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A membrane-immunofluorescent-viability staining technique for the detection of *Salmonella* spp. from fresh and processed meat samples

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G. DUFFY, B. KILBRIDE, J.J. SHERIDAN, I.S. BLAIR AND D.A. MCDOWELL. 2000. A direct staining technique was investigated for the detection of viable *Salmonella* in fresh and processed meats. The technique involved overnight enrichment in BPW, extraction of *Salmonella* cells onto a polycarbonate membrane, followed by detection of the pathogen using anti-*Salmonella* monoclonal antibody coupled with an antibody linked-fluorescent stain (Texas Red) and a viability stain (Sytox Green). The technique was applied to the detection of *Salm. enteritidis* inoculated into broth culture or minced beef and then subjected to a variety of stresses including freezing (− 20 °C), heating (2 or 4 min at 56·9 °C), low pH (5 or 3·5) or high salt (2 or 4%). The correlation between traditional plate counts and the rapid count varied widely ($r^2 = 0{\cdot}98$–$0{\cdot}03$), depending on the type and level of stress applied to the cells. The reason for the disparity in results obtained, and the potential application of the method as a diagnostic tool, are discussed.

INTRODUCTION

In the developed world, frequent reports of food poisoning have increased public concern in relation to the potential presence of pathogenic organisms in food, leading to greater demand for effective systems for their detection and control (Kilsby 1999). Current best practice recommends the use of Hazard Analysis Critical Control Point (HACCP) systems in food producing and processing (Tompkin 1994; Notermans and Louve 1995). Rapid microbial methods are essential to allow timely and effective monitoring of the critical control points within such systems. Current systems for the detection and/or enumeration of food-borne pathogens are not sufficiently rapid and/or sensitive to support such monitoring within food production and service activities. One significant group of food-borne pathogens, *Salmonella* spp., remains a leading cause of food poisoning in the developed world, resulting in multiple cases of absenteeism, illness, hospitalization and death each year (Scott 1996). The traditional method for detection of *Salmonella* is time consuming, taking 4 days to obtain a presumptive positive result and a further 1–2 days to speciate the pathogen (Duffy *et al*. 1999). Considerable efforts have been made in the development of rapid methods for the detection of *Salmonella* spp., many of which are based on immunological or DNA (Polymerase Chain Reaction) techniques (Swaminathan and Feng 1994). The speed and sensitivities of these techniques vary, and are significantly affected by the duration of the enrichment period necessary to achieve detectable amounts of target cells or cell components, the complexity of the sample, and the specificity of the reporting mechanism. As many of these developed methods detect discrete cellular components, antigens or genetic material, rather then evidencing metabolism, they are unable to distinguish between viable and non-viable cells, which may yield false positive results for samples containing only dead cells (De Boer 1999). This is not a significant problem in the application of these techniques to those fresh foods in which most of the bacteria present are viable, but it is a potential problem in processed and preserved foods (cooked, frozen, low pH, low a_w, etc.) which can contain large numbers of stressed or dead cells. Therefore, to obtain an accurate assessment of the numbers of bacteria in a product, it is essential to be able to differentiate between viable and non-viable cells (Duffy and Sheridan 1998).

This paper reports on the modification of an immunofluorescent technique for the detection of *Salmonella* spp. previously reported by Cloak *et al*. (1999). The technique is based on combining an anti-*Salmonella* antibody labelled with Texas Red to give a differential stain while a nucleic

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acid viability stain (Sytox Green) is employed to distinguish between live and dead cells. The application of this modified technique was investigated in inoculated broths and minced beef, and in a small number of naturally contaminated retail samples.

