CONFIDENTIAL R&D REPORT NO. 28

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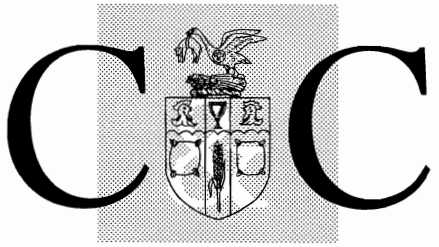
May 1996



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Use of the BAX™ System, a Commercial PCR-based System for the Detection of *Salmonella* in Foods

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SUMMARY

The increased occurrence of human salmonellosis, and therefore the requirement to be able to detect *Salmonella* in food samples in the quickest possible time, has led to the development of numerous rapid methods. Over the last decade or so, the polymerase chain reaction (PCR) has emerged as an extremely powerful technique with many applications for rapid diagnostic microbiology. The BAX™ system for screening *Salmonella* is one of the first commercial PCR-based systems for the detection of foodborne pathogens. The PCR technique targets a specific sequence of the nucleic acid of the chosen organism. This sequence is exponentionally amplified by a biochemical thermocycling reaction, resulting in multiple copies of the original sequence. Detection of amplified product indicates the presence of the target organism. The speed of amplification overcomes the reliance on bacterial multiplication to reach detection threshold levels. The BAX™ *Salmonella* system is able to give a definitive result just 28 hours after initiating analysis.

In the current study, the system has been shown to be a specific and sensitive detection method. There was shown to be 98.6% and 95.8% agreement between the BAX™ system and conventional cultural analysis, for the detection of *Salmonella* in artificially inoculated, and uninoculated, food samples respectively. In both cases, the BAX™ system generated more positive detections than the cultural analysis.

The speed of assay, ease of use and high specificity and sensitivity of BAX™ system for the detection of foodborne *Salmonella* make it an attractive method for routine food microbiology laboratories.

CONTENTS

1. INTRODUCTION

The ability to reliably detect pathogenic microorganisms in food is recognised as an important tool for the control of foodborne disease. Some food manufacturers will only release certain foods for retail sale on the assurance that a product is "pathogen-free", based on the results of microbiological analysis. With other manufacturers, and especially with short-life produce, microbiological test results are known only after sale and are utilized as validation that safety and quality assurance systems are adequate throughout a manufacturing process (e.g. verification of Hazard Analysis Critical Control Point (HACCP) plans).

Salmonella is a major cause of food poisoning throughout the world. Incidence of infection by this organism is on the increase. For example, in 1980 there were approximately 10,000 reported cases of salmonellosis in England and Wales, this had risen to over 30,000 in 1994. (Anon, 1994). Many foods are routinely tested, therefore, for the presence of *Salmonella.* The potentially low infective dose of this organism renders it necessary to test for the presence of a single *Salmonella* cell in a food sample and traditionally this is achieved by the use of cultural methods. A food sample (typically 25g) is pre-enriched in a non-selective liquid medium to enable recovery of stressed/injured cells and begin cell multiplication. A sample is then exposed to selective enrichment to allow an increase in the number of target *Salmonella* cells whilst inhibiting growth of competitor organisms. Isolation and identification of target cells is achieved by sub-culturing enrichments onto differential and selective solid media. Colonies characteristic of *Salmonella* which subsequently grow are confirmed as *Salmonella* by a series of biochemical and serological tests. This procedure will generate a negative result for *Salmonella* after three or four days but can take up to seven days for a confirmed positive result to be obtained for the presence of *Salmonella*. Such approaches are commonly integrated into standard methods (Anon, 1993; Andrews *et al,* 1995).

The laborious and lengthy nature of these approaches has led to the development of numerous rapid methods for the detection of *Salmonella* in foods. Such developments

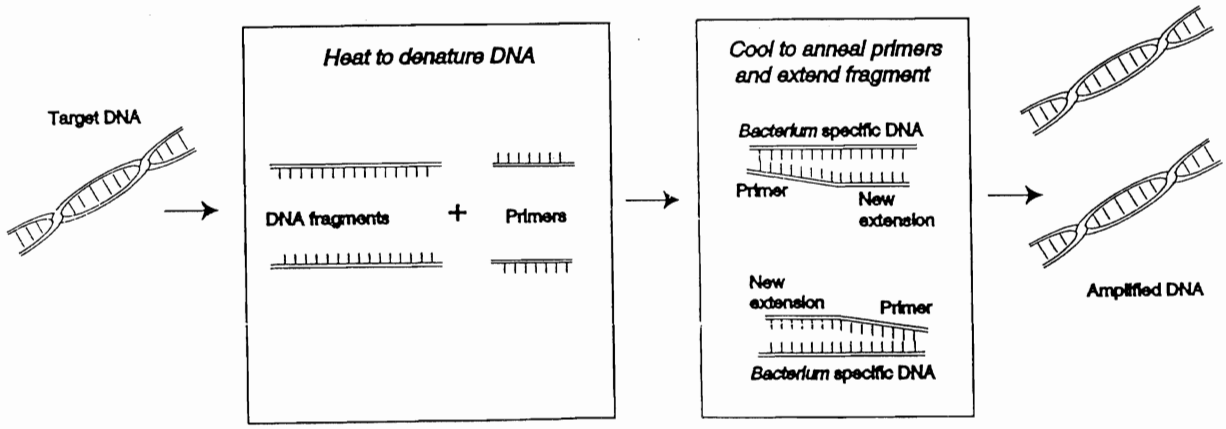
include electrical techniques, immunoassays and nucleic acid probe analyses. Traditional and rapid analytical microbiological methods available to the food microbiologist have been the subject of many reviews (e.g. Betts, 1992; Fung, 1994).

With both rapid and traditional approaches, analysis times are long, due to the poor sensitivity of the detection system. The potential single *Salmonella* cell present in the sample must be multiplied to high enough numbers to pass detection thresholds. In traditional microbiology, and as preparation for many of the rapid detection systems, this is achieved by cell multiplication, which is a lengthy process. With rapid detection methods, multiplication must typically result in a cell concentration of 10^4 -10^6 cells per ml to give a positive result. There has been great interest in the use of separation/concentration techniques to reduce total analysis time and also improve reliability of detection systems. These approaches aim to remove the target organism from food debris and competing microflora which can interfere with the isolation and/or detection system. Separated cells can be rapidly concentrated, preferably above detection thresholds, resulting in reduced enrichment times. A review of this approach has been written by Betts (1994).

A third, organism-specific approach to reducing test time by shortening the time required to reach detection threshold levels, utilizes molecular amplification systems. With such approaches, the target for detection is changed from the cell to a specific region of nucleic acid. Current conventional and rapid methods can be influenced by environmental conditions, whilst genetic methods, based on detection of nucleic acid, are not. Techniques exist to very rapidly amplify target sequences of nucleic acid to detectable levels. Such techniques include the polymerase chain reaction (PCR), nucleic acid sequence-based amplification (NASBA), strand displacement amplification (SDA), Qß replicase amplification of probes to the target sequence, and the ligase chain reaction (LCR) (Carrino and Lee, 1995). Of these amplification techniques, it is PCR that has been most widely investigated for diagnostic food microbiology. This technique was first developed in 1983 (Mullis, 1990) and since that time has revolutionized many fields of biological science.

The PCR method (Figure 1) is a temperature cycling reaction to exponentially amplify target nucleic acid. Target deoxyribonucleic acid (DNA) is amplified by use of two short oligonucleotide primers that hybridise to opposite strands of DNA that flank the region of interest in the target DNA. PCR proceeds by heating the DNA to denature it, i.e. separate strands, followed by cooling to allow the primers to anneal (hybridise) to the complementary region of the target DNA. At the same, or a different, temperature that determines the stringency of the reaction, the primer sequences are extended by DNA polymerase in the presence of the four deoxyribonucleoside triphosphates using the original DNA target sequence as template. This process produces two copies of target DNA from the original template. The temperature is then increased to denature the newly formed DNA and the process is then repeated. This cycle is continuously repeated using products of each round of extension as templates for the next round. Each cycle results in a doubling of the target sequence, resulting in the exponential amplification of product. This method can result in a 10^7-fold amplification of the target sequence in just 2 - 3 hours.

Figure 1. Schematic representation of the polymerase chain reaction (PCR)



For diagnostic tests, amplified product must be detected. The presence of amplified product indicates the presence of the target organism in the original sample. Various approaches exist to detect amplified product, ranging from gel electrophoresis to microtitre plate-based, ELISA-like procedures.

