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SUMMARY

The EiaFoss *Listeria* Detection System is an ELISA which uses immunomagnetic separation combined with automated ELISA for the rapid detection of *Listeria* species in foods.

This study has investigated the sensitivity and specificity of the system, and compared its ability to detect *Listeria* in foods with detection of *Listeria* by cultural procedures.

Sensitivity of the system ranged from 1.5×10^4 cfu/ml to 1.4×10^6 cfu/ml depending on the strain tested, and specificity of the system was found to be satisfactory with cross reactivity only noted with one strain of *Erysipelothrix rhusiopathiae*.

Detection of *Listeria* from foods was more successful with the EiaFoss system, when using the ISO-based protocol for non-dairy foods, and the EiaFoss dairy protocol for dairy products, than by the USDA-based cultural method. Both cultural analysis and EiaFoss analysis from these enrichment protocols detected *Listeria* on more occasions than the USDA-based cultural method, although the Eiafoss and USDA cultural methods failed to detect *Listeria* from positive samples on 9 and 14% of occasions respectively.

CONTENTS

INTRODUCTION

The genus *Listeria* comprises six recognised species: *L. monocytogenes, L. innocua, L. seeligeri, L. ivanovii, L. welshimeri* and *L. grayi*. The species *L. grayi* incorporates the former species *L. murrayi* (Rocourt *et al*, 1992). *L. monocytogenes* is an important pathogen in humans and animals; *L. ivanovii* is pathogenic to animals and has occasionally been reported to cause disease in humans. *L. seeligeri* and *L. welshimeri*, however, have also occasionally been implicated in human infection (Lund, 1990).

Listeria are widely distributed in the environment, having been found in many sources including rivers, lakes, soil, sewage, animal fodder, fertiliser, insects and kitchen premises (Lacey, 1992). In addition, *Listeria* species have been isolated from a wide variety of foods such as raw meat, poultry, fish, milk and vegetables, and also from cooked and cured meats and ready-to-eat meals (Lund, 1990). Of particular concern is the fact that high numbers of *L. monocytogenes* have been found in raw meats, soft cheese and paté (Lund, 1990).

There has been an increase in the number of reported cases and outbreaks of human listeriosis and, in several cases, specific foods have been implicated as the vehicle of infection. Until 1980, the reported incidence of *Listeria* infection in the UK was 50-60 cases per year but this increased to 291 in 1988. The number of reported cases has since declined to approximately 100 per year (Legan, 1997).

There is concern over the presence of *Listeria* in foods as human listeriosis can be of foodborne origin, and the severity of the ensuing infection can be high (abortion or still birth if pregnant women are infected; meningitis; septicaemia; etc.). Due to this concern, a number of methods have been developed for the isolation and detection of *Listeria* and *L. monocytogenes* in food.

Conventional cultural methods for the detection of *Listeria* in foods are long, usually involving one or two enrichment steps, and can take up to 5 days to obtain a presumptive result. This, together with the requirement of many laboratories to test large numbers of samples with minimum labour time, has led to the development of a variety of rapid methods. Immunological methods are commonly used in the food industry, in particular the enzyme linked immunosorbant assay (ELISA). Many ELISAs are done in microtitre plates. Additional equipment is usually necessary, such as microtitre plate washers and plate readers. Care is needed to avoid cross-contamination of reagents when carrying out the test, and the total test time after enrichment is approximately 2.5 hours.

Immunomagnetic separation (IMS) methods have also been developed. In these systems, target cells (e.g. *Listeria*) from an enriched food sample are captured onto antibody-coated magnetic beads. The beads are removed from the culture by application of a magnet.

The separation of the cells from food debris and competing organisms enables easier detection of the target organism. The captured cells can be concentrated by resuspending in a volume smaller than that from which they were captured, thus increasing the sensitivity of subsequent detection/isolation methods, e.g. conventional cultural methods or ELISA. Commercial IMS systems have been reported for the isolation of foodborne pathogenic microorganisms such as *Salmonella* (e.g. Bennett and Betts, 1993) and *Escherichia coli* O157 (e.g. Bennett *et al*, 1996).

Foss Electric has developed an automated detection system which utilises immunomagnetic separation combined with automated ELISA for the rapid detection of *Listeria* species in foods (the EiaFoss *Listeria* Detection System). The EiaFoss system is already established for the detection of *Salmonella* in foods (Jones and Betts, 1994). Pre-enriched food samples are heat treated to denature the flagella antigen of any contaminating *Listeria* cells. Monoclonal antibodies specific to the denatured flagella antigen are covalently linked to the magnetic particles (paramagnetic beads; IDG®). The particles are added to the treated sample, target cells are captured from the sample, and non-bound material is washed away, the particles being held on the side of the test tube by magnetic force.

A second flagella specific monoclonal antibody labelled with alkaline phosphatase is added, which binds to the captured antigen. The substrate, 4-methylumbelliferylphosphate, reacts with alkaline phosphatase to produce 4-methylumbelliferone. The end product is measured fluorimetrically.

The assay is completely automatic and takes approximately 2 hours. Enrichment of the food sample prior to the assay is in 3 steps, with a total incubation time of 51 hours. Thus a presumptive result can be obtained on the second day after initiating analysis.