MATERIALS AND METHODS

Meat samples

Beef samples were obtained from the abattoir on-site at The National Food Centre. Diaphragm meat used in inoculation experiments was prepared by aseptic removal of fat tissue and mincing through a sterile 5 mm steel plate (Crypto Peerless Mincer, model EB 12F, London, UK). All meat samples used in inoculation experiments were screened for the presence of naturally occurring *Salmonella* using the cultural detection techniques described below. Processed meat samples [cooked chicken ($n = 10$), frozen chicken ($n = 10$), cured meats ($n = 10$) and fermented meats ($n = 10$)] were purchased from retail outlets in the greater Dublin area.

Cultural detection of *Salmonella*

A 25 g portion of sample was placed in a filter bag (Seward Medical, London, UK) with 225 ml buffered peptone water (Oxoid) and incubated at 37 °C for 24 h. A 0·1 ml aliquot of this pre-enriched culture was transferred to 10 ml Rappaport-Vassilidias (R.V.) broth (Oxoid) and incubated at 42 °C for 24 h, and a further 10 ml of the pre-enrichment culture was transferred to 100 ml selenite cystine broth (Oxoid) and incubated at 37 °C for 24 h. Following incubation, both broths were streaked onto Brilliant Green Agar (Oxoid) and mannitol lysine crystal violet brilliant green (M.L.C.B.) agar (Oxoid) and incubated at 37 °C for 24 h. Suspect *Salmonella* colonies were confirmed using biochemical tests (Anon. 1993).

Preparation of double mutant of *Salmonella enteriditis*

A double antibiotic-resistant mutant strain of *Salmonella enteritidis* (NCTC 5765) was prepared in accordance with the method of Blackburn and Davies (1994). In this procedure, an isolate of *Salm. enteriditis* (NCTC 5765) was sequentially incubated in the presence of nalidixic acid (100 μg ml^{-1}; Sigma) and streptomycin sulphate (2000 μg ml^{-1}; Sigma). Double resistant mutants were recovered on nutrient agar containing nalidixic acid (50 μg ml^{-1}) and streptomycin sulphate (1000 μg ml^{-1}). The mutant was maintained on Protect beads (Technical Service Consultants Ltd, Haywood, Lancs, UK) and stored at −20 °C. The mutant was streaked at regular intervals onto tryptone soya agar (TSA, Oxoid) supplemented with the aforementioned antibiotics, to maintain viability. Being antibiotic resistant, this mutant could be effectively recovered and enumerated from inoculated meat samples containing high levels of competitive flora.

Anti-*Salmonella* polyclonal antibody

Anti-*Salmonella* polyclonal antibody (Biogenesis Ltd, Poole, UK) was diluted 1/50 (v/v) in a 1·0% solution of skim milk powder (Marvel, Stafford, UK) containing 0·1% Tween-80 (Sigma), dispensed in 5 ml volumes and stored at −20 °C for up to 12 months.

Texas Red

Texas Red-labelled anti-rabbit antibody (Molecular Probes, Leiden, The Netherlands) was diluted 1/100 (v/v) in a 1·0% solution of skim milk powder (Marvel) containing 0·1% Tween-80, dispensed into 10 ml volumes and stored at −20 °C for up to 12 months.

Sytox Green

Sytox Green nucleic acid stain (Molecular Probes) was prepared by diluting 40 μl of the stain in 100 ml sterile distilled water. The solution was stored at −20 °C.

Salmonella enteritidis inoculum suspension

The double antibiotic-resistant *Salmonella* strain was streaked onto TSA (Oxoid) containing nalidixic acid (50 μg ml^{-1}) and streptomycin sulphate (1000 μg ml^{1}) and incubated at 37 °C for 20 h. A single colony from the agar plate was inoculated into 10 ml Brain Heart Infusion broth (BHI, Oxoid) and incubated for 20 h at 37 °C.

Broth culture

Broth cultures were prepared by inoculating 50 ml peptone water (Oxoid) with 5 ml of an overnight culture of *Salm. enteritidis* to achieve final concentrations of approximately $\log_{10}$ 8·0 cfu ml^{-1}. The cells were then treated in one the four following ways.