The application of PCR to diagnostic microbiology has been widely researched but only now are commercial tests becoming available. There have been many research reports on the use of PCR for the rapid detection of pathogenic microorganisms in food. Among others, these include reports on the detection of *Listeria* (e.g. Niederhauser *et al,* 1992), *Campylobacter* (e.g. Wegmüller *et al,* 1993), *Staphylococcus* (e.g. Tsen and Chen, 1992), verocytotoxigenic *E. coli* (e.g. Gannon *et al,* 1992) and *Salmonella* (e.g. Bej *et al,* 1994; Cano *et al,* 1993; Aabo *et al,* 1995; Kwang *et al,* 1996).

The current study evaluated a commercially available PCR system for the detection of foodborne pathogens. The BAX™ system for screening *Salmonella* is produced by DuPont subsidiary Qualicon, L.L.C. The system is reported to be extremely specific and sensitive. Jensen *et al* (1994) reported 99.7% of 1400 *Salmonella* strains to be detected with less than 1% of over 100 non-*Salmonella* Gram negative enteric bacterial strains yielding an amplification product. The 0.3% of *Salmonella* which did not yield detectable amplification products was due to isolates of *S. alachua, S. havana* and *S. arizonae,* although many other isolates of these serotypes did give characteristic bands. In addition, 100% exclusivity was not achieved due to a *Hafnia alvei* isolate which gave a characteristic amplification product. The system was also shown to detect *Salmonella* at a concentration of 10^4 cfu/ml. In the current study the BAX™ system was evaluated for its ability to detect *Salmonella* in a range of meat, poultry and dairy products within 28 hours. Further studies investigated the sensitivity and specificity of the system.

2. MATERIALS AND METHODS

2.1 Microorganisms

Microorganisms used in this evaluation are listed in Table 2.1. Wherever possible isolates of food origin were used. All organisms were obtained from the CCFRA culture collection.

The organisms were grown on Nutrient Agar (NA) (Oxoid CM3) at 37°C for 18-24h to ensure purity and then subcultured as appropriate.

2.2 Foods

Foods were purchased from local retail outlets or obtained from a food service kitchen; raw milk was obtained from a local dairy farmer. All foods were stored at 4°C prior to use.

2.3 Sensitivity

Salmonella strains (CRA 1944, 1009, 1019, 1012, 1042, 1028, 1952, 1946, 1050, 1096, 1081, 1352, 1405, 1090, 1379, 1402, 1934, 1049, 1070 and 1378) were inoculated separately into Buffered Peptone Water (BPW) (Lab M 46) and incubated at 37°C for 20 h. Each culture was then diluted in BPW to levels between 10^1 and 10^5 cfu/ml as determined by the plate count technique. Aliquots (0.1ml) of serial dilutions of a sample were subcultured onto NA by the standard spread plate technique. After incubation (37°C/24h) the concentration of *Salmonella* (cfu/ml) was calculated from the number of colonies that developed on the medium. All dilutions were tested with the BAX™ system as detailed in Section 2.5.2. Brain Heart Infusion Broth (BHIB) (Lab M 49) cultures were enumerated on NA following "grow-back" by the spread plate technique. The minimum concentration at which the BAX™ system gave a positive result was then determined.

2.4 Inclusivity/Exclusivity

Salmonella and non S*almonella* organisms, listed in Table 3.2, were inoculated separately into BPW and incubated at 37°C for 20h. After incubation, test organisms were at a minimum concentration of 10^7 cfu/ml. All samples were tested with the BAX™ PCR detection system (Section 2.5.2).

2.5 Inoculated Foods

Salmonella strains listed in Table 3.3 were inoculated separately into BHIB and incubated at 37°C for 24h. The concentration of each 24h broth culture was estimated using a counting chamber and confirmed by enumeration on NA (spread plate technique) at 37°C for 24h. An appropriate dilution of each broth was made in Maximal Recovery Diluent (MRD) (Lab M 103) to inoculate food samples (25g) in duplicate with <50 cells of *Salmonella* per 25g. A third, uninoculated sample of each food was tested as a control. Where possible, foods were inoculated with serotypes that had originally been isolated from a similar food type. Uninoculated control samples were included for each food type.

BPW (225ml) was aseptically added to each food sample. Samples were stomached for 1 minute. Dried foods were soaked in BPW for 60 minutes to prevent osmotic shock to cells, prior to mixing. Samples were incubated at 37°C for 20h (Figure 2.1). In addition to pre-enrichment with BPW, milk powder samples (25g) were enriched with 225ml Brilliant Green Water (BGW) (BS 4285 : Section 3.9 : 1987) (Figure 2.2). Liquid milk and cream samples were transferred directly to selective enrichment broths without pre-enrichment, and 25ml/25g samples were enriched in 225ml Selenite Cystine Broth (SC) (Oxoid CM699 + L121) at 37°C for 18-24h and 48h and in 225ml Müller-Kauffmann Tetrathionate Broth (MK) (Oxoid CM 343) at 43°C for 18-24h and 48h (BS 4285 : Section 3.9 : 1987) (Figure 2.3).

2.5.1 Conventional Method for the detection of *Salmonella* (Figures 2.1, 2.2 and 2.3)

For the majority of samples, following pre-enrichment, 10ml of the BPW enriched sample was subcultured into 100ml of SC broth, and 0.1ml into 10ml of Rappaport Vassiliadis Broth (RV) (Oxoid CM669). SC broths were incubated at 37°C for 24h and 48h. RV broths were incubated at 42°C for 24h. When testing milk powder, 10ml of the BGW enriched sample was subcultured into 100ml of SC broth and 10ml into 100ml of MK broth. MK broths were incubated at 43°C for 24h and 48h.

After incubation, a loopful of each selective enrichment broth was streaked onto Brilliant Green Agar (BGA) (Oxoid CM263) and Xylose Lysine Decarboxylase Agar (XLD) (Lab M32). Plates were incubated at 37°C for 18-24h. Up to three typical *Salmonella* colonies were taken from each plate for confirmation. Colonies were subcultured on NA at 37°C for 24h, then tested for agglutination with *Salmonella* polyvalent O and H agglutinating sera (Murex Diagnostics Ltd.). Agglutinating isolates from uninoculated food samples were confirmed biochemically by Vitek GNI (Biomérieux).

2.5.2 BAX™ system for the detection of *Salmonella* (Figure 2.4)

Following pre-enrichment, 1ml of the BPW enriched sample was subcultured into 9ml of pre-warmed (37°C) BHI broth. BHI broth samples were incubated at 37°C for 3 hours ("grow back"). When testing milk powder, 1ml of the BGW enriched sample was subcultured into 9ml of prewarmed BHI broth. For liquid milk and cream samples, 1ml of the 225ml SC broth enriched sample and 1ml of the 225ml MK broth enriched sample were each subcultured into separate 9ml of prewarmed BHI broths. Following "grow-back", a 5µl aliquot of each BHI broth was added to a lysis tube containing 200µl lysis reagent with added protease enzyme, both of which are provided with the BAX™ system. Tubes were incubated in a waterbath at 37°C for 20 minutes to lyse cells and degrade cellular proteins, and then incubated at 95°C for 10 minutes to complete lysis and destroy the protease. Following lysis, 50µl of lysate

was transferred into a sample tube containing a PCR tablet and 50µl was transferred into a tube containing a positive control tablet (both provided with the BAX™ system kit). Tablets contain all the reagents necessary for the PCR reaction: *Taq* polymerase, deoxyribonucleosides, primers, etc. In addition, the positive control tablet contains target sequence for the specific PCR. This tablet tube should always yield a positive result irrespective of whether *Salmonella* is present or not in the food sample, and is used as a control to establish that nothing inhibits the PCR reaction. Tubes were then taken to a separate work area. Pre- and post-amplification operations are kept separate to prevent contamination. Tubes were loaded into a thermocycler (Perkin Elmer 9600) and the PCR reaction initiated. A thermocyler automatically cycles the temperature required for PCR. The reaction proceeded via an initial hold period of 2min at a temperature of 94°C, followed by 35 cycles of 94°C/15sec and 72°C/3.0min. After the 35 cycles were complete, tubes were held at 72°C for 7min to complete the reaction before finally being held at 4°C to await analysis.

Amplified samples were analysed by agarose gel electrophoresis using 2.0% SeaKem® Gold Reliant® Agarose gels prestained with ethidium bromide (FMC, Maine, USA). Sample and positive control amplified products were prepared for gel loading by adding 1.7µl of loading dye provided with the BAX™ kit, to 8.3µl of amplified product. The resulting 10µl aliquots were transferred to the wells in the gel and electrophoresed at 100V for 25 minutes. After electrophoresis, the gels were photographed under UV light using a Foto/Phoresis® UV Documentation System (Fotodyne Incorporated, Wisconsin, USA). Photographs were examined for the presence/absence of a *Salmonella*-specific band in each lane. A *Salmonella*-specific band was indicated by a fluorescent band at the 725 base pair level. The specific *Salmonella* sequence that is amplified is 725 bp in size. This amplified sequence is indicated by a band at a position corresponding to the third band of six in the lane on the gel that is loaded with a moleculer weight marker (Figure 2.5). For a food sample to be deemed positive for *Salmonella,* this characteristic band must be present in the test sample lane, but can be present or absent in the corresponding positive control lane. A negative sample was one in which no band appeared in the sample lane, but a band was present in the sample control lane. If there was no band in the sample or

control lane, the result was deemed indeterminate and further action taken as detailed in the manufacturer's instructions. There is potential for a positive control to yield a negative result, if the original food sample contains substances that are inhibitory to the PCR reaction.