Enrichment procedures have been studied in detail at CCFRA (data not shown), showing that with the exception of dairy foods the optimum results from the EiaFoss assay were achieved using an ISO-based enrichment. A dairy protocol based on the Food and Drug Administration Bacteriological Analytical Manual (FDA-BAM) enrichment was preferable for dairy products. The enrichment procedures were modified in order to optimise expression of the *Listeria* flagella antigen and growth of all *Listeria* species. Therefore, incubation of the secondary enrichment broth was at 30°C not, as recommended in the conventional procedures, at 35°C or 37°C.

The EiaFoss *Listeria* system for the detection of *Listeria* from foods utilising these optimised enrichment procedures has been evaluated with respect to its sensitivity, specificity and ability to detect *Listeria* from a range of inoculated foods and potentially naturally contaminated foods. All foods were tested in parallel by a cultural method based on USDA recommended procedures.

MATERIALS AND METHODS

Sensitivity and specificity

Five strains of each *Listeria* species (*L. monocytogenes, L. innocua, L. seeligeri, L. ivanovii, L. welshimeri* and *L. grayi*) were obtained from the CCFRA Culture Collection. Organisms were maintained on storage beads (LabM) at -80°C, and cultured in Tryptone Soya Broth (Oxoid CM 129) + 0.6% (w/w) yeast extract (TSYEB) incubated overnight at 30°C. Cultures were serially diluted in TSYEB and aliquots (500µl) of each dilution were tested for the presence of *Listeria* with the EiaFoss *Listeria* Detection System. Cell concentrations were confirmed by plate counts on Tryptone Soya Agar (Oxoid CM 131) + 0.6% (w/w) yeast extract (TSYEA) with incubation at 30°C for 24h.

Exclusivity

Ten non-*Listeria* organisms (Table 1) which potentially could survive and grow in the enrichment system were obtained from the CCFRA Culture Collection. Organisms were cultured in TSYEB incubated at 30°C for 24h. An aliquot (500µl) of each culture was tested with the EiaFoss *Listeria* Detection System. Cell concentrations were confirmed by plate counts on TSYEA, as above.

Inoculated foods

A wide variety of foods (including soft cheese, hard cheese, milk powder, processed meats and meat products, fish and fish products and potato salad) were obtained from local retail outlets. Inocula were prepared by culturing *L. monocytogenes* CRA 5553, *L. innocua* CRA 5897, *L. grayi* CRA 3001, *L. ivanovii* CRA 1120 and *L. seeligeri* CRA 1139 in Nutrient Broth (NB; Oxoid CM1) incubated overnight at 30°C. Samples (25g) of food were inoculated individually with each organism at levels of <50 cfu/25g sample. Inoculum levels were confirmed by plate counts on TSYEA. Uninoculated control samples were set up for each food.

Foods were tested for *Listeria* by cultural procedures based on the recommended USDA method (McClain and Lee, 1989) (Figure 1), and by the EiaFoss *Listeria* system using an ISO *Listeria monocytogenes* recommended enrichment procedure (BS 5763: 18, 1997, ISO 11290 - 1: 1997) or the FDA-BAM recommended enrichment procedure for dairy products (Hitchins, 1992) (Figure 2).

It should be noted that the ISO and USDA methods are targeted at *Listeria monocytogenes*. The enrichment protocols in this study were modified in order to optimise expression of the *Listeria* flagella antigen and growth of all *Listeria* species. Therefore, incubation of the secondary enrichment broth, Fraser Broth, was done at 30°C not, as recommended in the conventional methods, at 35°C or 37°C.

Naturally contaminated foods

Foods (50 samples) which potentially could be contaminated with *Listeria* were obtained from retail outlets. Foods were tested for the presence of *Listeria* by the methods detailed below.

Cultural USDA-based method for detection of *Listeria* (Figure 1).

Food samples (25g) were enriched in 225ml *Listeria* Primary Selective Enrichment Broth (UVM1; Oxoid CM863 and SR142) incubated at 30°C for 24h. Aliquots (0.1ml) of enrichment cultures were subcultured into Fraser Broth (FB; Oxoid CM895 and SR156) and incubated at 30°C for 24h. After incubation, a loopful of FB was streaked onto Oxford Agar (Oxoid CM856) containing 10mg/l colistin and 15mg/l moxalactam (Sigma) (MOX), and Palcam Agar (Oxoid CM877 and SR150). Plates were incubated at 35°C for 48h. Grey, brown or black colonies with a black/brown halo on MOX agar, and grey-green colonies, with or without black sunken centres, and with a black halo, on Palcam Agar, were presumed to be *Listeria* species.

Up to three presumptive *Listeria* colonies per agar were taken for confirmation. Colonies were streaked onto TSYEA and plates were incubated at 30°C for 24h. Isolates from inoculated foods were confirmed by Gram-stain, catalase and oxidase tests, and isolates from naturally contaminated foods were further biochemically confirmed with the MICRO-ID *Listeria* Identification System (Organon-Teknika).

EiaFoss methods for detection of *Listeria* (Figure 2)

Dairy products (25g samples) were enriched in 225ml modified *Listeria* Enrichment Broth (mLEB; Oxoid CM 862 and SR 141 + 2.5g/l K_2HPO_4), and incubated at 30°C for 24h. Aliquots (1ml) of enrichment broth were subcultured into 9ml FB and incubated at 30°C for 24h.

