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SUMMARY

The EiaFoss *Campylobacter* System is an automated ELISA for the rapid detection of *Campylobacter* in foods.

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

Sensitivity of the system was approximately 10^5 cfu/ml. Specificity of the system was found to be satisfactory; all *C. jejuni*, *C. coli*, *C. lari* and *C. fetus* subsp. *fetus* tested were detected. *C. hyointestinalis*, *C. upsaliensis*, *C. helveticus* and *C. mucosalis* strains tested either failed to grow or grew poorly in the EiaFoss enrichment system.

Detection of *Campylobacter* from foods by the EiaFoss System was equivalent to detection of *Campylobacter* by the ISO cultural method. The EiaFoss detected *Campylobacter* in foods on more occasions than the FDA-BAM cultural method. False positive results from certain foods may be overcome by centrifugation of the samples prior to the EiaFoss assay.

CONTENTS

INTRODUCTION

Campylobacter has emerged as the most frequent cause of bacterial gastroenteritis in man. In the UK in 1981 there were 12,168 reported cases, rising to 43,912 in 1995. *C. jejuni* and, to a lesser extent, *C. coli*, are the species most commonly identified as causing infection, with *C. lari* being implicated on rare occasions. The bacteria are commensals in the intestinal tract of cattle, sheep, pigs and birds, and consequently foods of animal origin can become contaminated. The majority of *Campylobacter* infections are acquired through the consumption of contaminated food or water; identified vehicles of infection include untreated or contaminated water, raw and inadequately pasteurised milk, and undercooked meats, particularly poultry. The infectious dose of *Campylobacter* is thought to be as low as 500 organisms.

Campylobacter species require an oxygen-reduced atmosphere (5% oxygen) for growth, and the thermophilic species, *C. jejuni, C. coli* and *C. lari* do not grow below 30°C, having an optimum temperature of 42-43°C. Mesophilic species are able to grow at 25-30°C, but not at 42°C. It is unlikely, therefore, that *Campylobacter* are able to grow in raw foods held under normal storage conditions. They can, however, survive in refrigerated foods, and indeed are inactivated much more rapidly in foods held at ambient temperature than in those held at lower temperatures (Betts, 1997a). *Campylobacter* is sensitive to freezing, drying, heat, organic acids, in particular lactic acid, and common disinfecting agents (Betts, 1997a). A major risk factor appears to be the increased use of modified atmosphere packaging and vacuum packaging. Survival of *C. jejuni* in foods, especially at 4°C, is greatly enhanced when oxygen is removed from the atmosphere (Tomancova *et.al.*, 1991).

Conventional methods for detection of *Campylobacter* in foods involve sample enrichment followed by plating onto selective media and biochemical confirmation. Enrichment media and selective agars have continued to develop during the last two decades. Enrichment media contain antibiotics to suppress the growth of competing organisms such as enterococci. Typically cefoperazone, cycloheximide, trimethoprim, rifampocin, vancomycin and polymyxin B are used in various combinations. In an attempt to aid resuscitation of sub-lethally damaged cells, enrichment procedures have developed where exposure to selective agents (antibiotics) is delayed until after a period of pre-enrichment at 37°C (Humphrey, 1986a and 1986b, and Hunt, 1992). More recently Bolton Broth has been developed, designed both to aid recovery of injured cells and to avoid the need for a microaerobic atmosphere (Post).

In 1977 Skirrow developed a selective isolation medium for *Campylobacter.* Further developments have led to a range of available agars; for example, modified Charcoal *Campylobacter* Deoxycholate Agar (CCDA) and Abeyta-Hunt Agar are recommended by the FDA-BAM (8th edition) method (Hunt and Abeyta, 1995), whilst the ISO method (Anon, 1995) recommends Karmali, modified Butzler, Skirrow, CCDA and Preston Agars.

In more recent years, rapid methods have developed for the detection of *Campylobacter* in foods, e.g. nucleic acid hybridisation probes such as Gene Trak and Gen-Probe Accuprobe, latex agglutination tests, and ELISAs (Betts, 1997b).

Foss Electric has developed the EiaFoss *Campylobacter* System for the detection of *Campylobacter*. The EiaFoss is an automated detection system which utilises immunomagnetic separation combined with automated ELISA, and is already established for the detection of *Salmonella* (Jones and Betts, 1994) and *Listeria* species (MacPhee *et al.*, 1997). Pre-enriched food samples are heat-treated to denature the *Campylobacter* antigens. Polyclonal antibodies specific to *Campylobacter* species are bound to magnetic microspheres (magnetic beads). The beads are added to the treated sample, target cells are captured from the sample, and non-bound material is washed away, the particles being immobilised on the side of the reaction tube by magnetic force.

A second polyclonal antibody labelled with alkaline phosphatase is added, which binds to the captured antigen. The substrate, 4-methyl-umbelliferylphosphate, reacts with alkaline phosphatase to produce 4-methylumbelliferone. The end product is measured fluorimetrically. The assay is completely automated and takes approximately 2 hours.

In this study, sensitivity, specificity and exclusivity of the EiaFoss *Campylobacter* System has been investigated, using a wide range of *Campylobacter* species and other microorganisms. Additionally, the ability of the system to detect *Campylobacter* from inoculated and naturally contaminated foods has been compared with the ISO and FDA-BAM recommended cultural procedures.

MATERIALS AND METHODS

Sensitivity

Three strains of *Campylobacter jejuni* (NCTC 11351, 11168 and 11392) and two strains of *C. coli* (NCTC 11366 and 11350) were cultured in blood-free *Campylobacter* Enrichment Broth (CEB; LabM LAB135 and X131) incubated microaerobically at 41°C±1°C for 24-48h. Cultures were serially diluted in CEB, and 5ml aliquots of each dilution were transferred to glass universal bottles and boiled for 15 minutes. Aliquots (200µl) of each heated culture were tested with the EiaFoss *Campylobacter* assay according to the manufacturer's instructions.