2.6 Uninoculated Foods

Thirty four potentially naturally contaminated foods were tested with the PCR detection system and standard cultural procedures. Methods used are those detailed in Section 2.5.

TABLE 2.1

Micro-organisms Used in the Evaluation

Organism	CRA Code	Source
Biochemically typical *Salmonella* strains		
S. enteritidis	1944	chicken
S. typhimurium	1009	milk
S. hadar	1019	turkey
S. virchow	1012	chicken
S. newport	1042	pork
S. heidelberg	1028	beef
S. infantis	1952	liquid egg
S. montevideo	1946	chicken
S. agona	1050	chicken
S. braenderup	1096	chicken
S. thompson	1081	pork
S. reading	1405	NCTC 5720
S. saint-paul	1090	chicken
S. javiana	1379	NCTC 6495
S. oranienberg	1402	NCTC 5743
S. indiana	1934	chicken
S. panama	1049	ham
S. brandenberg	1070	beef
S. java	1378	NCTC 5706
S. heidelberg	1029	turkey
S. anatum	1062	chicken
S. infantis	1036	chicken
S. saint-paul	1093	milk powder
S. brandenberg	1074	milk
S. infantis	1035	milk plant environment
S. typhimurium	3510	cream
S. berta	1068	uncooked chicken
S. montevideo	1032	chicken
S. bedford	1418	pork
S. berta	1069	chicken
S. binza	1436	dried spice
S. blockley	1088	human
S. braenderup	1097	dried egg
S. champaign	1327	liver
S. columbo	1337	NCTC 9922
S. corvallis	1755	cocoa bean
S. derby	1352	NCTC 5721

Table 2.1 continued

Organism	CRA Code	Source
S. dublin	1356	bovine
S. enteritidis	3815	human
S. enteritidis	3505	fish cakes
S. enteritidis	1001	egg
S. hadar	1017	milk sock
S. heidelberg	1964	chicken
S. panama	1045	pork sausages
S. anatum	1060	egg
S. bredeney	1076	pork
S. ealing	1362	dried milk
S. kedougou	1966	chicken
S. senftenberg	1940	chicken
S. stanley	1055	meat pie
S. kentucky	1382	NCTC 5799
S. kimberley	1423	beef
S. kottbuss	4179	
S. lille	1851	cocoa bean environment
S. livingstone	1963	chicken
S. locarno	1386	NCTC 10272
S. madelia	1388	NCTC 6482
S. malawi	1659	
S. napoli	1624	chocolate environment
S. ohio	1459	
S. santiago	3728	bourgignon powder
S. shangani	1409	NCTC 5784
S. agona	1053	animal feed
S. albany	1275	NCTC 9869
S. anatum	1061	shrimp
S. arizona	3265	maize
S. infantis	1037	prawns
S. kedougou	1024	turkey
S. manchester	1429	yeast
S. manila	3939	sesame seed
S. mbandaka	1935	chicken
S. montevideo	1031	sunflower pellets
S. newport	1041	duck
S. ohio	3266	flavouring powder
S. panama	1047	sausage casing
S. pretoria	1404	pig
S. saint-paul	1092	beansprouts
S. saint-paul	1091	egg
S. santiago	3727	dried onions
S. rubislaw	3263	black pepper
S. senftenberg	2075	cooked beef

Table 2.1 continued

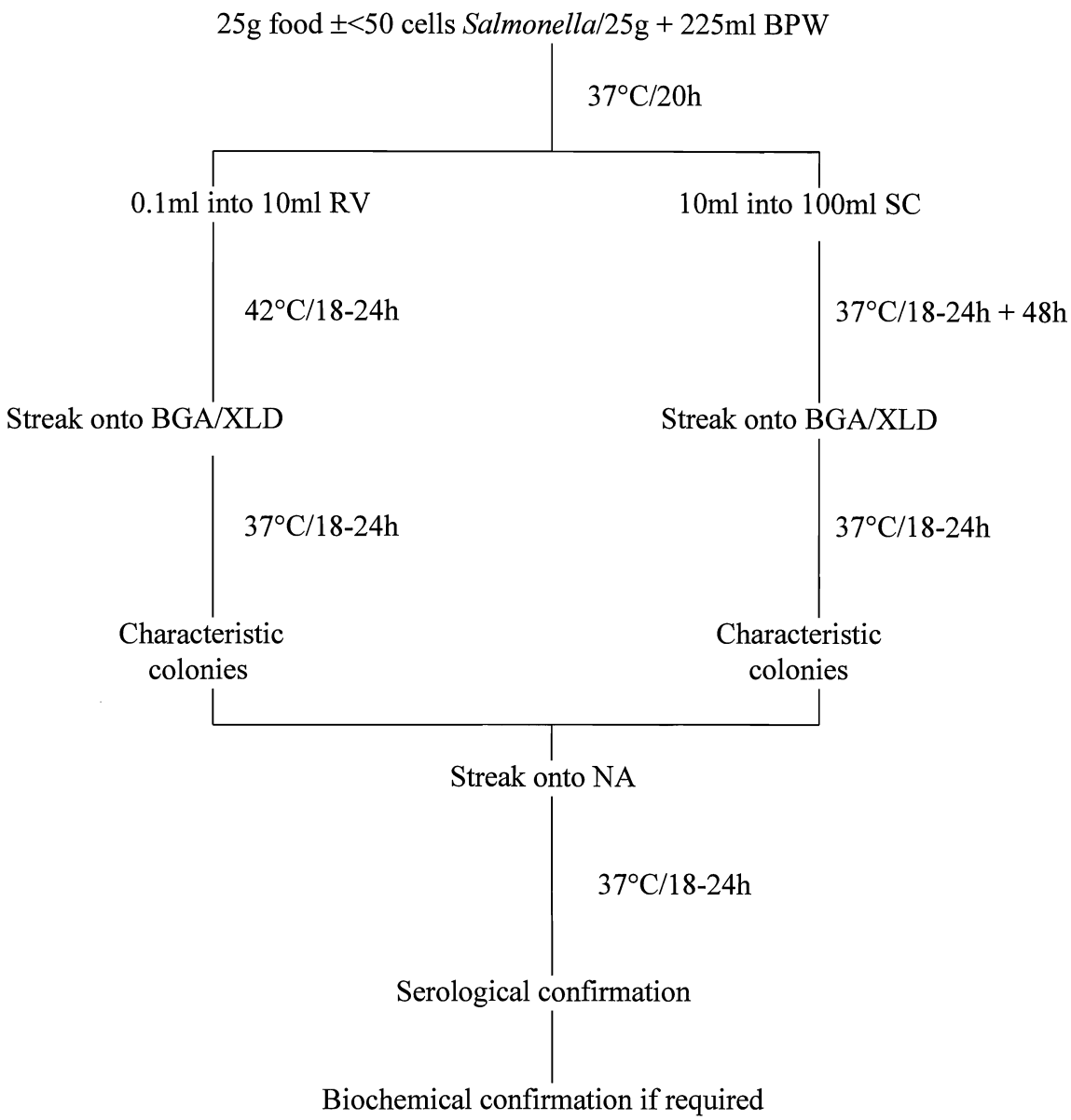
Organism	CRA Code	Source
S. stanley	1056	chicken
S. stanley	1059	oysters
S. tennessee	3946	sesame seed
S. thompson	1082	chicken
S. thompson	1083	egg
S. typhimurium	1960	chicken
S. typhimurium	1008	pork
S. typhimurium	1006	sausage
S. virchow	1014	turkey
S. virchow	1011	prawns
S. agona	1433	dairy product
S. anatum	1064	chicken
S. brandenberg	1072	roast pork
S. brandenberg	1073	milk
S. driffield	1430	beef
S. hadar	1015	spiced chicken
S. ibadan	1578	cocoa bean
S. orion	1936	chicken
S. anatum	1063	paprika
S. berta	1065	sausage
S. blockley	1086	frozen chicken
S. bovis morbificans	1306	NCTC 5754
S. mbandaka	1391	NCTC 7892
S. meunchen	1849	cocoa bean
S. ohio	3270	
S. senftenberg	1573	creamed coconut
S. poona	725	
S. reading	1405	
Atypical *Salmonella* strains		
S. indiana	71	turkey
S. gallinarum	1656	
S. eastbourne	1363	NCTC 3378
S. senftenberg	1939	
S. typhimurium	1949	
S. dublin	1953	
S. brandenberg	1959	
S. vietnam	3232	
S. bredeney	6721	
S. anatum	6807	DD 3532
S. wassenaar	7044	
S. virchow	7045	
S. abortusequi	7046	
S. arizonae	7047	
S. africana/thompson	2002	