Freezing. Aliquots (10·0 ml) of inoculated broth culture were added to 100 ml volumes of peptone water (final concentration approximately $\log_{10}$ 7·00 cfu ml^{-1}) and frozen in a domestic freezer at −20 °C. Prior to testing of the samples, they were defrosted overnight in a 4 °C refrigerator.

Heat. Aliquots (10·0 ml) of inoculated broth culture were added to 100 ml volumes of peptone water (Oxoid) in sterile 500 ml bottles (Duran Schott, Germany) (final concentration approximately $\log_{10}$ 7·00 cfu ml^{-1}). The bottles were fully submerged in a water-bath (Grant Y28, Grant Instruments, Cambridge, UK) and heated to 57 °C. The cultures were held at this temperature for 2 or 4 min. The temperature of the broths was monitored using thermocouples inserted into uninoculated peptone water, linked to *ellab* equipment (ELLAB A/S, Denmark).

Low pH. Aliquots (5·0 ml) of inoculated broth culture were added to 50 ml volumes of peptone water (final concentration approximately $\log_{10}$ 7·00 cfu ml^{-1}) which had been adjusted to pH 3·5 or 5 with 1 mol l^{-1} HCl The pH was measured using an Orion 210 pH meter (Orion). The samples were then incubated at 37 °C for 3 h.

Salt. Aliquots (5·0 ml) of inoculated broth culture were added to 50 ml volumes of peptone water (final concentration approximately $\log_{10}$ 7·00 cfu ml^{-1}) containing NaCl (Sigma) at a final concentration of 2% (w/v) or 4% (w/v). The samples were incubated at 37 °C for 3 h.

Minced beef studies

Aliquots (10 ml) of an overnight culture of *Salm. enteritidis* were thoroughly mixed through 100 g minced beef with a sterile spatula to achieve a final concentration of 10^7 cfu g^{-1}. The inoculated mince was then treated in one of the following ways.

Freezing. Inoculated minced beef samples (100 g) were frozen in a domestic freezer at −20 °C. Prior to testing of the samples, they were defrosted overnight in a 4 °C refrigerator.

Heat. Samples (10·0 g) of inoculated minced beef were aseptically added, in duplicate, to vacutainers (Becton Dickinson, Meylan Cedex, France). The vacutainers were fully immersed in a water-bath (Grant) and heated to 57 °C. The meat samples were held at this temperature for 2 or 4 min. The core temperature of the meat was monitored using thermocouples inserted into an uninoculated meat sample, linked to *ellab* equipment (ELLAB A/S, Denmark).

Low pH. The pH of inoculated minced beef was estimated by placing a 2 g sample of beef mince in 10 ml of a solution of 5 mmol l^{-1} sodium iodoacetate and 150 mmol l^{-1} potassium chloride (pH 7·00) and then homogenizing in a laboratory mixer emulsifier (Silverson Machines Ltd, Chesman, UK) for 2 × 15 s bursts with a 5 s interval. The pH was measured using an Orion 210 pH meter (Orion Research Corp., Boston, MA, USA) with a combined electrode. Duplicate 100 g samples of minced beef were adjusted to pH 5 or 3·5 by the addition of 1 mol l^{-1} lactic acid. The samples were then incubated for 3 h at 37 °C.

Salt. NaCl (Sigma) was added to 100 g inoculated beef mince to achieve a final concentration of 2% (w/w) or 4% (w/w). The minced beef was then incubated at 37 °C for 3 h.

Isolation of *Salmonella* by surface adhesion onto a membrane

Polycarbonate membranes (0·6 μm, Poretics, 25 mm in diameter, Livermore, CA, USA) were attached to the surface of glass microscope slides (76 × 32 mm) (Cloak *et al.* 1999). Duplicate slides were completely immersed in each of the sample cultures for 15 min.