Cell numbers were confirmed by plating the unheated portion of each culture onto Blood Agar Base No. 2 plus 5% defibrinated horse blood (BA; Oxoid CM271 and SR50). Plates were incubated microaerobically at 41°C±1°C for 48h.

Specificity and exclusivity

C. jejuni and *C. coli* from 25 Penner serotypes (Table 2), *C. lari* (NCTC 11352 and 11937), *C. upsaliensis* (NCTC 11541 and 11540), *C. helveticus* (NCTC 12470 and 12471), *C. fetus* subsp. *fetus* (NCTC 10842 and 5850), *C. mucosalis* (NCTC 11000 and 11001), *C. hyointestinalis* (NCTC 11608 and 11562) and 48 non-*Campylobacter* organisms (Table 3) were cultured in CEB incubated microaerobically at 41°C±1°C for 24-48h.

Cultures were heat-treated and tested with the EiaFoss *Campylobacter* System. Cell concentrations were confirmed by plate counts from the unheated portion of culture on BA or Nutrient Agar (NA; Oxoid CM3).

Organisms which failed to grow in the enrichment system were cultured in CEB without antibiotics or Nutrient Broth No. 2 (NB No. 2; Oxoid CM67), under optimum growth conditions, and retested.

Detection of *Campylobacter* from food samples

Inoculated foods

Campylobacter jejuni (NCTC 11392) was cultured in CEB incubated microaerobically at 41°C±1°C for up to 48h. Appropriate dilutions of the culture were inoculated into foods (Appendix 3) at target inoculum levels of approximately 5 cfu/25g and 50 cfu/25g sample. Inoculum levels were confirmed by plate counts on BA. Uninoculated control samples were also prepared.

Naturally contaminated foods

Foods which potentially could contain *Campylobacter* (raw poultry, raw chicken livers, other raw meats, meat products, offal and cottage cheese) were obtained from local retail outlets. Foods were stored at 2-8°C prior to analysis. A total of 60 food samples were tested.

Detection Methods

All food samples were tested for the presence of *Campylobacter* by the following methods:-

- EiaFoss *Campylobacter* System (Fig. 1)
- FDA-BAM cultural method (Fig. 2)
- ISO cultural method (Fig. 3)

EiaFoss method for detection of Campylobacter from foods (Fig. 1)

Food samples (25g) were stomached in CEB (225ml) for 2 minutes, then transferred to a 300ml jar. Samples were enriched at 41°C±1°C for 48h. An aliquot (10ml) was removed and placed in a glass Universal bottle, and heated at 100°C for 15 minutes. After allowing samples to cool to room temperature, the EiaFoss assay was carried out according to the manufacturer's instructions. Aliquots (1ml) of a proportion of heated samples were centrifuged at 6500rpm for 3 minutes prior to testing with the EiaFoss assay.

A loopful of the unheated enrichment culture was streaked onto CCDA. Plates were incubated microaerobically at 42°C for 48h. After incubation, plates were examined for characteristic colonies:- grey, green or cream, circular or spreading, opaque or slightly translucent. Up to 5 typical colonies were selected for confirmation. Colonies were streaked onto Blood Agar (BA) and incubated at 37°C for 24-48 hours. Colonies on BA were confirmed as *Campylobacter* by Gram-stain, catalase and oxidase tests and API Campy (bioMérieux).

FDA-BAM cultural method for detection of Campylobacter from foods (Hunt, J.M. 1992) (Fig. 2)

Food samples (25g) were weighed into filter stomacher bags and 225ml *Campylobacter* Enrichment Broth (Nutrient Broth No. 2 + 0.6% yeast extract + ferrous sulphate-bisulphite-pyruvate (FBP) supplement (Oxoid SR84) + 5% defibrinated horse blood (Oxoid SR50)) was added, together with 15mg/l sodium cefoperazone, 12.5mg/l trimethoprim lactate, 10mg/l vancomycin and 100mg/l cycloheximide (Appendix 1). For dairy foods the vancomycin was omitted and rifampicin (10mg/l) was added. Samples were stomached and transferred to a 300ml jar. Broths were incubated at 37°C for 18-24h, then at 42°C for 24h, under microaerobic conditions.

After incubation, a loopful of each broth was streaked onto CCDA. Plates were incubated at 42°C for 48h. Up to 5 characteristic colonies were chosen for confirmation by Gram-stain, catalase and oxidase tests, and API Campy, as previously described.