Other food products (25g samples) were enriched in 225ml Half Fraser Broth. Enrichment broths were incubated at 30°C for 24h. Aliquots (0.1ml) of enrichment culture were subcultured into 10ml FB, and incubated at 30°C for 24h.

Aliquots (2ml) of FB derived from either approach were subcultured into TSYEB (8ml). Broths were incubated in a water bath at 30°C for 3h. After incubation, TSYEB cultures were heated at 100°C for 15 minutes, and cooled to <35°C. An aliquot (600µl) was tested for the presence of *Listeria* using the EiaFoss *Listeria* Detection System, according to the manufacturer's instructions.

Positive ELISA results were confirmed by streaking FB cultures onto MOX and Palcam Agars. Samples generating negative ELISA results were also streaked onto the confirmation agars to assess false-negative detections by the EiaFoss system. Plates were incubated at 35°C for 48h. Up to 3 characteristic colonies were taken for confirmation by Gram-stain, catalase and oxidase tests and/or MICRO-ID, as previously described.

Figure 1 Cultural USDA-based protocol for detection of *Listeria*

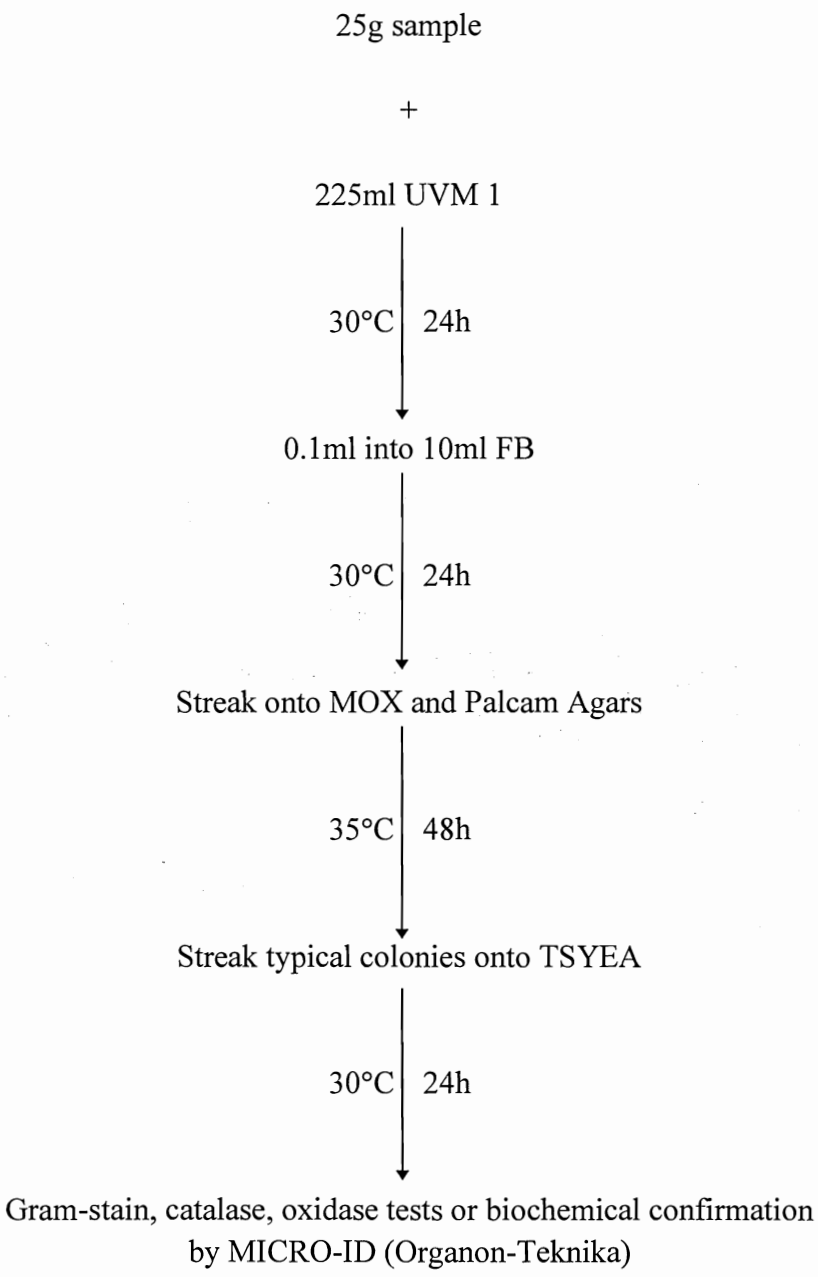
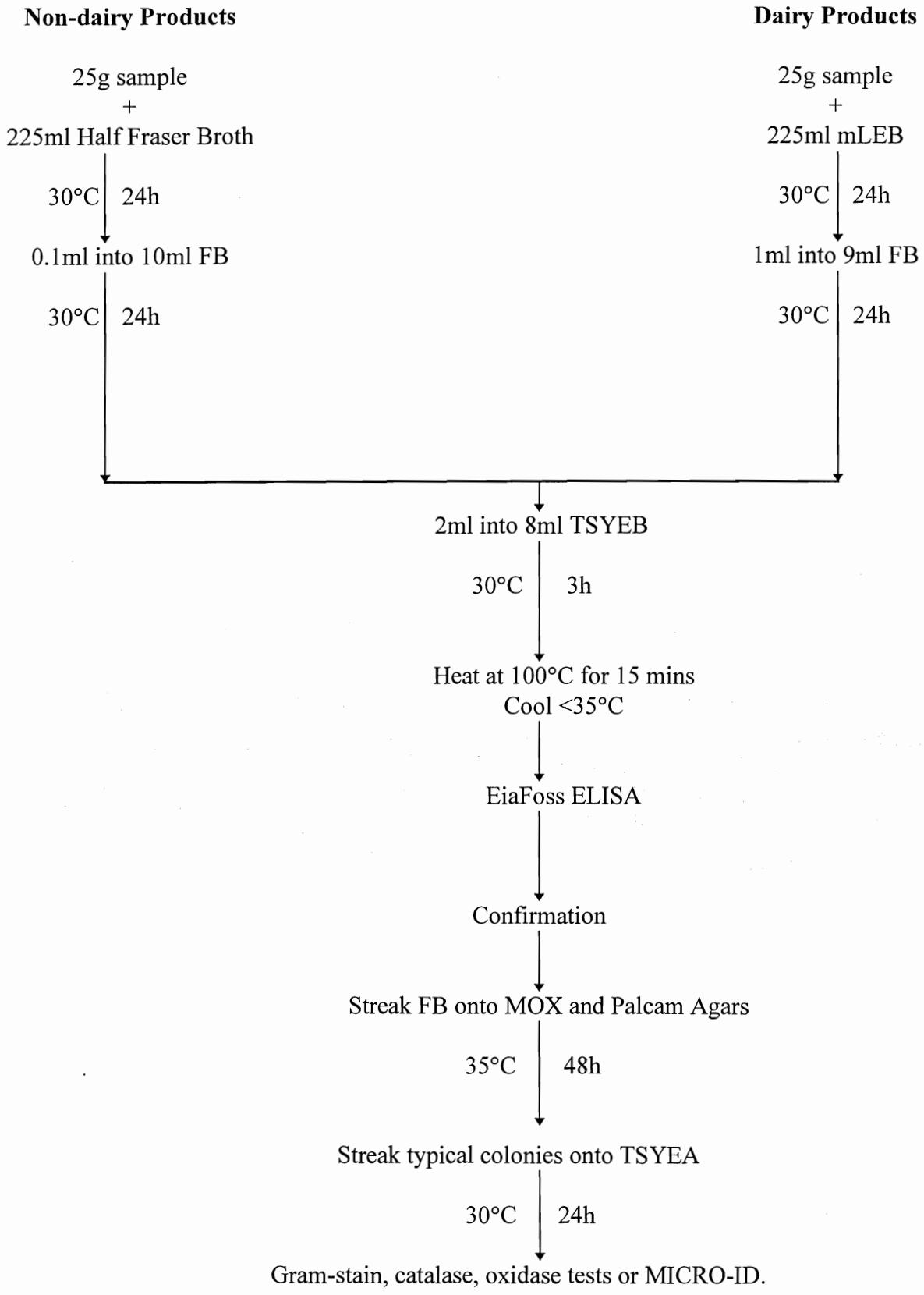