Enumeration and detection

Plate counts. Aliquots (0·1 ml), or derived 10-fold dilutions in 0·1% peptone water, from the enrichment broths and minced beef cultures were plated in duplicate onto TSA (Oxoid) containing nalidixic acid (50 μg ml^{-1}) and streptomycin sulphate (1000 μg ml^1) and incubated at 37 °C for 24 h.

Immunofluoresence microscopy. The membrane was placed in a sterile Petri dish and then coated with 0·5 ml anti-*Salmonella* polyclonal antibody before incubating at 37 °C for 30 min. The membrane was then placed on a vacuum manifold and washed under vacuum (25 psi) using 30 ml sterile water to remove any unbound antibody. The membranes were replaced in a sterile Petri dish and coated with 0·5 ml Texas Red, re-incubated at 37 °C for 30 min, and washed as above. The membranes were then coated with 0·5 ml Sytox Green, incubated at 37 °C for 10 min, washed as above, dried under vacuum and mounted in oil on a glass slide. A Nikon Optiphot microscope fitted with an epifluorescent attachment, a 100 W mercury vapour light source and a 60× oil immersion plan objective was used to examine the bacteria on the membrane. Live *Salmonella* cells were stained with a characteristic red cell outline where the antibody–dye complex attached, while dead *Salmonella* cells also displayed the red fluorescent cell outline but with a distinctive green centre. The total number of live cells were determined by counting 20 fields of

vision and multiplying the number of cells by a working factor of 1·25 (Sheridan *et al.* 1998) to determine the number of *Salmonella* per square millimetre of membrane.

All the above samples were carried out in duplicate and repeated five times.

Retail samples (naturally contaminated)

Retail samples including fermented meat ($n = 10$), cooked chicken ($n = 10$), frozen chicken ($n = 10$) and cured meat ($n = 10$) were examined for the presence of *Salm. enteritidis* using the membrane-imunofluorescent-viabilty staining technique and by the traditional cultural detection method. Traditional cultural detection of *Salmonella* was carried out as described above. A 100 ml aliquot of the overnight enrichment culture (BPW) was withdrawn and the membrane extraction/immunofluorescent staining technique carried out as described above.

Statistical analysis

Linear regression analysis was employed to determine the relationship between the number of *Salm. enteritidis* detected by traditional plate counts and by immunofluorescence.

RESULTS

The mean numbers of *Salm. enteritidis* in broth cultures following exposure to freezing, heat, low pH and salt are shown in Table 1. The four treatments resulted in different levels of cell death. The initial inoculum in all samples was $\log_{10}$ 7·00 cfu ml^{-1}, and following treatment, the numbers of *Salmonella* cells remaining in the sample, as determined by the plate count method, was considerably reduced, with heat being most detrimental followed by NaCl, pH and freezing. While heat resulted in a $\log_{10}$ 2·00–4·00 cfu ml^{-1} reduction, freezing yielded no significant reduction in cell numbers. When the samples were examined by the immunofluorescence-viability stain, a reduction in the number of *Salmonella* attached to the membrane was observed following the four treatments, with heat resulting in the greatest reduction in attached cells followed by pH, NaCl and freezing.

Linear regression analysis was applied to determine the relationship between *Salm. enteritidis* numbers in broth cultures and the numbers attaching to the membrane in untreated and treated cultures (Table 2). Although it was observed that the numbers of *Salmonella* per millilitre of broth and attaching to the membrane decreased in a similar pattern, the correlation between the two counting methods varied widely depending on the type and level of treatment applied ($r^2 = 0{\cdot}26$–$0{\cdot}98$). As expected, the correlation between methods in untreated broths was excellent ($r^2 = 0{\cdot}89$). Broths subjected to the most severe treatment (heat) showed very poor correlation between methods ($r^2 = 0{\cdot}27$). Surprisingly, the staining technique was also very poor ($r^2 = 0{\cdot}28$) in frozen broths in which no significant cell death was shown to occur. The counting methods correlated well in broths exposed to low pH (5·0) and high salt (2%) ($r^2 = 0{\cdot}73$–$0{\cdot}98$), although this decreased to $r^2 = 0{\cdot}26$ and 0·54 when the levels of exposure were increased to pH 3·5 and 4% NaCl.