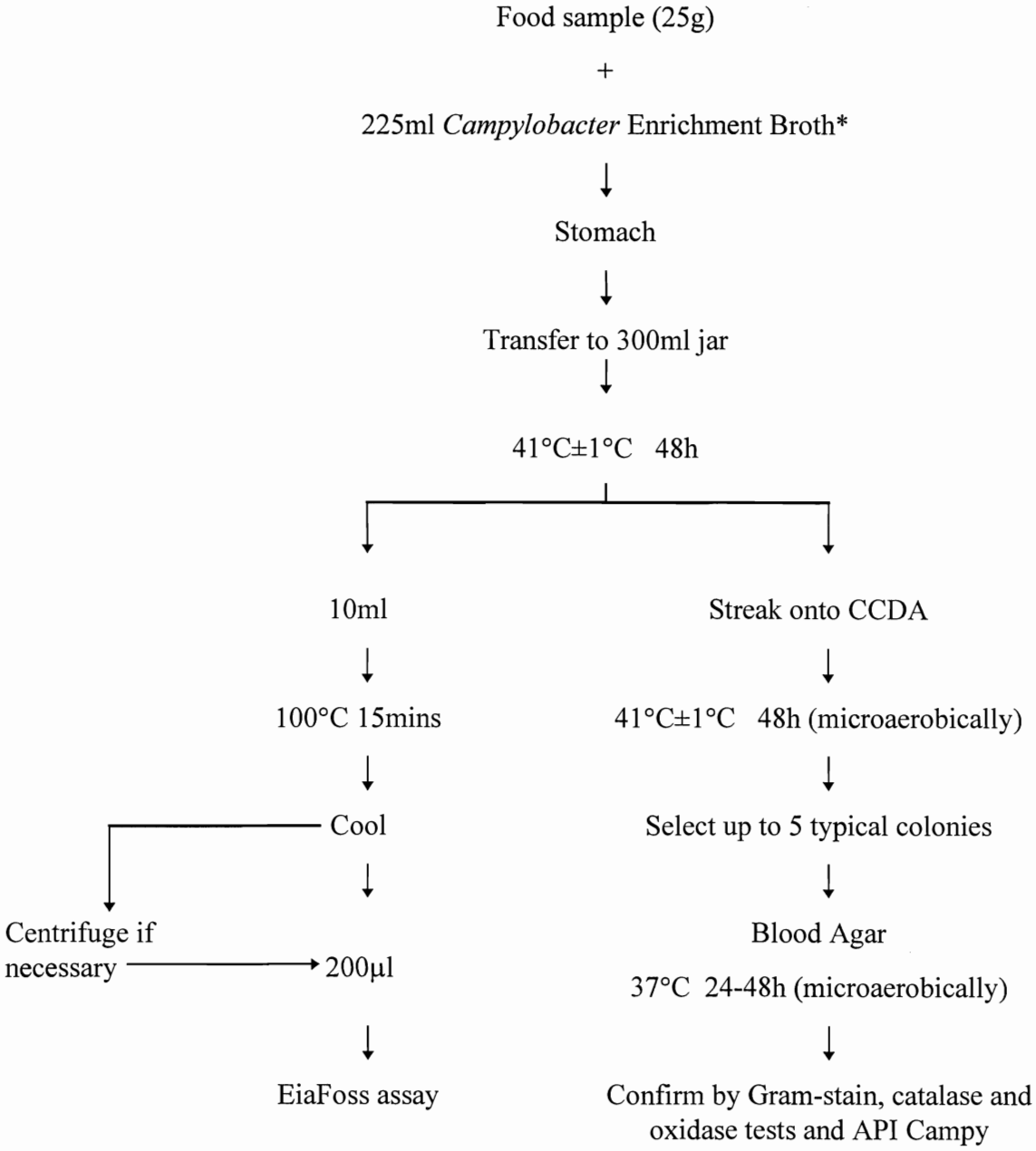
ISO cultural method (part method) for detection of Campylobacter from foods (ISO/CD 10272) (Fig. 3)

Food samples (25g) were stomached in 225ml Preston Broth (Appendix 2), then transferred to 300ml jars. Broths were incubated at 42°C for 18h, under microaerobic conditions.

After incubation, a loopful of Enrichment Broth was streaked onto Karmali Agar (Oxoid CM908 and SR139) and Butzler Agar (Oxoid CM331 and SR85 + 5% defibrinated horse blood). Plates were incubated microaerobically at 42°C for 48-72h.
Following incubation, up to 5 typical colonies per agar were selected for confirmation. Colonies were isolated on Blood Agar and confirmed as described previously.

Figure 1

EiaFoss method for detection of *Campylobacter* from foods



* *Campylobacter* Enrichment Broth (LabM LAB135) + supplement (LabM X131) without blood.