Figure 2 EiaFoss protocols for detection of *Listeria*



Key **Figures 1 and 2**

UVM1	Listeria Primary Selective Enrichment Broth.
FB	Fraser Broth.
MOX	Listeria Selective Agar (Oxford Formulation) + 10mg/l colistin + 15mg/l moxalactam
TSYEA	Tryptone Soya Agar + 0.6% yeast extract.
TSYEB	Tryptone Soya Broth + 0.6% yeast extract.
mLEB	Modified *Listeria* Enrichment Broth (based on FDA/BAM 7th-Edition).

*Note: In the current study, enrichment cultures yielding negative EiaFoss results were also subcultured onto isolation media to assess the extent of false negative results.

RESULTS AND DISCUSSIONS

Sensitivity

The sensitivity of the EiaFoss *Listeria* Detection System was determined by testing serially diluted pure cultures of *Listeria*. Five strains of each *Listeria* species were tested. A summary of results is shown in Table 1. A positive result was equivalent to an analyser signal of ≥ 0.10.

The sensitivity levels ranged from 1.5×10^4 cfu/ml (*L. ivanovii* and *L. grayi*) to 9.1×10^6 cfu/ml (*L. monocytogenes*). One strain of *L. seeligeri* (CRA 1820) was detected at apparent levels of 1.4×10^3 cfu/ml and 1.4×10^2 cfu/ml, but not at 1.4×10^4 cfu/ml. In this case it was considered that the two detections at 10^2 and 10^3 were probably erroneous. Similarly, *L. welshimeri* CRA 6175 was detected at 1.4×10^3 but not at 1.4×10^4. Generally the sensitivity of the system was found to be approximately 10^5 cfu/ml. The EiaFoss system was less sensitive to *L.* monocytogenes than the other *Listeria* species, but since ten-fold dilutions of each culture were tested, only approximate sensitivity levels can be concluded from these experiments. One strain of *L. innocua* (CRA 6132) was not always detected. This could be due to poor expression of flagella antigen in this particular strain. Other strains of *L. innocua*, however, were detected at a level of 10^5 cfu/ml.

