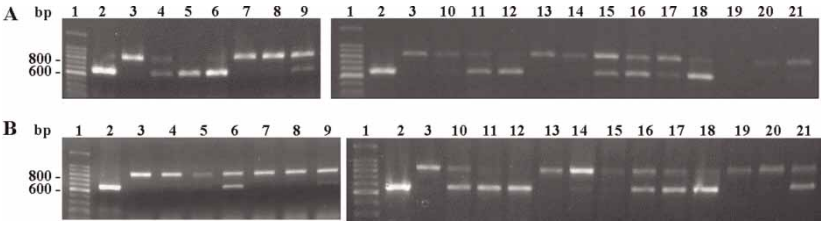

Figure 3. Detection limit of *S.* Typhimurium in experimentally contaminated meat samples by IMS associated to PCR. Agarose gel electrophoresis of PCR products amplified with DNA extracted from different enrichment protocols of experimentally contaminated chicken meat (A) and pork meat (B). Lanes: 1-100 pb DNA Ladder; 2-Positive control; 3-IAC only; 4, 5, and 6-Protocol 1; 7, 8, and 9-Protocol 4; 10, 11 and 12-Protocol 2; 13, 14, and 15-Protocol 5; 16, 17, and 18-Protocol 3; 19, 20, and 21-Protocol 6. The lanes in each protocol were from samples contaminated with 1 to 10, 10^2 to 10^3, and 10^2 to 10^3 CFU/mL, respectively.

use IMS, was of approximately 20, 26, and 28 h, respectively. All samples were positive by conventional methodology, but 48 h of incubation at 37°C were required to observe characteristic colonies in the XLD and BPLS agar plates, and the number was extremely low, making the total time of analysis approximately 6 days (144 h).

DISCUSSION

A rapid method for the detection of *S.* Typhimurium in meat samples based on IMS with in-house MAb-coated magnetic beads associated with PCR amplification of the *fimA* gene was developed. *Salmonella*-specific genes coding for surface antigens were initially selected with the aim of cloning them in a eukaryotic expression vector for use in production of MAbs by genetic immunization. Because primers have shown high specificity and sensitivity in preliminary tests, they were evaluated for use in the development of a PCR to detect salmonellae in foods.

The *fimA* gene encodes the major subunit of *Salmonella* type 1 fimbriae.[11] It has already been shown that all *Salmonella* serovars possess a closely related *fimA* gene, even though some strains produce type 2 antigenically unrelated fimbriae or do not produce fimbriae at all.[12–14] Only short regions of this gene are conserved among other members of the *Enterobacteriaceae*.[12] Our alignment studies also have shown a high degree of identity of the *fimA* gene of *S.* Enteritidis with target regions of several salmonellae, such as *S.* Typhimurium, *S.* Paratyphi, *S.* Pullorum, *S.* Diarizone e *S.* Typhi, and low similarity with other bacteria.

Other studies designed primers to amplify small internal fragments of the *fimA* gene, but they have shown limitations. Doran et al.,[12] who

sequenced the *S.* Enteritidis *fimA* gene used in the present study, designed three pairs of primers based on the alignment of *fimA* sequences from *S.* Enteritidis, *S.* Typhimurium, *E. coli*, *K. pneumoniae*, and *S. marcescens*. One of these primers yielded a false-positive response with *C. freundii* and another yielded non-specific bands with DNA from other bacteria. In addition, these primers were used in association with other pairs of primers (to gene *tctC*) and were not evaluated with food samples. Cohen et al.[14] designed primers to amplify regions of *S.* Typhimurium *fimA* gene (GenBank accession number M18283) based on the alignment of this gene with only *E. coli* and *K. pneumoniae* strains, besides other *Salmonella*. The PCR developed in the present study did not show non-specific bands with DNA from *Salmonella*, or a band of target size with DNA from other closely related organisms likely to be present in food samples. Herewith, it was concluded that the primers evaluated in this study are specific to the salmonellae tested and suitable for detecting these bacteria by PCR.

In order to prevent false-negative results that might be caused by inhibitory substances present in the sample, an IAC is required when detecting bacteria in foods by PCR. The presence of an IAC product in the absence of the target product allows the differentiation between true and false-negative results. In this study, an IAC construction was amplified with the same pair of primers used to amplify the target DNA. This strategy, known as a competitive method, due to competition between target and IAC DNA for reaction components, allows amplification of both DNA under the same conditions avoiding the risk of undesirable interactions between primers.[15] Another advantage of this methodology is that there is no formation of heteroduplexes between products of target DNA and IAC because the sequence of the IAC internal to primers is completely different from the target sequence.[9,16] Also, since, in theory, the PCR reaction kinetics are driven towards the smaller product, the IAC product was designed to be larger than the target product.[9] The IAC sequence was cloned in a plasmid that was stored in *E. coli* cells. Plasmids can be safely stored in convenient quantities for long periods using this method, allowing better control of stability, size, and copy number and guaranteeing the quality and continuous availability of the IAC.[15]

The concentration of IAC is critical for the reliability of PCR assays. Too much IAC will compete with the target DNA inhibiting its amplification and hiding weak inhibition of extremely low concentrations of target DNA, resulting in an increase in the detection limit or in false-negative results.[17] The optimal concentration of the IAC established in this study was similar to those of other PCR for detection of salmonellae.[18,19] The detection limit of purified DNA by *fimA* PCR was not influenced by the presence of the IAC and was better than that found by Cohen et al.[14] using a different pair of primers. The detection limit of *S.* Typhimurium was determined from a 6 h culture of *S.* Typhimurium in BHI broth to

ensure a population in logarithmic growth, thus avoiding the amplification of dead cells.