Figure 2

FDA-BAM cultural method for detection of *Campylobacter* from foods

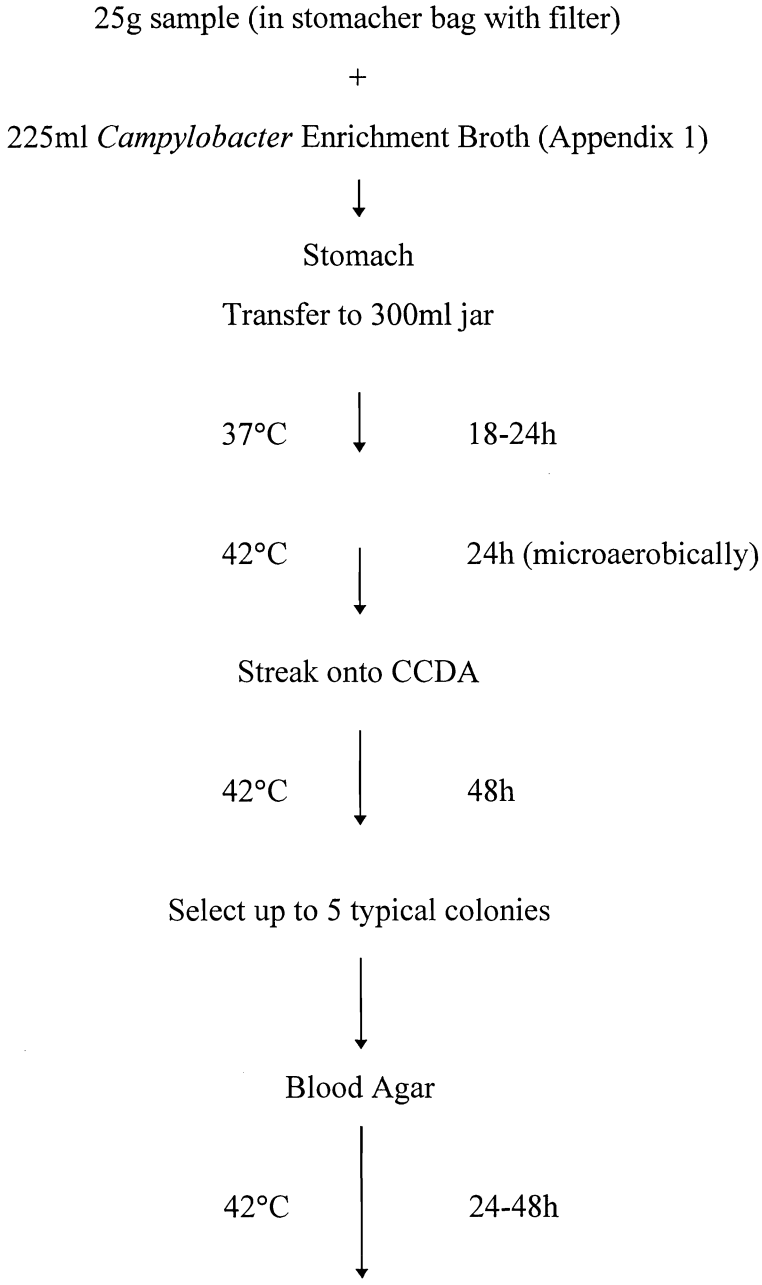
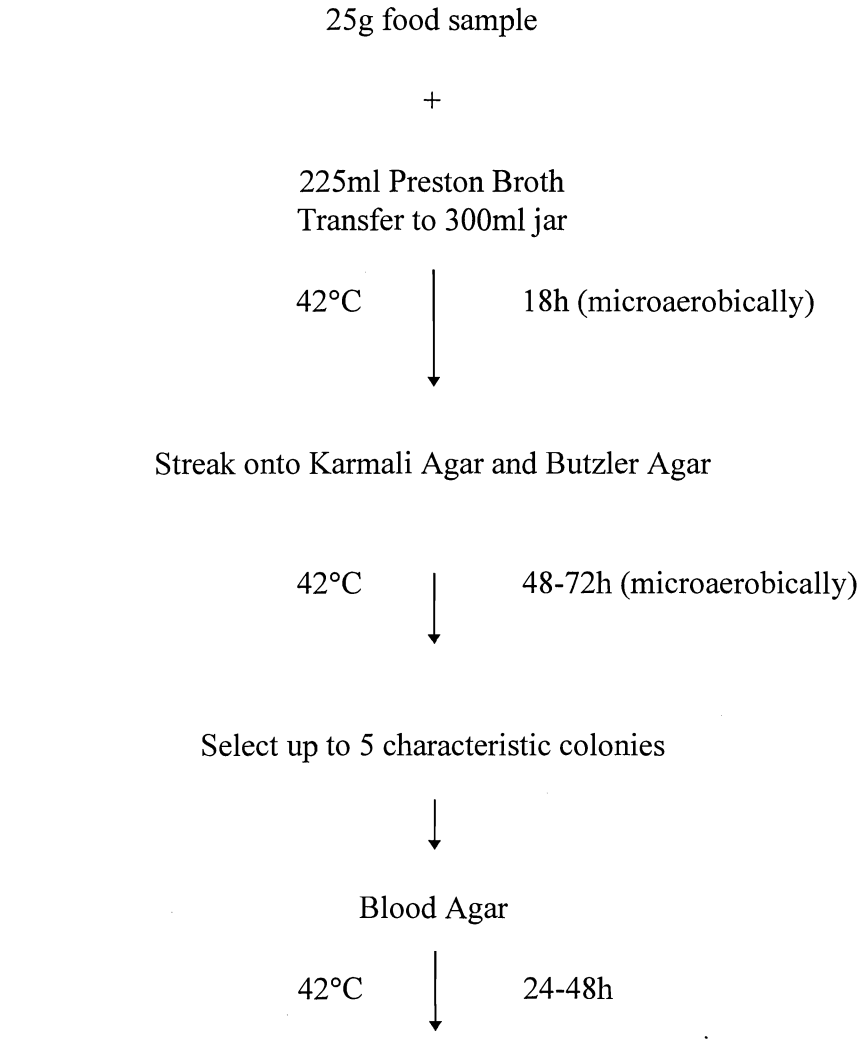


Figure 3

ISO/CD 10272 Cultural method (part method) for detection of *Campylobacter* from foods



RESULTS AND DISCUSSION

Sensitivity

The sensitivity of the EiaFoss *Campylobacter* detection system was determined by testing serially diluted pure cultures of *Campylobacter.* Three strains of *C. jejuni* and two strains of *C. coli* were tested. Results are shown in Table 1. The sensitivity of the system was found to be approximately 10^5 cfu/ml.

Specificity

C. jejuni (12 strains), *C. coli* (15 strains) and two strains of each of *C. lari, C. fetus* subsp. *fetus*, *C. hyointestinalis, C. upsaliensis, C. helveticus* and *C. mucosalis* were cultured in the EiaFoss enrichment broth (CEB) at 42°C, and tested with the EiaFoss *Campylobacter* detection system. Results are shown in Table 2.

All *C. jejuni, C. coli, C. lari* and *C. fetus* subsp. *fetus* tested were detected. *C. helveticus* and *C. mucosalis* failed to grow in the enrichment system. *C. hyointestinalis* and *C. upsaliensis* were not detected by the EiaFoss at cell concentrations of $5.0x10^5$ and $2.0x10^5$ cfu/ml respectively. These cell concentrations may have been just below the sensitivity threshold of the EiaFoss. The organisms were cultured again for repeat testing, but failed to grow in the enrichment broth. The other strains of *C. hyointestinalis* and *C. upsaliensis* tested also failed to grow in the EiaFoss enrichment system (Table 2). The EiaFoss enrichment has been developed to recover thermophilic strains of *Campylobacter* (*C. jejuni, C. coli* and *C. lari*), and therefore may not favour growth of the mesophilic strains which have a lower optimum temperature. In order to detect the mesophilic strains of *Campylobacter* with the EiaFoss *Campylobacter* system, it may be necessary to modify the enrichment of the food sample using a lower incubation temperature.