Table 2.1 continued

Organism	CRA Code	Source
S. typhimurium	3425	
S. senftenberg	7048	
S. typhimurium	3426	
S. pullorum	7049	
S. indiana	7050	
Non *Salmonella* organisms		
Citrobacter freundii	3664	black forest gateau
Citrobacter freundii	1489	raw mince
Citrobacter freundii	4030	raw poultry
Citrobacter freundii	4169	
Citrobacter freundii	4122	
Proteus mirabilis	4602	
Proteus mirabilis	4609	
Proteus mirabilis	4171	raw chicken
Proteus vulgaris	1581	
Proteus vulgaris	1580	mince
Serratia marcescens	1521	
Serratia marcescens	4190	
Serratia fonticola	3977	
Serratia liquefaciens	1560	
Serratia liquefaciens	1498	
Escherichia coli	3991	
Escherichia coli	4611	
Escherichia coli	1545	mince
Escherichia coli	3025	frozen turkey
Escherichia coli	2077	
Pseudomonas aeruginosa	4636	
Pseudomonas fluorescens	1503	
Pseudomonas fluorescens	373	
Pseudomonas fluorescens	1499	
Providencia alcalifaciens	4170	
Morganella morganii	5120	
Morganella morganii	1542	
Hafnia alvei	4007	
Hafnia alvei	4009	
Hafnia alvei	4011	
Hafnia alvei	3642	cured pork
Hafnia alvei	1561	mince
Klebsiella pneumoniae	1483	mince
Klebsiella aerogenes	243	
Klebsiella ozaenae	4273	

FIGURE 2.1

CONVENTIONAL PROCEDURE FOR DETECTION OF *SALMONELLA*
(based on BS5763/ISO 6579)

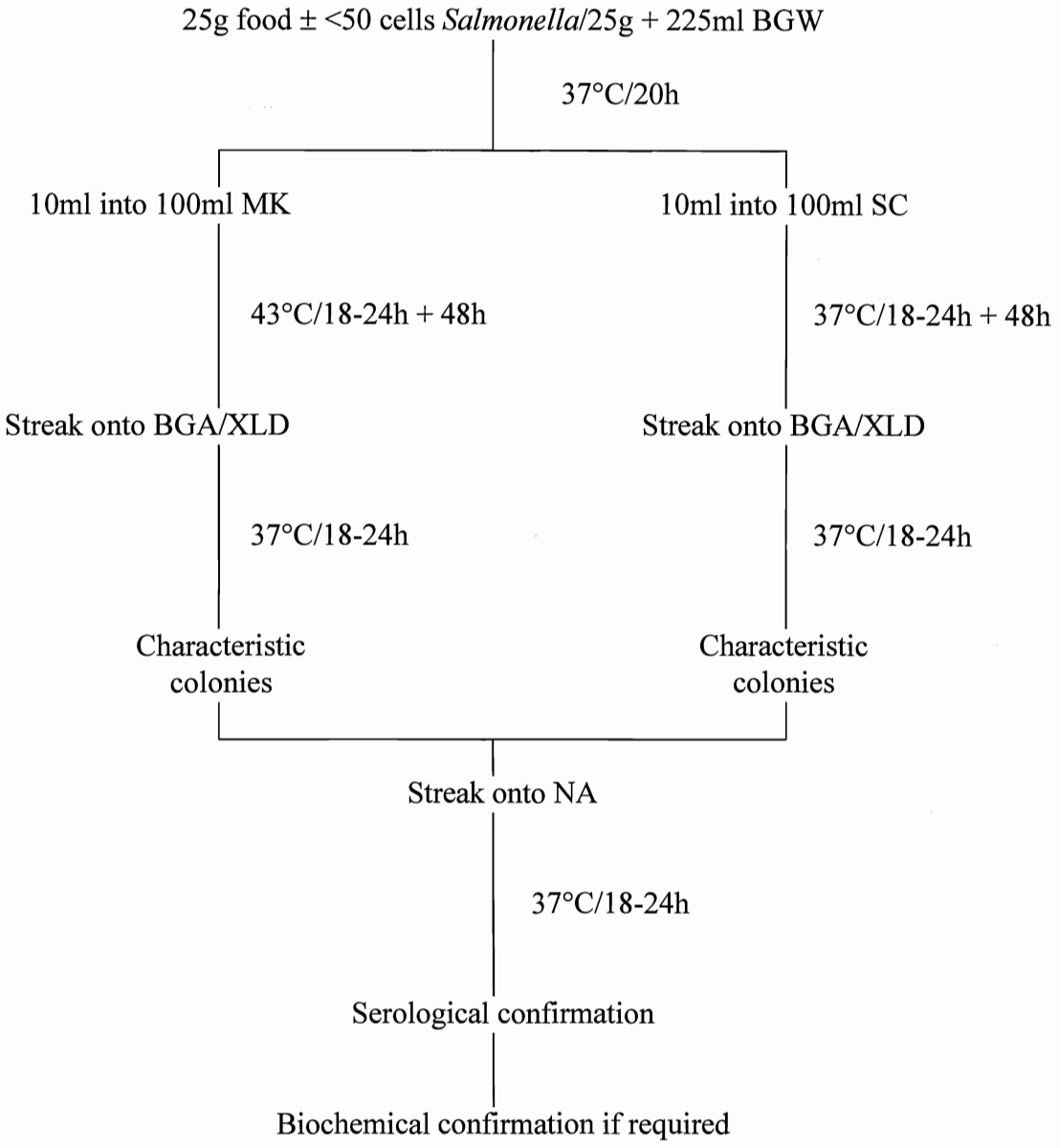


BPW - Buffered Peptone Water
RV - Rappaport Vassiliadis Broth
SC - Selenite Cystine Broth
BGA - Brilliant Green Agar
XLD - Xylose Lysine Decarboxylase Agar
NA - Nutrient Agar

FIGURE 2.2

CONVENTIONAL PROCEDURE FOR DETECTION OF *SALMONELLA* IN DRIED MILK

(Based on BS4285; Section 3.9 : 1987)