The mean numbers of *Salm. enteritidis* in minced beef following exposure to freezing, heating, low pH and high salt are shown in Table 3. As observed in inoculated broths, the numbers of *Salm. enteritidis* in the meat and attaching to the membrane decreased in the four treated meat samples. However, it was noted that despite the same level of treatment being applied, the decrease in *Salm. enteritidis*

Table 1 The mean number of *Salmonella enteritidis* in broth cultures following exposure to freezing, heat, low pH and salt as determined by plate counts and membrane-immunofluorescent-viability counts

Treatment	Treatment level	Plate counts ($\log_{10}$ cfu ml^{-1})	Immunofluorescent membrane count ($\log_{10}$ cfu mm^{-2})
Control	None	6·62	2·88
Freezing	− 20 °C	7·11	2·42
Heat at 57 °C	2 min	2·09	1·36
	4 min	2·11	0·81
NaCl	2%	6·82	2·63
	4%	5·73	2·16
pH	5·0	7·10	2·29
	3·5	3·89	0·80

The results shown are the mean of five replicates and the mean initial inoculum in all samples was $\log_{10}$ 7·00 cfu ml^{-1}.

Table 2 The relationship between *Salmonella enteritidis* numbers in broth cultures following exposure to freezing, heat, low pH and salt as determined by plate counts and membrane-immunofluorescent-viability count

Treatment	Level of treatment	Intercept	Slope	S.E.	r^2	rsd
Control	None	3·64	1·058	0·60	0·89	0·21
Freezing	− 20 °C	8·77	− 0·68	0·64	0·28	0·46
Heat at 57 °C	2 min	3·44	− 0·99	0·94	0·27	1·25
	4 min	4·33	− 1·31	1·56	0·41	1·32
NaCl	2%	− 11·96	7·13	0·65	0·98	0·19
	4%	8·63	− 1·34	0·72	0·54	0·32
pH	5·0	3·85	1·42	0·50	0·73	0·25
	3·5	3·54	0·44	0·43	0·26	0·50

Table 3 The mean number of *Salmonella enteritidis* in minced beef following exposure to freezing, heat, low pH and salt as determined by plate counts and membrane-immunofluorescent- viability count

Treatment	Level of treatment	Plate counts ($\log_{10}$ cfu ml^{-1})	Immunofluorescent membrane count ($\log_{10}$ cfu mm^{-2})
Control	None	7·27	4·26
Freezing	− 20 °C	5·48	2·27
Heat at 57 °C	2 min	5·94	1·90
	4 min	4·88	1·80
NaCl	2%	5·82	2·34
	4%	6·08	2·24
pH	5·0	5·99	2·47
	3·5	3·97	1·55

The results shown are the mean of five replicates and the mean initial inoculum in all samples was $\log_{10}$7·00 cfu g^{-1}

numbers in the meat sample was, in general, lower than had been observed in broth. A different pattern in cell death *vs* treatment was also observed in the meat samples with low pH, resulting in the highest number of dead *Salm. enteritidis* cells followed by salt, freezing and heat.

The linear relationship between *Salm. enteritidis* numbers in minced beef following freezing, heat, low pH and salt as determined by plate counts and immunofluoresent-viability staining are shown in Table 4. Only the untreated meat sample and the sample exposed to salt (2%) yielded good correlations between the two counting methods ($r^2 = 0{\cdot}83$ and 0·72). There was no correlation between the counting methods in the other treated samples.

To investigate the application of the staining technique as a qualitative test, a small number of retail samples was examined for the presence of naturally occurring *Salmonella* using both the cultural and rapid staining techniques. One sample of cooked sliced chicken and two samples of frozen chicken were positive for *Salmonella* using the rapid method. Using the cultural methods, only one of the samples (frozen) was positive (Table 5).