Exclusivity

Non-*Campylobacter* organisms (48 species) were cultured in the EiaFoss enrichment system, and tested with the EiaFoss *Campylobacter* assay. Results are shown in Table 3.

A total of 17 organisms grew in the enrichment system to cell levels ranging from $1.2x10^6$ cfu/ml (*E. coli*) to $9.5x10^8$ cfu/ml (*S. typhimurium*), but none were detected by the EiaFoss assay. Organisms which failed to grow in the enrichment system were cultured by one of the following methods until growth was evident (up to 3 days):-

- In EiaFoss CEB at optimum temperature.
- In CEB without supplement at 42°C.
- In CEB without supplement at optimum temperature.
- In NB No. 2 at optimum temperature.

All organisms grew to cell levels greater than 10^6 cfu/ml, but none were detected by the EiaFoss assay (Table 3).

EiaFoss non-centrifuged method

Inoculated foods

Food samples were inoculated with *C. jejuni* at inoculum levels ranging from 0.45 cfu/ml to 47.3 cfu/ml. Results of detection of *Campylobacter* by all detection methods are summarised in Table 4.

The FDA-BAM cultural method gave 30 presumptive positive results, i.e. typical colonies on CCDA, and 20 were confirmed as *Campylobacter.* The ISO cultural method produced 33 presumptive positive results and 27 were confirmed (Table 4).

The EiaFoss assay gave positive results from 32 food samples, and 30 were confirmed culturally. Two samples were positive with the EiaFoss assay and typical colonies were isolated on CCDA, but the isolates were not confirmed as *Campylobacter* (Table 4). Cultural analysis of the EiaFoss enrichment broth resulted in presumptive positive isolates on CCDA from 41 samples, and 36 were confirmed as *Campylobacter*. Therefore, the EiaFoss failed to detect *Campylobacter* from 6 enrichment broths which contained viable organisms.

Results from inoculated foods indicate that the EiaFoss enrichment procedure is preferable for recovery of *Campylobacter.* Consequently the EiaFoss detection system recovered *Campylobacter* from more inoculated samples than the other test methods assessed.

Uninoculated foods

Results of detection of *Campylobacter* from potentially naturally contaminated foods (60 samples) together with results from uninoculated control samples analysed in the inoculated foods study (25 samples) are summarised in Table 4. A total of 85 uninoculated food samples were tested.

The FDA-BAM cultural method gave presumptive positive isolates from 23 samples, and 15 were confirmed as *Campylobacter*. The ISO cultural method gave presumptive positive results from 25 samples, 20 of which were confirmed as *Campylobacter*.

The EiaFoss assay gave positive results from 30 samples, but only 15 were confirmed positive. A further 4 samples which were negative by the EiaFoss assay were found to contain *Campylobacter* by cultural analysis.

Considering all food samples analysed (135 samples), the ISO and FDA-BAM cultural methods and the EiaFoss detected *Campylobacter* in 47, 35 and 45 samples respectively (Table 4). Cultural analysis of the EiaFoss Enrichment Broth, however, detected *Campylobacter* in a further 10 samples which were negative with the EiaFoss assay. The EiaFoss enrichment system was more effective at recovering *Campylobacter* than the other enrichments tested, but in ten samples cell numbers did not exceed the detection threshold of the EiaFoss assay (approximately 10^5 cfu/ml); however, *Campylobacter* was detected from the broths culturally. Method agreement between the EiaFoss and each reference method is shown in Tables 5a and 6a. The false-positive and false-negative rates of the EiaFoss and reference methods were calculated and are summarised in Tables 5b and 6b. All confirmed positive results from each method were assumed to be true positive results. The significance of the results was assessed using Fisher Exact Probability test. The BAM method had a significantly higher false-negative rate ($p=0.013$) than the EiaFoss method (35% and 15% false-negative rate respectively Table 5b). When the ISO and EiaFoss methods were compared, the false-negative rates were 18.5% and 18.8% respectively (Table 6b).

The EiaFoss false-positive rate was significantly higher than the BAM and ISO methods ($p=0.01$). This would be expected since only confirmed results from the BAM and ISO methods were used in the calculations. From these methods, however, a further 18 and 11 samples respectively produced presumptive positive results which were not confirmed, i.e. colonies on agar typical of *Campylobacter* in appearance which were confirmed as non-*Campylobacter* (Table 4).

EiaFoss Centrifuge Method

Results from foods indicated a potential problem of "false positive" results from the EiaFoss assay. This problem had previously been encountered by Foss Electric (Inge Knap, personal communication) and seemed to occur in foods with a high fat content, such as sausages, minced beef and pork rashers. Further experiments were carried out in order to investigate

the effect of centrifuging the enrichment broths prior to testing with the EiaFoss assay. Samples of minced beef, sausages, bacon, liver and turkey were tested for the presence of *Campylobacter* by the EiaFoss method (Fig. 1) and by the FDA-BAM method (Fig. 2). In addition, after heat treatment of the EiaFoss Enrichment Broths, aliquots (1ml) were centrifuged at 6500rpm for 3 minutes, and tested with the EiaFoss assay. Results are shown in Table 7. Without centrifuging, 24 of the initial 25 samples tested were positive with the EiaFoss assay (cut-off signal = 0.25). After centrifuging, only 2 of these samples were positive with the EiaFoss assay and results were confirmed culturally. *Campylobacter* was also detected from these two samples by the FDA-BAM method. Thus 22 samples were negative with the assay after centrifuging and 21 were confirmed negative by cultural analysis. One sample gave a "false negative" result after centrifuging; *Campylobacter* was detected from this sample by both the EiaFoss and FDA-BAM cultural procedures.