Commercial ELISA systems, e.g. Listeria-TEK (Organon-Teknika) and VIDAS (bioMérieux), are reported to have detection limits of 10^5 to 10^6 cfu/ml (Nørrung *et al.*, 1991; Beumer and Brinkman, 1989; Haines and Patel, 1989; and Bennett *et al.*, 1993). The Tecra Listeria Visual Assay (Tecra Diagnostics) has detected *Listeria* at a cell concentration of 1×10^3 cfu/ml (Betts *et al.*, 1992).

The detection limits of the EiaFoss *Listeria* Detection System appear, therefore, to be equivalent to those of most other ELISAs.

Specificity and exclusivity

Detection of all *Listeria* species by the EiaFoss was demonstrated in the sensitivity experiments. Thirty strains of *Listeria* (5 of each species) were tested and 29 were detected (Table 1). The exception was one strain of *L. innocua* (CRA 6132) as discussed above.

Results of 10 non-*Listeria* species tested with the EiaFoss *Listeria* Detection System are shown in Table 2. *Erysipelothrix rhusiopathiae* (CRA 2069) gave a positive signal when tested at a cell concentration of 1.8×10^8 cfu/ml in TSYEB. No organisms were recovered on TSYEA from the broth and attempts to re-culture the organism were unsuccessful. The test was repeated with another isolate obtained from the same mother culture, and the result was negative.

Cross-reaction of *E. rhusiopathiae* with the EiaFoss *Listeria* has been recorded (F. Hansen, formerly Foss Electric, Denmark personal communication). The organism is closely related to *Listeria*, but it is not commonly reported in foods. Therefore this result should not be of great concern.

The remaining organisms tested gave negative signals at cell concentrations of approximately 10^8 cfu/ml (Table 2).

Inoculated foods

Ten foods were inoculated singly with one strain of each of five *Listeria* species, at inoculum levels of <50 cfu/per 25g sample. One sample was repeated (milk powder inoculated with *L. innocua*) because inoculum levels in the initial experiment were found to be very low (<1 cfu/25g). Thus a total of 51 inoculated foods and 11 uninoculated control samples were tested. Results of *Listeria* detected from inoculated foods by all detection methods are shown in Table 3. Presumptive and confirmed results from each method are summarised in Table 5.

The EiaFoss protocols detected *Listeria* in 42 inoculated samples and all were confirmed. A further 4 samples were found to contain *Listeria* by conventional cultural analysis of the EiaFoss enrichment cultures, but failed to give a positive signal from the EiaFoss. *Listeria* was detected in 45 samples by the USDA-based cultural method. *Listeria* was detected in the uninoculated samples of four foods (cooked ham, smoked sausage, lasagne and prawns) but was only detected in the cooked ham using the USDA-based protocol. The EiaFoss failed to detect *Listeria* in any inoculated samples from the potato salad and *Listeria* was only detected from one sample by cultural methods.

Naturally contaminated foods

A total of 61 potentially naturally contaminated foods were tested, including 11 control samples from the inoculated foods study. Results of *Listeria* detected by all protocols are shown in Table 3 (uninoculated control samples) and Table 4, and presumptive and confirmed results are summarised in Table 5.

The EiaFoss system detected *Listeria* in 22 of these samples. One sample (smoked haddock), gave a positive signal from the EiaFoss but the result could not be confirmed culturally (Table 4). Five samples generated negative ELISA results yet *Listeria* was found to be present by conventional analysis of the EiaFoss enrichment cultures. The USDA-based cultural procedure detected *Listeria* in 15 naturally contaminated food samples.

It is interesting to note that on 11/27 (41%) occasions where *Listeria* was isolated from naturally contaminated foods, more than one species was present (Tables 3 and 4).

Results, particularly from naturally contaminated foods, suggest that the EiaFoss enrichment procedures are superior to the USDA-based enrichment procedure. In order to study more closely the effect of the enrichment procedures on separate food types, results from various food categories have been summarised in Table 5. For this purpose, results from inoculated and naturally contaminated foods have been combined, and the number of presumptive and confirmed *Listeria* detections from each method are summarised for each food category.

Although the EiaFoss gave fewer positive signals from the *Listeria*-inoculated samples compared with USDA cultural procedures, overall the EiaFoss assay gave more positive *Listeria* detections, particularly with naturally contaminated samples. Cultural procedures generally tend to be more sensitive than immunological methods. For example, 10 colonies resulting from a loopful of broth streaked onto an agar plate equates to approximately 10^3 cfu/ml in the broth. The sensitivity of the EiaFoss *Listeria* detection system has been shown to be approximately 10^5 cfu/ml. Cultural methods, however, are lengthy and laborious, taking up to 5 days to obtain a presumptive positive result, whereas the EiaFoss can give a presumptive result on the second day of analysis.

Analysis of results

Method agreement between the EiaFoss and cultural methods was calculated as the percentage of samples giving equivalent results by both methods (Table 6). Positive deviation by the EiaFoss was calculated as the percentage of cultural negative samples which were positive by the EiaFoss. Negative deviation by the EiaFoss was calculated as the percentage of cultural positive samples which were negative by the EiaFoss.