DISCUSSION

The membrane-immunofluorescent-viability technique reported in this paper is a modification of a membrane immunofluorescent technique previously reported for *Listeria* spp. (Sheridan *et al.* 1997), *Salmonella* spp. (Cloak *et al.* 1999) and *Yersinia enterocolitica* (Sheridan *et al.* 1998). These methods worked well on fresh meat samples, but it was considered that their inability to distinguish between live and dead cells would be a distinct disadvantage when applying them to processed food samples or foods containing stressed cells, and they could potentially

Table 4 The relationship between *Salmonella enteritidis* numbers in minced beef following exposure to freezing, heat, low pH and salt as determined by plate counts and membrane-immunofluorescent-viabilty counts

Treatment	Level of treatment	Intercept	Slope	S.E.	R^2	rsd
Control	none	3·01	1·00	0·11	0·83	0·78
Freezing	− 20 °C	− 1·0	2·87	1·63	0·51	0·47
Heat at 57 °C	2 min	5·87	0·04	0·12	0·03	0·09
	4 min	5·58	− 0·39	0·63	0·11	0·55
NaCl	2%	− 0·53	2·71	0·99	0·72	0·33
	4%	5·97	0·05	0·08	0·12	0·04
pH	5·0	4·76	0·50	1·19	0·06	0·27
	3·5	4·16	0·55	1·47	0·04	1·03

Table 5 The number of retail samples containing *Salmonella* spp. as determined by cultural detection methods and membrane-immunofluorescent-viability count

Sample type	Number tested	Cultural detection methods	Membrane counts
Fermented meat	10	0	0
Cooked sliced chicken	10	0	1
Frozen chicken	10	1	2
Cured meat	10	0	0

result in false negative results. This is a problem that occurs with all commercial immunological methods, and a viability method would have tremendous advantages and potential applications.

The technique investigated was based on the premise that *Salmonella* could be specifically detected on the membrane using anti-*Salmonella*-Texas Red-labelled antibody, and Sytox Green could distinguish between viable and non-viable cells. The antibody targets an outer membrane protein on the *Salmonella* membrane, and the *Salmonella* appear as very distinct rod-shaped organisms with a red fluorescent cell outline. Sytox Green (S-7020) is a high-affinity nucleic acid stain that readily penetrates cells that have compromised plasma membranes but does not cross the intact membrane of live cells. Once Sytox Green has entered the cell, it binds to the nucleic acids causing them to fluoresce bright green. Live *Salmonella* cells were stained with a red outline while dead cells also had the red outline but had a green-stained cell centre. A possible problem with the technique is the detection of injured cells which have temporarily compromised membranes. These cells can potentially allow the dye to enter, thus falsely recording them as dead cells. This would be of public heath significance as these cells may subsequently recover their viability and virulence. It is expected that some of the variability in correlations between the plate count and staining technique observed in this study is due to the presence of injured *Salmonella* in the samples. It can be seen from the plate counts that the four treatments examined (freezing, heat, low pH and salt) resulted in different levels of *Salmonella* being killed and would also result in different types of damage to the cell.

The plate count and immunofluorescent-viability stain counts obtained for *Salmonella* cells exposed to freezing (− 20 °C) correlated poorly for both broth and minced beef samples ($r^2 = 0{\cdot}28$, $r^2 = 0{\cdot}51$). It was noted from the plate counts that there was no cell death in broth culture and very little cell death in minced beef (approximately $\log_{10}$ 1·50 cfu g^{-1}). Freezing results in physical damage and causes a direct increase in viscosity of the cell as a result of ice crystal formation. It may also cause some denaturation of cellular proteins (Jay 1996). Precisely how this is

achieved is unclear, but it is known that some -SH groups disappear upon freezing. It is also known that lipoproteins may break apart on freezing. It is reported that the level of cell injury as a result of freezing is very high, with cells been able to recover and regenerate upon thawing (Budu-Amoako *et al*. 1992). Clearly, this type of treatment may have sub-lethally damaged both the antibody binding site and the membrane integrity, resulting in erroneous staining of the cell, and would account for the poor correlation between the plate and immunofluorescent-viability stain counts.