Results clearly showed the advantage of centrifuging in order to reduce the number of "false positive" results from the EiaFoss assay, assumed to be attributable to particular food types. One positive sample, however, was not detected after centrifuging. In order to investigate the effect of centrifuging on true positive results and with other food types, a wider selection of foods (20 samples) were tested for the presence of *Campylobacter* by the EiaFoss assay incorporating centrifuging, and by the FDA-BAM method as described previously. Processed foods (12 samples) were inoculated with *C. jejuni* at an inoculum level of $7.9x10^1$ cfu/25g sample; the remaining foods (8 samples) were tested for naturally occurring *Campylobacter*. Results are shown in Table 7.

Six of the 8 uninoculated samples tested were positive by the EiaFoss assay without centrifuging. After centrifuging, 3 samples gave a positive signal from the EiaFoss assay, and only these 3 samples were confirmed as containing *Campylobacter*. The FDA-BAM cultural method also detected *Campylobacter* only in these 3 samples.

Campylobacter was not detected from any of the inoculated samples. The inoculum level was assumed to be $7.9x10^1$ cfu/25g sample, calculated from plate counts of the inoculum on Blood Agar. Results suggest that the inoculated organisms may have been rendered non-viable after inoculation into food.

Overall the correlation between the EiaFoss assay after centrifuging and the FDA-BAM method was good, although the assay failed to detect one positive sample. This sample gave a positive result without centrifuging. A total of 24 samples, however, gave "false positive" results when samples were not centrifuged prior to the assay.

Table 1. Sensitivity of the EiaFoss *Campylobacter* detection system

Organism	cfu/ml in *Campylobacter* Enrichment Broth	EiaFoss Result
C. jejuni (NCTC 11351)	$1.1x10^8$	+
	$1.1x10^7$	+
	$1.1x10^6$	+
	$1.1x10^5$	+
	$1.1x10^4$	-
	$1.1x10^3$	-
	$1.1x10^2$	-
	$1.1x10^1$	-
C. jejuni (NCTC 11168)	$1.0x10^8$	+
	$1.0x10^7$	+
	$1.0x10^6$	+
	$1.0x10^5$	+
	$1.0x10^4$	-
	$1.0x10^3$	-
	$1.0x10^2$	-
	$1.0x10^1$	-
C. jejuni (NCTC 11392)	$3.4x10^7$	+
	$3.4x10^6$	+
	$3.4x10^5$	+
	$3.4x10^4$	-
	$3.4x10^3$	-
C. coli (NCTC 11350)	$2.8x10^8$	+
	$2.8x10^7$	+
	$2.8x10^6$	+
	$2.8x10^5$	+
	$2.8x10^4$	-
	$2.8x10^3$	-
	$2.8x10^2$	-
	$2.8x10^1$	-
C. coli (NCTC 11366)	$9.7x10^8$	+
	$9.7x10^7$	+
	$9.7x10^6$	+
	$9.7x10^5$	+
	$9.7x10^4$	-

Table 2 Specificity of the EiaFoss *Campylobacter* detection system

Organism	Penner Serotype	Code	cfu/ml in *Campylobacter* Enrichment Broth	EiaFoss Result
C. jejuni	1	NCTC 12500	$3.6x10^8$	+
"	2	NCTC 10983	$3.0x10^8$	+
"	3	NCTC 12502	$5.8x10^7$	+
"	4	NCTC 12561	$1.3x10^8$	+
"	12	NCTC 12511	$3.4x10^7$	+
"	17	NCTC 12515	$4.0x10^8$	+
"	40	NCTC 12541	$7.0x10^7$	+
"	50	NCTC 12559	$3.8x10^8$	+
"	63	NCTC 12556	$3.0x10^6$	+
"		NCTC 11626	$5.2x10^7$	+
C. jejuni subsp. *jejuni*	1	NCTC 11322	$2.3x10^7$	+
"	15	NCTC 11385	$2.3x10^7$	+
C. coli	4	NCTC 11366	$1.2x10^8$	+
"	5	NCTC 12525	$4.2x10^7$	+
"	8	NCTC 12110	$>10^6$	+
"	14	NCTC 12526	$3.4x10^7$	+
"	16	NCTC 11350	$3.6x10^8$	+
"	16/21	NCTC 11353	$7.1x10^7$	+
"	20	NCTC 12527	$8.2x10^7$	+
"	24	NCTC 12528	$4.8x10^8$	+
"	25	NCTC 12529	$2.9x10^7$	+
"	26	NCTC 12530	$5.1x10^8$	+
"	28	NCTC 12531	$2.1x10^7$	+
"	30	NCTC 12532	$3.7x10^7$	+
"	34	NCTC 12533	$3.9x10^7$	+
"	39	NCTC 12534	$3.4x10^7$	+
"	47	NCTC 12535	$1.2x10^8$	+
C. lari		NCTC 11352	$6.7x10^5$	+
"		NCTC 11937	$9.0x10^6$	+
C. fetus subsp. *fetus*		NCTC 5850	$1.7x10^7$	+
"		NCTC 10842	$9.6x10^7$	+
C. hyointestinalis		NCTC 11608	$5.0x10^5$	-
"	(repeat test)	"	no growth	
"		NCTC 11562	no growth	
C. upsaliensis		NCTC 11540	$2.0x10^5$	-
"	(repeat test)	"	no growth	
"		NCTC 11541	no growth	
C. helveticus		NCTC 12470	no growth	
"		NCTC 12471	no growth	
C. mucosalis		NCTC 11000	no growth	
"		NCTC 11001	no growth	