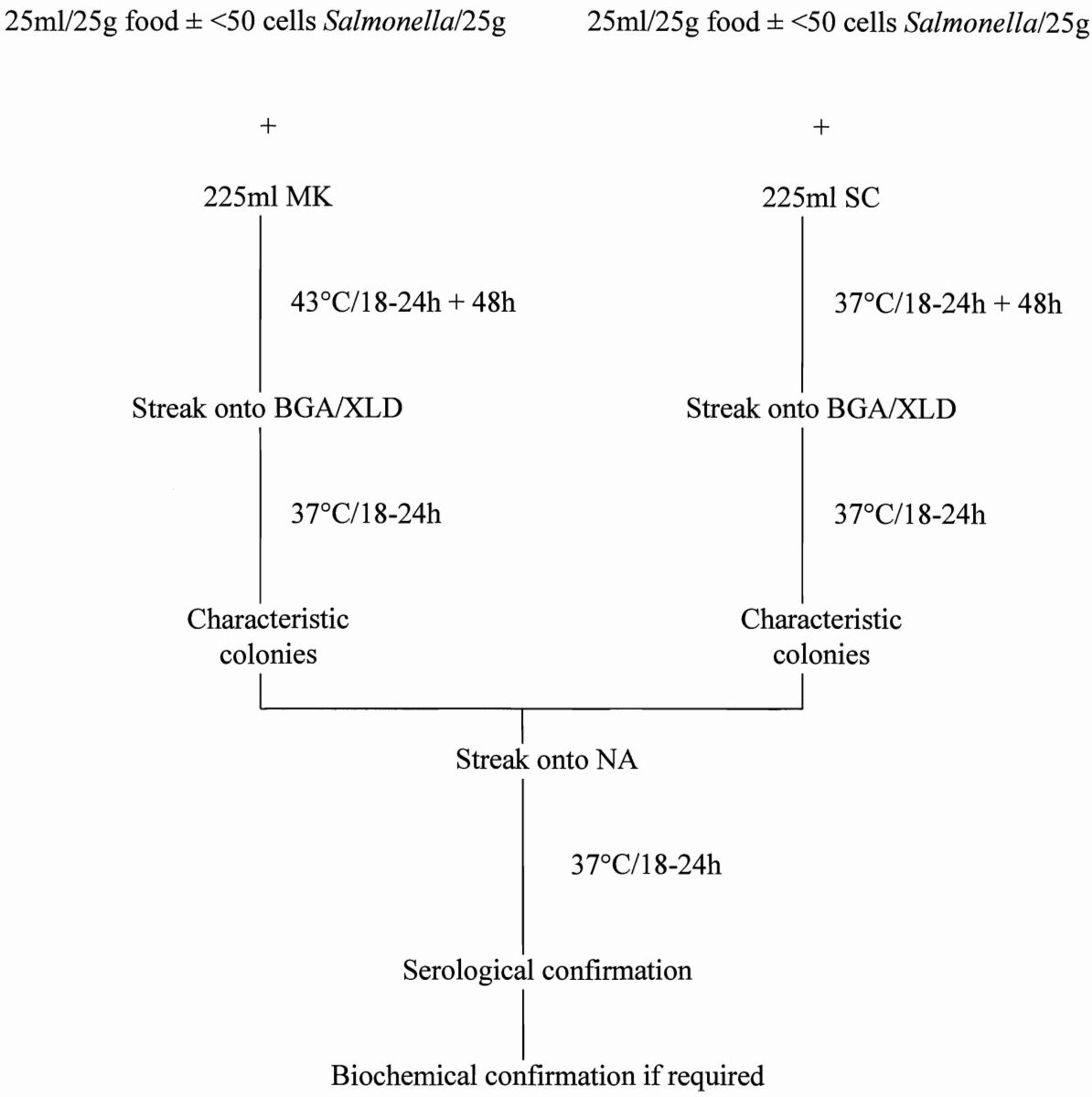


BGW - Brilliant Green Water
(1ml of 0.5% Brilliant Green Solution in 225ml water)
MK - Müller-Kauffmann Tetrathionate Broth
SC - Selenite Cystine Broth
XLD - Xylose Lysine Decarboxylase Agar
BGA - Brilliant Green Agar
NA - Nutrient Agar

FIGURE 2.3

CONVENTIONAL PROCEDURE FOR DETECTION OF *SALMONELLA* IN LIQUID MILK AND CREAM

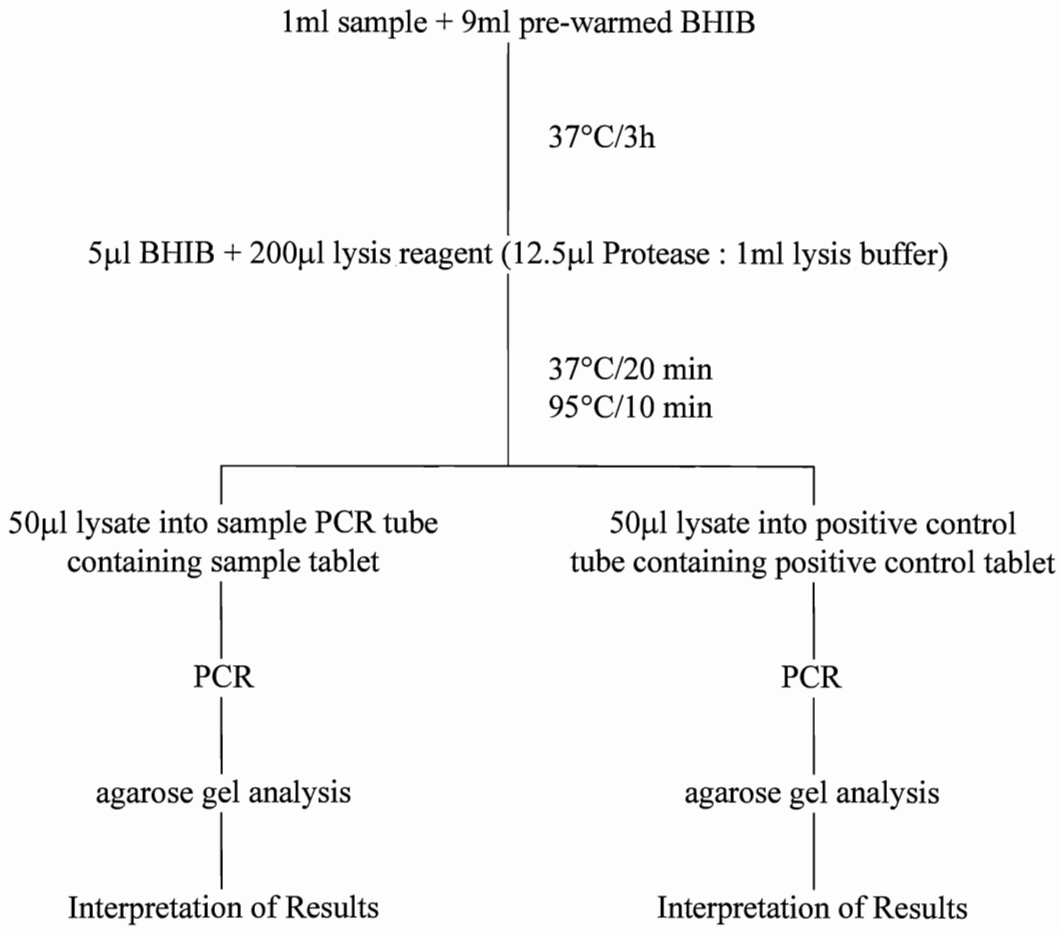
(Based on BS4285; Section 3.9 : 1987)



MK - Müller-Kauffmann Tetrathionate Broth
SC - Selenite Cystine Broth
XLD - Xylose Lysine Decarboxylase Agar
BGA - Brilliant Green Agar
NA - Nutrient Agar

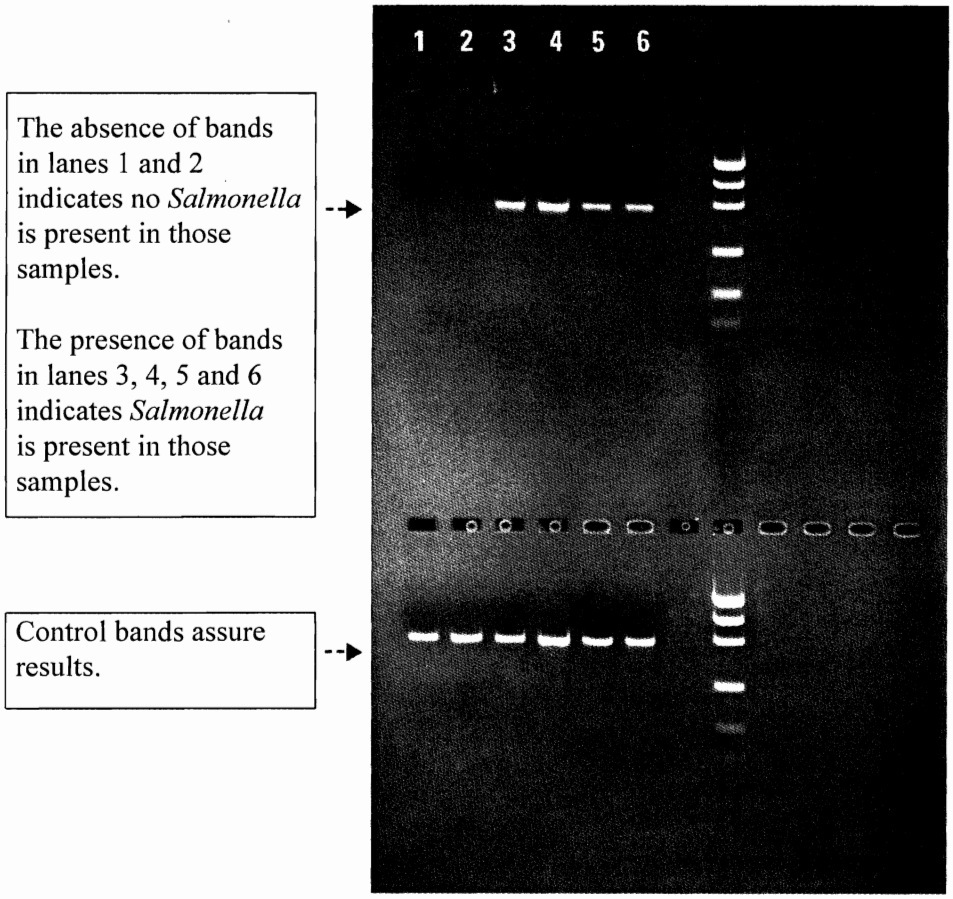
FIGURE 2.4

BAX™ PCR DETECTION SYSTEM



BHIB - Brain Heart Infusion Broth

FIGURE 2.5 Interpretation of BAX™ System Agarose Gel Electrophoresis Results of PCR Amplified Product.