Considering all food samples, method agreement between the EiaFoss and cultural USDA-based methods was 85.7% (Table 6). There were 10 (19.2%) EiaFoss positive deviations. Of these 10 samples, by cultural analysis of EiaFoss enrichment cultures, 9 were confirmed positive results. The EiaFoss gave 6 negative deviations (10%).

Thus, overall, the EiaFoss detected *Listeria* in more samples than the USDA-based cultural method.

TABLE 1 SENSITIVITY OF THE EIAFOSS *LISTERIA* DETECTION SYSTEM WITH FIVE TEST ISOLATES OF EACH SPECIES

Listeria species	Range of target cell concentrations (cfu/ml) necessary to generate a positive EiaFoss signal, depending on isolate	Mean minimum concentration of target cells (cfu/ml) necessary to generate a positive EiaFoss Signal
L. monocytogenes	1.3×10^5 - 9.1×10^6	2.3×10^6
*L. innocua**	1.7×10^4 - 1.6×10^5	1.1×10^5
L. welshimeri #	1.2×10^5 - 1.4×10^6	5.2×10^5
L. seeligeri $	1.3×10^5 - 1.9×10^5	1.5×10^5
L. ivanovii	1.5×10^4 - 9.7×10^5	2.4×10^5
L. grayi	1.5×10^4 - 1.6×10^5	9.5×10^4

* Data excludes results from *L. innocua* CRA 6132 which was inconsistently detected by the EiaFoss (see text).

\# *L. welshimeri* CRA 6175 gave a positive result at a concentration of 1.4×10^3 cfu/ml but not at 1.4×10^4 cfu/ml. This data is not shown.

$ *L. seeligeri* CRA 1820 gave positive results at concentrations of 1.4×10^2 and 1.4×10^3 cfu/ml but not 1.4×10^4 cfu/ml. These results are not shown on this table.

TABLE 2 EXCLUSIVITY OF THE EIAFOSS *LISTERIA* DETECTION SYSTEM

Organism	CCFRA code	TSBYE cfu/ml	EiaFoss result
Bacillus mycoides	1510	1.3×10^8	-
Carnobacterium divergens	2072	1.2×10^8	-
Carnobacterium gallinarum	2071	1.6×10^8	-
Carnobacterium pisicola	2058	1.3×10^8	-
Erysipelothrix rhusiopathiae	2069	1.8×10^8	+/-*
Erysipelothrix rhusiopathiae	6983	1.0×10^8	-
Lactobacillus casei	3373	1.2×10^8	-
Lactobacillus lactis	751	1.5×10^8	-
Lactobacillus plantarum	561	1.2×10^8	-
Micrococcus species	149	1.3×10^8	-
Staphylococcus xylosus	1529	1.1×10^8	-

*Positive result but negative on repeat analysis using another culture of the same strain.

TSBYE = Tryptone Soya Broth + 0.6% yeast extract

TABLE 3: RESULTS OF *LISTERIA* DETECTED FROM INOCULATED FOODS BY EIAFOSS AND CULTURAL DETECTION METHODS

FOOD	INOCULUM		EIAFOSS DAIRY PROTOCOL				CULTURAL USDA-BASED PROTOCOL		
	SPECIES	cfu/ 25g	EIAFOSS ELISA	MOX	PAL	CONFIRMED	MOX	PAL	CONFIRMED
Dairy Foods									
Milk Powder	*L. monocytogenes*	17	+	+	+	+	+	+	+
	L. innocua	<1	+	+	+	+	+	+	+
	L. grayi	13	+	+	+	+	+	+	+
	L. ivanovii	12	+	+	+	+	+	+	+
	L. seeligeri	17	-	-	+b	+	-	-	NA
	Uninoculated	0	-	-	-	NA	-	-	NA
Milk Powder	*L. innocua*	17	+	+a	+	+	+a	+	+
	Uninoculated	0	-	-	-	NA	-	-	NA
Camembert	*L. monocytogenes*	17	+	+	+	+	+	+	+
	L. innocua	<1	+	+	+	+	+	+	+
	L. grayi	13	+	+	+	+	+	+	+
	L. ivanovii	12	-	+	+	+	+	+	+
	L. seeligeri	17	+	+a	+	+	+a	+	+
	Uninoculated	0	-	-	-	NA	-	-	NA
Soft Blue	*L. monocytogenes*	5	+	+	+	+	-	-	NA
Cheese	*L. innocua*	8	+	+	+	+	+	+	+
	L. grayi	13	+	+	+	+	+	+	+
	L. ivanovii	15	-	-	-	NA	+a	+	+
	L. seeligeri	17	+	-	+	+	-	+a	+
	Uninoculated	0	-	-	-	NA	-	-	NA
Hard Cheese	*L. monocytogenes*	5	+	+	+	+	+	+	+
	L. innocua	8	+	+	+	+	+a	+a	+
	L. grayi	13	+	+	+	+	+	+	+
	L. ivanovii	15	+	+a	+	+	+a	+	+
	L. seeligeri	17	+	-	+	+	+	+	+
	Uninoculated	0	-	-	-	NA	-	-	NA
Processed Meat Products									
Cooked Ham	*L. monocytogenes*	19	+	+	+	+	+	+	+
	L. innocua	8	+	+	+	+	+	+	+
	L. grayi	19	+	+	+	+	+	+	+
	L. ivanovii	20	+	+	+	+	+	+	+
	L. seeligeri	22	+	+	+	+	+	+	+
	Uninoculated	0	+	+	+	+ (*L. innocua*)	+	+	+ (*L. innocua*)