The detection limit of the IMS-PCR method (10^4 CFU/mL) suggest that the IMS, using in-house prepared *S.* Typhimurium-specific MAb-coated magnetic beads, was efficient for concentrating and separating *S.* Typhimurium from liquid cultures. Although the same detection limit was obtained when using PCR only, the amplicons were more intense when PCR was associated to IMS. Nevertheless, this detection limit can still be considered very high and to decrease the detection limit of the method to a level ranging from 1 to 10 CFU/mL, an additional enrichment of 6 h in BHI broth between IMS and PCR was necessary. Other studies compared PCR sensitivity for detection of *Salmonella* after IMS, centrifugation or filtration of the enrichment broth, and the best results were obtained using IMS.[20,21]

The influence of natural flora on the performance of the IMS associated to *fimA* PCR was investigated using pork and chicken meats experimentally contaminated with different numbers of *S.* Typhimurium cells and six enrichment protocols. Protocols including IMS were more sensitive than those that did not include it, suggesting that IMS not only enables recovery of *S.* Typhimurium from meat samples, but also separates this bacterium from inhibitory substances and other bacteria that may greatly affect the detection limit of PCR assays and produce false negative results. Among the enrichment protocols tested, those including IMS and a post-enrichment period, (numbers 2 and 3, Table 1), were the ones showing more clear bands and the highest sensitivities. These protocols enabled detection of 1–10 CFU of *Salmonella* in 25 g of meats, result similar to those reported in other studies[21–23] and better than the one obtained by Civilini et al.[24] A minimum incubation time of 16 hours was required to obtain a number of *Salmonella* cells detectable by PCR.[21] Likewise, PCR-based commercial systems for detection of *Salmonella*, such as Probelia™ (Sanofi Diagnostics Pasteur, Marnes La Coquette, France), TaqMan™ (Perkin Elmer, Norwalk, CT, USA) and Bax™ (Qualicon, Wilmington, DE, USA), suggest a pre-enrichment time of 16–20 h before PCR. Therefore, protocol 2 that includes 16–18 h pre-enrichment in BPW at 37°C, IMS, and 6–8 h of post-enrichment in BHI broth at 37°C, was considered having the combination of enrichments appropriate to achieve the detection limit of the conventional method and an acceptable total analysis time of 25–27 hrs. The long pre-enrichment allows *Salmonella* cells to recover from sublethal damage and increase in number so that IMS is effective, and the short post-enrichment is sufficient to increase cell numbers to a level detectable by PCR.

The total time of the IMS-PCR method developed in the present study was approximately 26 h. Therefore, the method saves at least 5 days of work in comparison with the conventional methodology. The results of its evaluation in the detection of S. Typhimurium from experimentally contaminated meats demonstrated that the method is rapid, effective, sensitive,

specific, and reliable, and has potential for detecting this bacterium in various food matrices.

ACKNOWLEDGMENTS

This work was supported by a research grant from the Secretaria da Ciência e Tecnologia do Estado do Rio Grande do Sul (SCT no. 41/01). Author ANM was recipient of a scholarship from CAPES, Ministry of Education, Brazil.