3. RESULTS AND DISCUSSION

Although the potential use of PCR in diagnostic food microbiology is unarguably an attractive approach, many workers have reported on the inhibitory effect of food substances on the PCR reaction (e.g. Wernars *et al,* 1991; Rossen *et al,* 1992; Grant *et al,* 1993; Lantz *et al,* 1994; Bickley *et al,* 1996). This is not, however, a phenomenon observed with the optimized BAX™ system which is developed for the detection of *Salmonella* in meat, poultry and milk products. The BAX™ system for screening *Salmonella* is one of the first commercially available PCR systems for detection of pathogenic microorganisms in food. In addition it offers a novel approach to performing PCR. The requirement to hold and use carefully controlled stock solutions of chemicals and reagents is reduced by the provision of a "PCR tablet". The *Salmonella*-specific primers, deoxynucleotides, potassium chloride, magnesium chloride and *Taq* polymerase are all contained within a tablet, presented in a reaction tube for PCR. After addition of lysed test sample to the tube, PCR can be performed. The use of this approach makes the BAX™ system simple, optimized and standardized.

3.1 Sensitivity

The sensitivity of the system was investigated using dilutions of pure cultures of twenty *Salmonella* isolates (Table 3.1). The concentration of target cells following "grow-back" indicates the concentration of *Salmonella* that must be reached in order to give a positive detection by BAX™. Generally a level of 10^3-10^4 cfu/ml after "grow-back" would lead to a positive PCR result depending upon the isolate. Results indicate that it is this same level (10^3-10^4 cfu/ml) that is required after pre-enrichment in BPW, that is subsequently diluted and incubated to allow growth during "grow-back", that leads to a positive PCR result. Some isolates showed a greater sensitivity with as few as 10^2 cells/ml after pre-enrichment leading to a positive PCR result, whilst others required in excess of 10^4 cells/ml. For example, *S. braenderup* (CRA 1096) gave a positive PCR result from a BPW culture containing 3.1 x 10^2 *Salmonella* /ml which multiplied to 1.19 x 10^3 *Salmonella* /ml during "grow-back". *S. montevideo* (CRA 1946) gave a positive PCR result from a BPW culture containing 2.3 x 10^2 *Salmonella* /ml which only multiplied to 8.4 x 10^2 *Salmonella* /ml during "grow-back". The system, therefore, appears to be a more sensitive detection method when compared with other rapid detection systems. Electrical (impedance) methods (Easter and Gibson, 1989), immunoassays (ELISA) (Betts, 1992) and commercial

nucleic acid probe assays (e.g. Mozola *et al*, 1991) all commonly require 10^5 - 10^6 target cells/ml to detect a target pathogen in a food enrichment system. The BAX™ system appears more sensitive by 1 - 2 log values. Care should be taken when interpreting such sensitivity data as they are generated with pure cultures. The presence of food debris and competitor organisms will affect sensitivity by either affecting target organism growth or affecting the PCR. This should be considered when interpreting pure culture data from any method evaluation.

3.2 Specificity

The BAX™ *Salmonella* system showed excellent specificity and of the isolates tested, all *Salmonella* yielded a positive result (100% inclusivity; Table 3.2). One hundred *Salmonella* isolates, covering a range of serotypes, including those most commonly associated with human foodborne *Salmonella* gastroenteritis, were analysed in pure culture with the BAX™ system. This study included a number of isolates which are considered "atypical". The latter may produce colonies on differential agar which do not appear characteristic of *Salmonella* and so would be missed by conventional analysis. Such "atypical" isolates are believed to occur infrequently; however, this may simply reflect the difficulty of recognition of an atypical isolate on a selective agar plate. Their isolation is thus more likely from clinical specimens where a patient's symptoms indicate presence of *Salmonella* and thus a concerted effort to isolate this genus is undertaken. For example, Farmer *et al* (1985) reported that 5% of serotypes of salmonellae isolated from clinical specimens did not produce hydrogen sulphide, whilst Devenish *et al* (1986) reported that 1% of isolates belonging to *Salmonella* subgroup I, isolated mostly from human clinical specimens, were lactose fermenters. Production of hydrogen sulphide and inability to ferment lactose are diagnostic features of cultural *Salmonella* isolation. The BAX™ *Salmonella* system will detect these isolates in foods that would go undetected by conventional cultural procedures. These isolates could also be missed by other rapid techniques such as ELISAs where a positive immunoassay must be confirmed by isolating the organism on conventional solid differential media. The BAX™ system gives a definitive result that requires no confirmation, unless the testing laboratory requires an isolate for further analysis such as serotyping or sub-typing.

In addition, 35 non-*Salmonella* isolates were analysed with the BAX™ system. All gave negative results (100% exclusivity; Table 3.2) indicating an absence of false positive detections. Jensen *et al* (1994) found a single *Hafnia alvei* isolate to give a characteristic band with the BAX™ System. This was not found to be the case with the five *H. alvei* isolates tested in the current study. In summary, the choice of primers, and stringency of PCR conditions, has produced an extremely specific detection system.

3.3 Detection of *Salmonella* in Artificially Inoculated Foods

Seventy two inoculated foods were analysed. Each was set up in triplicate with two samples being inoculated with a low level of *Salmonella* and the third remaining uninoculated as a control. This included dairy samples, where different pre-enrichment regimes were analysed due to deviations in protocols between those recommended for the BAX™ system and international standards (section 2). A range of raw and cooked meat, poultry and dairy products were analysed. Results are shown in Table 3.3 and summarised in Tables 3.4 and 3.5. With inoculated foods, both conventional and BAX™ analysis detected *Salmonella* in 123 of 144 samples (85.4%). There was 98.6% agreement between results obtained from the standard method and the BAX™ method. The BAX™ system showed a 4.8% positive deviation rate (positive results where conventional results were negative) and a 0.8% negative deviation rate (BAX™ negative results where conventional analysis yielded a positive result) (Table 3.4). With the 72 uninoculated control samples, both conventional and BAX™ analysis detected *Salmonella* in 1 of 72 samples (1.4%). There was 93.1% agreement between results obtained from the two methods. The BAX™ system showed a 7.0% positive deviation rate (a sample positive by BAX™ but not conventionally) and a 0% negative deviation rate (Table 3.5).

Both methods detected a natural *Salmonella* contaminant from a raw chicken sample, whilst only the BAX™ system gave a positive *Salmonella* result in an uninoculated sample of raw pork steak, raw chicken, skimmed milk powder, liquid milk and cooked sliced turkey. These were not confirmed with the exception of the liquid milk sample and the raw pork steak sample where the BHI "grow-back" broths were shown to contain *Salmonella.* Both methods generally failed to detect inoculated *Salmonella* in

dairy samples where primary enrichment was done in Müller Kauffman tetrathionate broth (MK). It would appear that the selectivity of the broth was too great for the *Salmonella.* There are many reports of the toxicity of MK to *Salmonella* (e.g. Vassiliadis *et al,* 1974; van Schothorst *et al,* 1977). On one occasion, however, the BAX™ system was successful at detecting inoculated *Salmonella* from half-fat milk enriched in MK where conventional analysis failed. On another occasion, the reverse was true as inoculated *Salmonella* was conventionally detected from a raw beef steak where BAX™ analysis yielded a negative result.

As BAX™ results show more detections than conventional analysis with uninoculated samples, there is a temptation to class these results as "false positives". This is a possibility, but specificity trials have shown the BAX™ system to be a stringent and specific reaction. Additionally, the excellent sensitivity of the BAX™ system may allow detection at a low level of contamination that would make isolation on solid media unlikely. It is possible that the BAX™ system detected *Salmonella,* but that these could not be detected conventionally due to competition from background microflora in liquid, or on solid media, or due to atypical biochemical reactions of the *Salmonella* on the differential isolation media.

3.4 Detection of *Salmonella* in Uninoculated Foods

Thirty six potentially naturally contaminated food samples were tested in duplicate including dairy samples where different enrichment regimes were used. Each was analysed by conventional cultural methods and the BAX™ system for occurrence of naturally contaminating *Salmonella.* Results are shown in Table 3.6 and summarized in Table 3.7. There was 95.8% agreement between results obtained from the standard cultural method and the BAX™ method. BAX™ showed a 4.7% positive deviation rate and 0% negative deviation rate (Table 3.7).

Both methods detected *Salmonella* in duplicate samples of four pig's liver samples. The BAX™ system detected *Salmonella* in just one of the duplicate samples of another pig's liver sample that was not detected conventionally. This could not be isolated from enrichment media. In addition the BAX™ system detected *Salmonella* in one of the duplicates of two raw chicken samples. Again these were not detected

conventionally and *Salmonella* could not be isolated from enrichment broths. Again it is possible that *Salmonella* could not be culturally isolated because of competitor organism growth. For example, competitor growth on isolation agars can make it impossible to isolate *Salmonella* due to overcrowding on plates.