The correlation between the plate counts and immunofluorescent-viability counts for heat-treated cells in broth and minced beef was also poor, regardless of the length of heating (2 or 4 min at 57 °C). Exposure to high temperatures results in large numbers of killed cells, as observed from plate counts, but also results in large numbers of sub-lethally injured cells (Busch and Donnelly 1992). The initial damage caused by heat treatment is the disruption of membrane lipoprotein complexes and enzymes associated with the integrity of the cell membrane. This would again indicate that both antibody binding and membrane integrity would be affected, resulting in poor accuracy of the viability stain technique.

In low pH broth and minced beef systems, the correlation between counting methods was variable, with $r^2 = 0{\cdot}73$ for broth, pH 5·0, and $r^2 = 0{\cdot}04$ for meat at pH 3·5. Some of this variability between the broth cultures and meat samples may be related to the type of acid used to reduce the pH. An inorganic acid (HCl) was added to broth cultures and an organic acid (lactic acid) was used in the meat samples. Organic acids exhibit distinct anionic effects which have an influence on bacterial inactivation (Buchanan and Golden 1994). This anionic effect does not occur with inorganic acids (HCl), and they are reportedly less inhibitory to bacterial cells (Young and Foegeding 1993). As expected, as the pH was lowered from 5 to 3·5, the inhibitory effect of the acid was enhanced and poorer results were obtained with the viability technique.

The correlation between the plate counts and immunofluorescent-viability stain counts were good at NaCl levels of 2% in broth cultures ($r^2 = 0{\cdot}98$) and in minced beef samples ($r^2 = 0{\cdot}72$). At higher levels of salt (4%), the correlation was poor ($r^2 = 0{\cdot}54$, $r^2 = 0{\cdot}12$). The normal level of salt found in many processed meat products would normally be about 2% (Riordan *et al*. 1998). High NaCl concentration can cause plasmolysis in bacterial cells (Jay 1996). At medium levels of NaCl (2%), this did not affect the cell-staining process but obviously had a very significant affect at high NaCl concentrations (4%).

Although it is clear that as a quantitative technique the immunofluorescent-viability staining technique had considerable limitations, it was considered that it might still have potential application as a qualitative test for the presence of *Salmonella*. As enrichment is a standard part of any diagnostic test for *Salmonella* spp., cultural and/or rapid, the quantitative limitations would not be of significant importance. When the technique was applied to retail processed meat samples ($n = 40$), one sample was reportedly positive by the cultural technique and three by the immunofluorescent-viability stain technique. Cloak *et al*. 1999 obtained no false positives/negatives when they applied a similar technique based on immunofluorescence (without the viability stain) to detect *Salmonella* in fresh poultry and minced beef. With regard to the immunofluorescent-viability stain technique investigated, it would therefore appear that while it will no doubt perform well on fresh foods (as indicated by the excellent correlation for untreated samples), its use for processed samples is limited.

Other reports on the use of viability stains for the determination of total viable counts in meat have yielded excellent correlations with the total plate count technique ($r^2 = 0{\cdot}83$–$0{\cdot}92$) for a range of fresh and processed meat samples (cooked ham, bacon rashers, frozen burgers) (Duffy and Sheridan 1998). This would indicate that some of the problems encountered in the present study were related to poor antibody binding to sub-lethally injured cells because of temporary damage to the outer membrane proteins. It is possible that the incorporation of a recovery step in to the technique, i.e., a short incubation period of the membrane (with attached *Salmonella*) on a non-selective agar could address this problem. Further studies are needed to investigate this theory.