Table 3. Exclusivity of the EiaFoss *Campylobacter* detection system

Organism	Code	cfu/ml in *Campylobacter* Enrichment Broth	EiaFoss Result
Arcobacter cryaerophilus	NCTC 11885	$6.0x10^{7}$(a)	-
Arcobacter skirrowii	NCTC 12713	$5.8x10^{8}$(a)	-
Arcobacter butzleri	NCTC 12481	$3.2x10^{6}$	-
Vibrio parahaemolyticus	NCTC 11344	$3.1x10^{6}$	-
Salmonella enteritidis	CRA 1002	$1.5x10^{6}$	-
Salmonella typhimurium	CRA 3510	$9.5x10^{8}$	-
Salmonella infantis	CRA 3264	$1.2x10^{9}$(b)	-
Enterobacter amnigenus	CRA 7426	$2.6x10^{6}$	-
Enterobacter agglomerans	CRA 1490	$1.6x10^{8}$(b)	-
Enterobacter cloacae	CRA 1478	$2.8x10^{6}$	-
Enterobacter sakazakii	CRA 5533	$6.3x10^{6}$	-
Proteus mirabilis	CRA 4707	$8.4x10^{8}$(a)	-
Proteus vulgaris	CRA 4003	$2.5x10^{8}$(a)	-
Proteus rettgeri	NCTC 7475	$4.2x10^{8}$(a)	-
Citrobacter freundii	CRA 5382	$1.9x10^{6}$	-
Citrobacter amalonaticus	CRA 7458	$8.6x10^{8}$(b)	-
Citrobacter diversus	CRA 3411	$6.5x10^{8}$(b)	-
Klebsiella pneumoniae	CRA 1483	$4.9x10^{8}$(b)	-
Klebsiella aerogenes	NCTC 8167	$1.8x10^{8}$	-
Klebsiella oxytoca	CRA 6764	$6.4x10^{8}$(b)	-
Yersinia enterocolitica	CRA 3899	$1.9x10^{8}$(a)	-
Edwardsiella tarda	NCTC 10396	$5.1x10^{8}$(a)	-
Aeromonas hydrophila	CRA 1550	$1.6x10^{9}$(c)	-
Aeromonas salmonicida	NCTC 10402	$2.2x10^{7}$(a)	-
Aeromonas sobria	NCTC 11215	$4.0x10^{8}$(a)	-
Providencia rettgeri	CRA 5561	$3.1x10^{8}$(a)	-
Providencia alcalifaciens	CRA 5721	$3.2x10^{6}$	-
Pseudomonas fluorescens	CRA 1503	$1.0x10^{8}$	-
Pseudomonas alcaligenes	NCTC 10367	$1.9x10^{8}$(a)	-
Pseudomonas aeruginosa	CRA 4896	$2.3x10^{8}$(a)	-
Serratia marcescens	CRA 4190	$1.7x10^{7}$	-
Serratia liquefaciens	CRA 1498	$1.7x10^{6}$	-
Pasteurella avium	NCTC 11297	$6.7x10^{6}$(a)	-
Pasteurella bettii	NCTC 10535	$2.7x10^{8}$(a)	-
Escherichia coli	CRA 3490	$1.2x10^{6}$	-
Escherichia hermanii	CRA 3973	$4.9x10^{8}$(a)	-
Escherichia fergusonii	CRA 7522	$8.4x10^{8}$(b)	-
Bacillus cereus	CRA 6064	$4.6x10^{7}$(a)	-
Bacillus coagulans	CRA 216	$5.2x10^{7}$(a)	-
Listeria monocytogenes	CRA 6293	$3.3x10^{7}$(a)	-
Listeria ivanovii	CRA 5931	$2.4x10^{8}$	-
Listeria innocua	CRA 6291	$2.5x10^{8}$(a)	-
Staphylococcus aureus	CRA 1218	$2.3x10^{8}$(a)	-
Staphylococcus epidermidis	CRA 567	$9.0x10^{7}$(a)	-
Enterococcus faecium	CRA 1513	$1.8x10^{8}$(b)	-
Lactobacillus brevis	CRA 186	$1.6x10^{7}$(a)	-
Streptococcus lactis	CRA 1532	$2.3x10^{7}$	-
Micrococcus luteus	CRA 3503	$1.6x10^{7}$	-

(a) Cultured in NB No. 2 (no growth in *Campylobacter* Enrichment Broth)
(b) Cultured in *Campylobacter* Enrichment Broth without antibiotic supplement at 37°C.
(c) Cultured in *Campylobacter* Enrichment Broth without antibiotic supplement at 25°C.

Table 4. Summary of detection of *Campylobacter* by all detection methods

Sample type (No. samples tested)	Number of samples positive by detection method							
	EiaFoss (non-centrifuged method)				FDA-BAM		ISO	
	Assay	Confirmed	Cultural	Confirmed	Presumptive	Confirmed	Presumptive	Confirmed
Inoculated foods (50 samples)	32	30	41	36	30	20	33	27
Uninoculated foods (85 samples*)	30	15	34	19	23	15	25	20
All foods (135 samples)	62	45	75	55	53	35	58	47

* includes uninoculated control samples from inoculated foods study.

Table 5a. Method agreement between EiaFoss and FDA-BAM methods

Result from detection method		EiaFoss Analyser Signal	
		+	-
FDA-BAM method	+	27	8
	-	35*	65

Method agreement = $\frac{92}{135}$ x100 = 68%

* 19 of these 35 samples were true positives i.e. confirmed culturally from the EiaFoss enrichment broth. Therefore a total of 27+8+19 = 54 true positive results, and (35-19) + 65 = 81 true negative results.