3.5 Use of the BAX™ system for detection of *Salmonella* in a Routine Analytical Food Microbiology Laboratory

The slow evolution of PCR from a technique in the research laboratory to a routine tool for quality control laboratories has created an aura of apprehension with respect to this powerful technique. The BAX™ system, however, is an easy to use method requiring fewer and simpler manipulations than conventional microbiology procedures. Combining all optimized reagents for the PCR into a single tablet greatly increases ease of use and minimizes the chance of operator error. Anticipated problems with PCR inhibition from product interference are overcome by inclusion of a positive control for each sample. If a positive result is not obtained from positive control tubes, then the PCR reaction has failed, and the result is invalid. Invalid results were extremely rare in this study. It should be noted that electrophoretic detection of amplified product can add an element of subjectivity to analysis. It can be difficult, on occasions, to determine whether or not a band indicating *Salmonella* is present and this becomes a matter for determination by the operator. These occasions are, however, rare.

It should also be noted that care must be taken throughout the PCR process to avoid contamination. The power of the PCR technique renders it essential that amplified product does not re-enter subsequent PCR analyses. This is achieved by following instructions specified by the manufacturer. In addition to the BAX™ kit it is necessary to have some specialist equipment such as the thermocycler and electrophoretic apparatus.

Overall the system is easy to use, convenient and extremely rapid.

TABLE 3.1

Sensitivity of the BAX™ *Salmonella* System

Organism	cfu/ml in BPW culture	cfu/ml in BHIB following "grow-back"	BAX™ Result
S. enteritidis CRA 1944	2.40×10^4 1.22×10^4 2.60×10^3 1.80×10^3 1.08×10^3 190 30	2.91×10^4 1.40×10^4 3.55×10^3 1.45×10^3 245 123 64	+ + + - - - -
S. typhimurium CRA 1009	3.40×10^4 2.00×10^4 3.90×10^3 1.50×10^3 400 225 40	5.30×10^4 2.15×10^4 2.40×10^4 2.10×10^3 515 1.18×10^4 51	+ + + - - - -
S. hadar CRA 1019	2.85×10^4 5.90×10^3 3.40×10^3 530 310 260	1.90×10^5 4.65×10^3 3.10×10^3 855 1.45×10^3 156	+ + - - - -
S. virchow CRA 1012	1.14×10^5 2.61×10^4 4.05×10^3 3.60×10^3 490 300 50	1.63×10^5 6.70×10^4 6.20×10^3 3.25×10^3 7.45×10^3 330 43	+ + + + - - -
S. newport CRA 1042	6.00×10^4 2.90×10^4 5.80×10^3 2.20×10^3 880 310 40	1.04×10^5 4.15×10^4 8.70×10^3 3.70×10^3 1.03×10^3 1.53×10^4 65	+ + + - - - -

Table 3.1 continued

Organism	cfu/ml in BPW culture	cfu/ml in BHIB following "grow-back"	BAX™ Result
S. heidelberg CRA 1028	5.18×10^4 2.24×10^4 3.80×10^3 1.68×10^3 590 180 60	1.09×10^5 8.00×10^4 1.17×10^4 5.95×10^3 1.31×10^3 775 223	+ + + - - - -
S. infantis CRA 1952	3.90×10^3 2.10×10^3 800 350 100	1.83×10^3 1.72×10^3 690 305 65	+ + - - -
S. montevideo CRA 1946	5.32×10^4 2.20×10^4 5.23×10^3 2.64×10^3 230 120 100	7.45×10^4 6.35×10^4 1.08×10^4 3.64×10^3 841 710 80	+ + + + + - -
S agona CRA 1050	4.69×10^4 2.57×10^4 4.40×10^3 2.45×10^3 590 340 80	1.03×10^5 5.45×10^4 9.65×10^3 4.85×10^3 1.03×10^3 690 95	+ + + + - - -
S. braenderup CRA 1096	4.55×10^4 2.64×10^4 4.45×10^3 3.27×10^3 310 120 160	6.32×10^4 5.55×10^4 1.02×10^4 4.85×10^3 1.19×10^3 650 139	+ + + + + - -
S. thompson CRA 1081	7.18×10^4 3.91×10^4 8.82×10^3 4.05×10^3 640 350 160	1.56×10^5 9.05×10^4 1.65×10^4 1.17×10^4 1.66×10^3 870 125	+ + + + - - -

Table 3.1 continued

Organism	cfu/ml in BPW culture	cfu/ml in BHIB following "grow-back"	BAX™ Result
S. derby CRA 1352	7.91×10^4	2.05×10^5	+
	3.82×10^4	8.20×10^4	+
	7.59×10^3	1.72×10^4	+
	4.23×10^3	1.18×10^4	+
	260	2.33×10^3	+
	370	1.41×10^3	-
	100	265	-
S. reading CRA 1405	5.05×10^4	1.40×10^5	+
	2.24×10^4	6.70×10^4	+
	5.18×10^3	1.43×10^4	+
	2.09×10^3	9.20×10^3	+
	700	1.35×10^3	+
	350	720	-
	60	69	-
S. saint-paul CRA 1090	6.60×10^3	1.61×10^4	+
	3.50×10^3	9.40×10^3	+
	800	765	-
	250	565	-
	>20	140	-
S. javiana CRA 1379	6.82×10^4	1.78×10^5	+
	2.85×10^4	1.07×10^5	+
	6.18×10^3	2.18×10^4	+
	3.27×10^3	1.20×10^4	+
	1.40×10^3	2.47×10^3	+
	220	1.12×10^3	-
	300	215	-
S. oranienberg CRA 1402	8.27×10^4	1.33×10^5	+
	4.09×10^4	6.40×10^4	+
	8.14×10^3	1.35×10^4	+
	4.82×10^3	6.50×10^3	+
	840	1.36×10^3	-
	470	580	-
	80	150	-
S. indiana CRA 1934	2.58×10^4	9.73×10^4	+
	1.74×10^4	7.35×10^4	+
	3.73×10^3	1.74×10^4	+
	1.86×10^3	1.59×10^4	+
	300	2.00×10^3	-
	170	490	-
	40	150	-

Table 3.1 continued

Organism	cfu/ml in BPW culture	cfu/ml in BHIB following "grow-back"	BAX™ Result
S. panama CRA 1049	6.70×10^3	1.43×10^4	+
	3.25×10^3	7.30×10^3	+
	700	1.40×10^3	-
	400	750	-
	200	155	-
S. brandenberg CRA 1070	5.40×10^3	1.62×10^4	+
	2.70×10^3	7.75×10^3	+
	250	2.00×10^3	-
	100	1.35×10^3	-
	250	195	-
S. java CRA 1378	7.77×10^4	1.65×10^5	+
	3.36×10^4	9.95×10^4	+
	6.95×10^3	2.06×10^4	+
	3.64×10^3	1.42×10^4	+
	540	2.04×10^3	+
	330	980	-
	130	306	-

TABLE 3.2

Inclusivity/Exclusivity trial of the BAX™ *Salmonella* System

Organism	CRA Code	BAX™ Result
Biochemically typical *Salmonella* strains		
S. agona	1053	+
S. agona	1433	+
S. albany	1275	+
S. anatum	1060	+
S. anatum	1061	+
S. anatum	1063	+
S. anatum	1064	+
S. arizona	3265	+
S. bedford	1418	+
S. berta	1065	+
S. berta	1069	+
S. binza	1436	+
S. blockley	1086	+
S. blockley	1088	+
S. bovis morbificans	1306	+
S. braenderup	1097	+
S. brandenberg	1072	+
S. brandenberg	1073	+
S. bredeney	1076	+
S. champaign	1327	+
S. columbo	1337	+
S. corvallis	1755	+
S. derby	1352	+
S. driffield	1430	+
S. dublin	1356	+
S. ealing	1362	+
S. enteritidis	1001	+
S. enteritidis	3505	+
S. enteritidis	3815	+
S. hadar	1015	+
S. hadar	1017	+
S. heidelberg	1964	+
S. ibadan	1578	+
S. infantis	1037	+

Table 3.2 continued

Organism	CRA Code	BAX™ Result
S. kedougou	1024	+
S. kedougou	1966	+
S. kentucky	1382	+
S. kimberley	1423	+
S. kottbuss	4179	+
S. lille	1851	+
S. livingstone	1963	+
S. locarno	1386	+
S. madelia	1388	+
S. malawi	1659	+
S. manchester	1429	+
S. manila	3939	+
S. mbandaka	1391	+
S. mbandaka	1935	+
S. meunchen	1849	+
S. montevideo	1031	+
S. napoli	1624	+
S. newport	1041	+
S. ohio	1459	+
S. ohio	3266	+
S. ohio	3270	+
S. orion	1936	+
S. panama	1045	+
S. panama	1047	+
S. poona	725	+
S. pretoria	1404	+
S. rubislaw	3263	+
S. saint-paul	1091	+
S. saint-paul	1092	+
S. santiago	3727	+
S. santiago	3728	+
S. senftenberg	1573	+
S. senftenberg	1940	+
S. senftenberg	2075	+
S. shangani	1409	+
S. stanley	1055	+
S. stanley	1056	+
S. stanley	1059	+
S. tennessee	3946	+
S. thompson	1082	+
S. thompson	1083	+