TABLE 3 CONTINUED: RESULTS OF *LISTERIA* DETECTED FROM INOCULATED FOODS BY EIAFOSS AND CULTURAL DETECTION METHODS

FOOD	INOCULUM		EIAFOSS ISO-BASED PROTOCOL				CULTURAL USDA-BASED PROTOCOL		
	SPECIES	cfu/ 25g	EIAFOSS ELISA	MOX	PAL	CONFIRMED	MOX	PAL	CONFIRMED
Lasagne	*L. monocytogenes*	17	+	+	+	+	+	+	+
	L. innocua	<1	+	+	+	+	+	+	+
	L. grayi	13	+	+	+	+	+	+	+
	L. ivanovii	15	+	+	+	+	+	+	+
	L. seeligeri	17	-	+	+	+	-	+	+
	Uninoculated	0	+	+	+	+ (*L. ivanovii & L. innocua)*	-	-	NA
Smoked Sausage	*L. monocytogenes*	19	+	+	+	+	-	+	+
	L. innocua	8	+	+	+	+	+	+	+
	L. grayi	19	+	+	+	+	+	+	+
	L. ivanovii	20	+	+a	+	+	-	+	+
	L. seeligeri	22	+	+	+	+	+	+	+
	Uninoculated	0	+	+	+	+ (*L. innocua & L. grayi)*	-	-	NA
Fish									
Prawns	*L. monocytogenes*	5	+	+	+	+	+	+	+
	L. innocua	8	+	+	+	+	+a	+a	+
	L. grayi	13	+	+	+	+	+a	+a	+
	L. ivanovii	15	+	+a	+	+	+a	+a	+
	L. seeligeri	17	+	+	+	+	-	+a	+
	Uninoculated	0	+	+	+a	+ (*L. innocua)*	-	-	NA
Fish Pie	*L. monocytogenes*	19	+	+	+	+	+	+	+
	L. innocua	8	+	+	+	+	+	+	+
	L. grayi	19	+	+	+	+	+	+	+
	L. ivanovii	20	+	+	+	+	+a	+	+
	L. seeligeri	22	+	-	+	+	-	+	+
	Uninoculated	0	-	-	-	NA	-	-	NA
Vegetables									
Potato Salad	*L. monocytogenes*	31	-	-	-	NA	-	-	NA
	L. innocua	17	-	+a	+a	+	+a	+a	+
	L. grayi	22	-	-	-	NA	-	-	NA
	L. ivanovii	17	-	-	-	NA	-	-	NA
	L. seeligeri	24	-	-	-	NA	-	-	NA
	Uninoculated	0	-	-	-	NA	-	-	NA

MOX MOX Agar
PAL Palcam Agar
+a <10 colonies per plate
+b <100 colonies per plate
NA Not applicable

TABLE 4: RESULTS OF *LISTERIA* DETECTED FROM POTENTIALLY NATURALLY CONTAMINATED FOODS BY EIAFOSS AND CULTURAL DETECTION METHODS

FOOD	EIAFOSS DAIRY PROTOCOL				CULTURAL USDA-BASED PROTOCOL		
	EIAFOSS ELISA	MOX	PAL	CONFIRMED	MOX	PAL	CONFIRMED
Dairy Foods							
Brie	+	+	+a	+ *L. innocua*	-	-	NA
Soft Blue Cheese	-	-	-	NA	-	-	NA
Stilton	-	-	-	NA	-	-	NA
Brie	-	-	-	NA	-	-	NA
Camembert	-	-	-	NA	-	-	NA
Danish Blue Cheese	-	-	-	NA	-	-	NA
Raw Meat Products	ISO-BASED PROTOCOL						
Minced Beef	+	+	+a	+ *L. welshimeri*	+	+	+ *L. monocytogenes*
Minced Beef	-	-	-	NA	-	-	NA
Minced Beef	+	+	+	+ *L. welshimeri*	+	+	+ *L. monocytogenes*
Minced Beef	+	+	+	+ *L. innocua*	+	+	+ *L. innocua*
Diced Beef	-	+	+	+ *L. innocua*	-	-	NA
Braising Steak	-	+	+	+ *L. monocytogenes*	-	-	NA
Minced Pork	+	+	+	+ *L. welshimeri*	+	+	+ *L. innocua*
Minced Pork	+	+a	+a	+ *L. innocua*	+a	+	+ *L. innocua*
Diced Pork	+	+	+	+ *L. welshimeri*	-	-	-
Pork Sausages	+	+	+	+ *L. welshimeri*	+	+	+ *L. innocua*
Pork Sausages	+	+	+	+ *L. innocua*	+	+	+ *L. innocua*
Pork Sausage Meat	-	+	+a	+ *L. monocytogenes*	-	-	NA
Bacon Burgers	+	+	+	+ *L. monocytogenes*	+	+	+ *L. welshimeri*
Chicken	-	-	-	NA	-	-	NA
Chicken Burgers	+	+	+	+ *L. innocua*	+	+	+ *L. monocytogenes*
Chicken Escalopes	-	+a	+a	+ *L. monocytogenes*	+	+	+ *L. monocytogenes*
Minced Turkey	+	+	+	+ *L. monocytogenes*	-	-	NA
Turkey and Cheese Burger	+	+	+	+ *L. seeligeri* + *L. ivanovii*	-	-	NA
Pig's Liver	-	-	-	NA	-	-	NA
Lamb's Kidney	-	-	-	NA	-	-	NA
Ox Kidney	+	+	+	+ *L. monocytogenes*	+	+	+ *L. monocytogenes*