REFERENCES

1. Andrews, W.H.; June, G.A.; Sherrod, P.; Hammack, T.S.; Amaguana, R.M. Salmonella. In *Food and Drug Administration Bacteriological Analytical Manual*, 8th Edn.; Merker, R.L. Ed.; AOAC International: Gaithersburg, 1998.
2. Anonymous. Microbiology of food and animal feeding stuffs. *Polymerase Chain Reaction (PCR) for the Detection of Food-borne Pathogens*; General method specific requirements (EN ISO 22174). Intl. Org. Standardizn. 2002, Geneva, Switzerland.
3. Gooding, C.M.; Choudary, P.V. Comparison of different primers for rapid detection of *Salmonella* using the polymerase chain reaction. Mol. Cell. Probes **1999**, *13*, 341–347.
4. Malorny, B.; Hoorfar, J.; Hugas, M.; Heuvelink, A.; Fach, P.; Ellerbroek, L.; Bunge, C.; Dorn, C.; Helmuth, R. Interlaboratory diagnostic accuracy of a *Salmonella* specific PCR-based method. Int. J. Food Microbiol. **2003**, *89*, 241–249.
5. Ziemer, C.J.; Steadham, S.R. Evaluation of the specificity of *Salmonella* PCR primers using various intestinal bacterial species. Lett. Appl. Microbiol. **2003**, *37*, 463–469.
6. Španová, A; Rittich, B.; Horák, D.; Lenfeld, J.; Prodelalová, J.; Suciková, J.; Strumcová, S. Immunomagnetic separation and detection of *Salmonella* cells using newly designed carriers. J. Chromatogr. A **2003**, *1009*, 215–221.
7. Centers for disease control and prevention (CDC). PHILIS surveillance data: *Salmonella* Annual Summaries [online]. Atlanta, CDC **2004**. Available from: http://www.cdc.gov/ncidod/dbmd/philsdata/salmonella.htm.
8. Humphrey, T. Review: *Salmonella* Typhimurium definitive type 104. A multi-resistant *Salmonella*. Intl. J. Food Microbiol. **2001**, *67*, 173–186.
9. Sachadyn, P.; Kur, J. The construction and use of a PCR internal control. Mol. Cell. Probes **1998**, *12*, 259–262.
10. Moreira, ÂN.; Conceição, F.R.; Conceição, R.C. S.; Goularte, F.L.; Carvalhal, J.B.; Dellagostin, O.A.; Aleixo, J.A. G. Monoclonal antibodies against serogroup B salmonellae: production, characterization and use in a sandwich ELISA (submitted for publication). Food Agric. Immunol. **2007**.
11. Purcell, B.K.; Pruckler, J.; Clegg, S. Nucleotide sequences of the genes encoding type 1 fimbrial subunits of *Klebsiella pneumoniae* and *Salmonella typhimurium*. J. Bacteriol. **1987**, *169*, 5831–5834.
12. Doran, J.L.; Collinson, S.K.; Kay, C.M.; Banser, P.A.; Burian, J.; Munro, C.K.; Lee, S.H.; Somers, J.M.; Todd, E.C.D.; Kay, W.W. *fimA* and *tctC* based DNA diagnostics for *Salmonella*. Mol. Cell. Probes **1994**, *8*, 291–310.

13. Swenson, D.L.; Clegg, S.; Old, D.C. Frequency of the *fim* genes among *Salmonella* serovars. Microb. Pathogen. **1991**, *10*, 497–498.
14. Cohen, H.J.; Mechanda, S.M.; Lin, W. PCR Amplification of the *fimA* gene sequence of *Salmonella typhimurium*, a specific method for detection of *Salmonella* spp. Appl. Envir. Microbiol. **1996**, *62* (12), 4303–4308.
15. Hoorfar, J.; Malorny, B.; Abdulmawjood, A.; Cook, N.; Wagner, M.; Fach, P. Minireview: practical Considerations in Design of Internal Amplification Controls for Diagnostic PCR Assays. J. Clin. Microbiol. **2004**, *42* (5), 1863–1868.
16. Jones, R.N.; Neale, M.L.; Beattie, B.; Westmoreland, D.; Fox, J.D. Development and application of a PCR-based method including an internal control for diagnosis of congenital cytomegalovirus infection. J. Clin. Microbiol. **2000**, *38*, 1–6.
17. Rosenstraus, M.; Wang, Z.; Chang, S.-Y.; Debonville, D.; Spadoro, J.P. An internal control for routine diagnostic PCR: design, properties, and effect on clinical performance. J. Clin. Microbiol. **1998**, *36*, 191–197.
18. Amavisit, P.; Browning, G.F.; Lightfoot, D.; Church, S.; Anderson, G.A.; Whithear, K.G.; Markham, P.F. Rapid PCR detection of *Salmonella* in horse faecal samples. Vet. Microbiol. **2001**, *79*, 63–74.
19. Cubero, J.; van der Wolf, J.; van Beckhoven, J.; López, M.M. An internal control for the diagnosis of crown gall by PCR. J. Microbiol Meth. **2002**, *51*, 387–392.
20. Kongmuang, U.; Luk, J.M.C.; Lindberg, A.A. Comparison of three stool-processing methods for detection of *Salmonella* serogroups B, C2, and D by PCR. J. Clin. Microbiol. **1994**, *32* (12), 3072–3074.
21. Rijpens, N.; Herman, L.; Vereccken, F.; Jannes, G.; de Smedt, J.; de Zutter, L. Rapid detection of stressed *Salmonella* spp. in dairy and egg products using immunomagnetic separation and PCR. Intl. J. Food Microbiol. **1999**, *46* (1), 37–44.
22. Trkov, M.; Majerikova, I.; Jerasek, B.; Stefanovicova, A.; Rijpens, N.; Kuchta, T. Detection of *Salmonella* in food over 30 h using enrichment and polymerase chain reaction. Food Microbiol. **1999**, *16* (4), 393–399.
23. Jeníková, G.; Pazlarová, J.; Demnerová, K. Detection of *Salmonella* in food samples by the combination of immunomagnetic separation and PCR assay. Intl. Microbiol. **2000**, *3*, 225–229.
24. Civilini, M.; Venuti, F.; de Bertoldi, M. Recovery of *Salmonella typhimurium* from compost with the IMS-PCR method. Waste Mgmt. Res. **2000**, *18*, 572–576.

Received April 20, 2007
Accepted July 5, 2007
Manuscript 3238