Table 5b. False-positive and False-negative rate of EiaFoss and FDA-BAM methods

Result from detection method	FDA-BAM	EiaFoss
False-positive	0% (0/81)	20% (16/81)
False-negative	35% (19/54)	15% (8/54)

Table 6a. Method agreement between EiaFoss and ISO methods

Result from detection method		EiaFoss Analyser Signal	
		+	-
ISO method	+	38	9
	-	24*	64

$$\text{Method agreement} = \frac{102}{135} \times 100 = 75.6\%$$

* 10 of these 24 samples were true positives i.e. confirmed culturally from the EiaFoss enrichment broth. Therefore a total of 38+9+10 = 57 true positive results, and (24-10) + 64 = 78 true negative results.

Table 6b. False-positive and False-negative rate of EiaFoss and ISO methods

Result from detection method	ISO	EiaFoss
False-positive	0% (0/78)	18% (14/78)
False-negative	18.5% (10/57)	18.8% (9/57)

Table 7. Summary of detection of *Campylobacter* from foods by the EiaFoss Method with and without centrifuging, and by the FDA-BAM method

Sample type (No. samples tested)	Number of samples positive					
	EiaFoss				FDA-BAM	
	Assay without centrifuging	Assay with centrifuging	Cultural	Confirmed	Cultural	Confirmed
Uninoculated *(25)	24	2	10	3	11	3
Uninoculated **(8)	6	3	4	3	3	3
Inoculated ***(12)	0	0	3	0	2	0
All foods (45)	30	5	17	6	16	6

* raw minced beef, sausages, bacon, liver and turkey

** raw poultry and seafood

*** ready meals, dairy foods and cooked seafood

CONCLUSION

The EiaFoss *Campylobacter* System is a convenient, automated rapid test for the detection of thermophilic *Campylobacter* in foods. The sensitivity of the assay is approximately 10^5 cfu/ml, and cross-reaction was not observed from 48 non-*Campylobacter* organisms tested.

The EiaFoss *Campylobacter* System was equivalent to the ISO method for detection of *Campylobacter* from foods; 45 (33%) samples were positive by the EiaFoss method, 35 (26%) by the FDA-BAM method and 47 (35%) by the ISO method.

False positive results from the EiaFoss assay occurred when testing certain (mainly fatty) foods. This was overcome by centrifugation of the samples prior to the assay.

The false negative rate of the EiaFoss assay was not significantly different to that of the ISO method, and significantly less than the FDA-BAM method.

The EiaFoss *Campylobacter* System provides a suitable rapid detection method for thermophilic *Campylobacter* species in foods.

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Appendix 1
FDA-BAM *Campylobacter* Enrichment Broth

Base

Nutrient Broth No. 2 (Oxoid CM67)	25.0g
Yeast extract	6.0g
Distilled water	950ml

Autoclave 121°C 15 mins.

Antibiotic solutions

1. Sodium cefoperazone (15mg/l)
   Dissolve 0.375g in 100ml distilled water. Filter sterilise. Store for 5 days at 4°C, or 3 weeks at -20°C (in polypropylene containers) or 5 months at -70°C.
2. Trimethoprim lactate (12.5mg/l)
   Dissolve 0.3125g in 100ml distilled water. Filter sterilise. Store at 4°C for up to 1 year.
3. Vancomycin (10mg/l)
   Dissolve 0.25g in 100ml distilled water and filter sterilise. Store at 4°C for up to 2 months.
4. Cycloheximide (100mg/l)
   Dissolve 2.5g in 20-30ml 70% ethanol. Make up to 100ml with distilled water. Store at 4°C indefinitely, but do not freeze.
5. Rifampicin (10mg/l)
   Dissolve 0.25g in 50ml ethanol. Make up to 100ml with distilled water and filter sterilise. Store at -20°C for up to 1 year.

Complete medium

To 950ml of base medium add ferrous sulphate-bisulphite-pyruvate (FBP) supplement (2 vials: Oxoid SR84) and 50ml lysed horse blood.
For dairy foods, add 4ml of each of antibiotic solutions 1, 2, 4 and 5.
For other foods, add 4 ml of each of antibiotic solutions 1, 2, 3 and 4.

Appendix 2

Preston Broth

Base	g/litre	
Meat extract	10.0	or Nutrient Broth No. 2
Peptone	10.0	(Oxoid CM67)
Sodium chloride	5.0	
Agar-agar	1.0	
Water	1 litre	

Autoclave 121°C 15 mins. pH 7.5 after autoclaving.

Antibiotic solution

B polymyxin	100,000 iu
Rifampicin	0.2g
Trimethoprim lactate	0.2g
Cycloheximide	2.0g
95% ethanol	50ml

Dissolve components in ethanol and make up to 200ml with water. Sterilise by filtration.

Complete medium

Base	940ml
Sterile lysed horse blood	50ml (Oxoid SR48)
Antibiotic solution	10ml or Oxoid Preston Supplement (SR117)

Appendix 3

Inoculated Foods

Cooked Prawns

Sausages

Cooked Turkey

Pepperoni

Chicken and Mushroom Pie

Cottage Cheese

Pasteurised Milk

Brie

Macaroni Cheese

Pasta Bake

Ham and Mushroom Pasta

Seafood Dressing

Seafood Cocktail

Chicken and Broccoli Pie

Beef Stroganoff

Roast Chicken

Duck Breast

Chicken Breast

Lasagne

Chicken Liver Paté

German Salami

Danish Salami

Smoked Ham

Soured Cream

Pork and Liver Paté