TABLE 4 CONTINUED: RESULTS OF *LISTERIA* DETECTED FROM POTENTIALLY NATURALLY CONTAMINATED FOODS BY EIAFOSS AND CULTURAL DETECTION METHODS

FOOD	EIAFOSS ISO-BASED PROTOCOL				CULTURAL USDA-BASED PROTOCOL		
	EIAFOSS ELISA	MOX	PAL	CONFIRMED	MOX	PAL	CONFIRMED
Processed Meat Products							
Cumberland Pie	+	+	+	+ *L. innocua*	+	+	+ *L. innocua*
Spaghetti Bolognese	-	-	-	NA	-	-	NA
Lasagne	-	-	-	NA	-	-	NA
Chicken Hot Pot	-	-	-	NA	-	-	NA
Salami	-	-	-	NA	-	-	NA
Fish							
Raw Cod	-	-	-	NA	-	-	NA
Raw Cod	-	-	-	NA	-	-	NA
Raw Cod In Batter	+	+	+	+ *L. monocytogenes*	+	+	+ *L. innocua*
Smoked Haddock	+	-	-	-	-	-	NA
Kipper	-	+a	+a	+ *L. innocua*	+	+	+ *L. monocytogenes*
Tuna and Pasta Bake	-	-	-	NA	-	-	NA
Raw Vegetables							
Mixed Vegetables	-	-	-	NA	-	-	NA
Mixed Vegetables	-	-	-	NA	-	-	NA
Mixed Vegetables	-	-	-	NA	-	-	NA
Mixed Vegetables	-	-	-	NA	-	-	NA
Mixed Vegetables	-	-	-	NA	-	-	NA
Salad	-	-	-	NA	-	-	NA
Salad	-	-	-	NA	-	-	NA
Beansprouts	+	+	+	+ *L. ivanovii*	-	-	NA
Beansprouts	-	+	+	+ *L. welshimeri*	-	-	NA
Mushrooms	-	-	-	NA	-	-	NA
Flour	-	-	-	NA	-	-	NA
Flour	-	-	-	NA	-	-	NA

MOX Mox Agar
PAL Palcam Agar
+a <10 colonies per plate
+b <100 colonies per plate
NA Not applicable

TABLE 5: RESULTS OF NUMBER OF SAMPLES WHERE *LISTERIA* DETECTED FROM DIFFERENT FOOD CATEGORIES BY EIAFOSS AND CULTURAL DETECTION METHODS

FOOD TYPE	NO. SAMPLES TESTED	EIAFOSS ANALYSIS				USDA-BASED	
		EIAFOSS		CULTURAL+		CULTURAL ANALYSIS	
		P	C	P	C	P	C
Dairy	32	19	19	21	21	19	19
Raw Meat	21	13	13	17	17	11	11
Processed Meat	23	18	18	19	19	17	17
Fish	18	13	12	13	13	12	12
Vegetables	16	1	1	3	3	1	1
Flour	2	0	0	0	0	0	0
Total Inoculated Foods	51	42	42	46	46	45	45
Total Naturally Contaminated Foods	61*	22	21	27	27	15	15
Total Foods (% positive)	112	64	63 (56%)	73	73 (65%)	60	60 (54%)

\+ Cultural analysis of EiaFoss enrichment cultures.

* Includes 11 control samples from inoculated foods study

P Presumptive positive result

C Confirmed positive result

TABLE 6: METHOD AGREEMENT BETWEEN EIAFOSS AND CULTURAL (USDA-BASED) METHODS

Result from Detection Method		**EiaFoss Analyser Signal**	
		+	-
USDA Based Cultural Method	+	54	6
	-	10	42

Method agreement = $\frac{96}{112}$ x 100 = 85.7%

EiaFoss +ve deviation = $\frac{10}{52}$ x 100 = 19.2%

EiaFoss -ve deviation = $\frac{6}{60}$ x 100 = 10.0%

CONCLUSION

The EiaFoss *Listeria* system is a convenient automated ELISA for the rapid detection of *Listeria* in foods. Presumptive positive results are obtained on the second day of analysis, compared with the fourth or fifth days for standard cultural methods.

The EiaFoss enrichment procedures overall produced more *Listeria* positive samples, both culturally and by the EiaFoss, than the USDA-based cultural method. However, both the EiaFoss and USDA cultural methods failed to detect between 9% (EiaFoss) and 14% (USDA) of all the positive samples. The EiaFoss *Listeria* system provides a suitable rapid detection method for *Listeria* in foods, provided the recommended ISO-based and dairy protocols are adhered to.

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