REFERENCES

Anon. (1993) BSI 5763, part 4; ISO 6579. London: British Standards Institution.

Blackburn, C. de W. and Davies, A.R. (1994) Development of antibiotic resistant strains for the enumeration of foodborne pathogenic bacteria in stored foods. *International Journal of Food Microbiology* **24**, 125–136.

Buchanan, R.L. and Golden, M.H. (1994) Interaction of citric acid concentration and pH on the kinetics of *Listeria monocytogenes* inactivation. *Journal of Food Protection* **57**, 567–570.

Budu-Amoako, E., Toora, S., Ablett, R.F. and Smith, J. (1992) Evaluation of the ability of primary selective enrichment to resuscitate heat injured and freeze injured *Listeria monocytogenes* cells. *Applied and Environmental Microbiology* **58**, 3177–3179.

Busch, S. and Donnelly, C.W. (1992) Development of a repair enrichment broth for resuscitation of heat injured *Listeria monocytogenes* and *Listeria innocua*. *Applied and Environmental Microbiology* **58**, 14–20.

Cloak, O.M., Duffy, G., Sheridan, J.J., McDowell, D.A. and Blair, I.S. (1999) Development of a surface adhesion immunofluorescent technique for the rapid detection of *Salmonella* spp.

from meat and poultry. *Journal of Applied Microbiology* **86**, 583–590.

De Boer, E. (1999) Methodology for detection and typing of food-borne micro-organisms. *International Journal of Food Microbiology* **50**, 119–131.

Duffy, G., Cloak, O.M., Sheridan, J.J., Blair, I.S. and McDowell, D.A. (1999) The incidence and antibiotic resistance profile of *Salmonella* spp. on Irish retail meat poultry products. *Food Microbiology* **16**, 623–631.

Duffy, G. and Sheridan, J.J. (1998) Viability staining in a direct count rapid method for the determination of total viable counts on processed meats. *Journal of Microbiological Methods* **31**, 167–174.

Jay, J.M. (1996) *Modern Food Microbiology* 5th edn. New York and London: Chapman and Hall.

Kilsby, D.C. (1999) Food microbiology: the challenges for the future. *International Journal of Food Microbiology* **50**, 59–65.

Notermans, S. and Louve, J.L. (1995) Quantitative risk analysis and HACCP: some remarks. *Food Microbiology* **12**, 425–429.

Riordan, D.C.R., Duffy, G., Sheridan, J.J. et al. (1998) Survival of *Escherichia coli* 0157:H7 during the manufacture of pepperoni. *Journal of Food Protection* **61**, 146–151.

Scott, E. (1996) Food borne disease and other hygiene issues in the home. *Journal of Applied Bacteriology* **59**, 93–95.

Sheridan, J.J., Duffy, G., McDowell, D.A. and Blair, I.S. (1997) Development of a surface adhesion immunofluorescent microscopy technique for the rapid detection of *Listeria monocytogenes and Listeria innocua* from raw meat. *Journal of Applied Microbiology* **82**, 225–233.

Sheridan, J.J., Logue, C.M., McDowell, D.A., Blair, I.S., Hegarty, T. and Toivanen, P. (1998) The use of a surface adhesion immunofluorescent (SAIF) method for the rapid detection of *Yersinia enterocolitica* serotype O:3 in meat. *Journal of Applied Microbiology* **85**, 737–745.

Swaminathan, B. and Feng, P. (1994) Rapid detection of food borne pathogenic bacteria. *Annual Review in Microbiology* **48**, 401–426.

Tompkin, R.B. (1994) HACCP in the meat and poultry industry. *Food Control* **5**, 153–159.

Young, K.M. and Foegeding, P.M. (1993) Acetic, lactic and citric acids and pH inhibition of *Listeria monocytogenes* Scott A and the effect on intracellular pH. *Journal of Applied Bacteriology* **74**, 515–520.