Table 3.2 continued

Organism	CRA Code	BAX™ Result
S. typhimurium	1006	+
S. typhimurium	1008	+
S. typhimurium	1960	+
S. virchow	1011	+
S. virchow	1014	+
Atypical *Salmonella* strains		
S. abortusequi	7046	+
S. africana/thompson	2002	+
S. anatum	6807	+
S. arizonae	7047	+
S. brandenberg	1959	+
S. bredeney	6721	+
S. dublin	1953	+
S. eastbourne	1363	+
S. gallinarum	1656	+
S. indiana	71	+
S. indiana	7050	+
S. pullorum	7049	+
S. senftenberg	1939	+
S. senftenberg	7048	+
S. typhimurium	1949	+
S. typhimurium	3425	+
S. typhimurium	3426	+
S. vietnam	3232	+
S. virchow	7045	+
S. wassenaar	7044	+
Non *Salmonella* strains		
Citrobacter freundii	1489	-
	3664	-
	4030	-
	4122	-
	4169	-
Escherichia coli	1545	-
	2077	-
	3025	-
	3991	-
	4611	-
Hafnia alvei	1561	-
	3642	-
	4007	-
	4009	-
	4011	-

Table 3.2 continued

Organism	CRA Code	BAX™ Result
Klebsiella aerogenes	243	-
Klebsiella ozaenae	4273	-
Klebsiella pneumoniae	1483	-
Morganella morganii	1542	-
	5120	-
Proteus mirabilis	4171	-
	4602	-
	4609	-
Proteus vulgaris	1580	-
	1581	-
Providencia alcalifaciens	4170	-
Pseudomonas aeruginosa	4636	-
Pseudomonas fluorescens	373	-
	1499	-
	1503	-
Serratia fonticola	3977	-
Serratia liquefaciens	1498	-
	1560	-
Serratia marcescens	1521	-
	4190	-

TABLE 3.3

Isolation/detection of *Salmonella* from inoculated foods and uninoculated controls

Food item	Inoculated Organism	Pre-enrichment medium	Inoculation level (cells/25g food)	Isolation/detection method	
				Confirmed standard cultural	BAX™ system
Raw beef steak	*S. locarno* CRA 1386	1:10 BPW	15	+	+
			15	+	+
			0	-	-
			12	+	+
			12	+	+
			0	-	-
	S. napoli CRA 1624	1:10 BPW	15	+	+
			15	+	+
			0	-	-
			10	-	-
			10	-	-
			0	-	-
Raw beef stewing steak	*S. senftenberg* CRA 1940	1:10 BPW	10	+	+
			10	+	+
			0	-	-
Raw beef braising steak	*S. driffield* CRA 1430	1:10 BPW	9	+	+
			9	+	-
			0	-	-
Raw beef steak and kidney	*S. stanley* CRA 1055	1:10 BPW	13	+	+
			13	+	+
			0	-	-

Table 3.3 continued

Food item	Inoculated Organism	Pre-enrichment medium	Inoculation level (ells/25g food)	Isolation/detection method	
				Confirmed standard cultural	BAX™ system
Raw beef mince	*S. heidelberg* CRA 1028	1:10 BPW	14	+	+
			14	+	+
			0	-	-
	S. indiana CRA 1934	1:10 BPW	14	+	+
			14	+	+
			0	-	-
	S. livingstone CRA 1963	1:10 BPW	10	+	+
			10	+	+
			0	-	-
Sliced processed beef	*S brandenberg* CRA 1070	1:10 BPW	13	+	+
			13	+	+
			0	-	-
Prepacked sliced processed beef	*S. kimberley* CRA 1423	1:10 BPW	7	+	+
			7	+	+
			0	-	-
Raw diced pork	*S. brandenberg* CRA 1072	1:10 BPW	12	+	+
			12	+	+
			0	-	-
Raw pork chop	*S. thompson* CRA 1081	1:10 BPW	18	+	+
			18	+	+
			0	-	-
Raw pork steak	*S. bedford* CRA 1418	1:10 BPW	13	+	+
			13	+	+
			0	-	-

Table 3.3 continued

Food item	Inoculated Organism	Pre-enrichment medium	Inoculation level (cells/25g food)	Isolation/detection method	
				Confirmed standard cultural	BAX™ system
Raw pork loin steak	*S. bredeney* CRA 1076	1:10 BPW	15	+	+
			15	+	+
			0	-	+
Raw belly pork	*S. panama* CRA 1049	1:10 BPW	14	+	+
			14	+	+
			0	-	-
Cooked pork	*S. newport* CRA 1042	1:10 BPW	19	+	+
			19	+	+
			0	-	-
	S. agona CRA 1050		38	+	+
			38	+	+
			0	-	-
Cooked sliced pork	*S. madelia* CRA 1388	1:10 BPW	22	+	+
			22	+	+
			0	-	-
			14	+	+
			14	+	+
			0	-	-
Prepacked cooked sliced pork	*S. typhimurium* CRA 1008	1:10 BPW	12	+	+
			12	+	+
			0	-	-

Table 3.3 continued

Food item	Inoculated Organism	Pre-enrichment Medium	Inoculation/level (cells/25g food)	Isolation/detection method	
				Confirmed standard cultural	BAX™ system
Raw lamb chop	*S. montevideo* CRA 1946	1:10 BPW	17	+	+
			17	+	+
			0	-	-
	S. montevideo CRA 1032		14	+	+
			14	+	+
			0	-	-
Raw lamb mince	*S. agona* CRA 1050	1:10 BPW	18	+	+
			18	+	+
			0	-	-
Raw lamb's liver	*S. panama* CRA 1045	1:10 BPW	10	+	+
			10	+	+
			0	-	-
Cooked lamb	*S. indiana* CRA 1934	1:10 BPW	12	+	+
			12	+	+
			0	-	-
	S. senftenberg CRA 1940		9	+	+
			9	+	+
			0	-	-
Raw chicken breast portion	*S. montevideo* CRA 1946	1:10 BPW	16	+	+
			16	+	+
			0	-	-
	S. saint-paul CRA 1090		16	+	+
			16	+	+
			0	-	-

Table 3.3 continued

Food item	Inoculated Organism	Pre-enrichment medium	Inoculation level (cells/25g food)	Isolation/detection method	
				Confirmed standard cultural	BAX™ system
Raw chicken quarter	*S. shangani* CRA 1409	1:10 BPW	16	+	+
			16	+	+
			0	+	+
			9	-	-
			9	-	-
			0	-	+
Raw fresh chicken portion	*S. virchow* CRA 1012	1:10 BPW	14	+	+
			14	+	+
			0	-	-
Raw chicken portion	*S. kimberley* CRA 1423	1:10 BPW	13	-	-
			13	-	-
			0	-	-
			10	+	+
			10	+	+
			0	-	-
	S. berta CRA 1068		12	+	+
			12	+	+
			0	-	-
Prepacked roast chicken breast	*S. infantis* CRA 1036	1:10 BPW	9	+	+
			9	+	+
			0	-	-
Cooked chicken portion	*S. braenderup* CRA 1096	1:10 BPW	19	+	+
			19	+	+
			0	-	-
Prepacked cooked sliced chicken	*S. anatum* CRA 1062	1:10 BPW	3	+	+
			3	+	+
			0	-	-

Table 3.3 continued

Food item	Inoculated Organism	Pre-enrichment medium	Inoculation level (cells/25g food)	Isolation/detection method	
				Confirmed standard cultural	BAX™ system
Raw diced turkey	*S. hadar* CRA 1019	1:10 BPW	23	+	+
			23	+	+
			0	-	-
Prepacked raw turkey breast steak	*S. malawi* CRA 1659	1:10 BPW	14	+	+
			14	+	+
			0	-	-
			12	+	+
			12	+	+
			0	-	-
	S. ohio CRA 1459		15	+	+
			15	+	+
			0	-	-
			14	+	+
			14	+	+
			0	-	-
Cooked sliced turkey	*S. enteritidis* CRA 1944	1:10 BPW	8	+	+
			8	+	+
			0	-	-
	S. newport CRA 1042		13	+	+
			13	+	+
			0	-	+
Sliced cooked turkey breast	*S. heidelberg* CRA 1029	1:10 BPW	16	+	+
			16	+	+
			0	-	-

Table 3.3 continued

Food item	Inoculated Organism	Pre-enrichment medium	Inoculation level (cells/25g food)	Isolation/detection method	
				Confirmed standard cultural	BAX™ system
Prepacked cooked sliced turkey	*S. kedougou* CRA 1024	1:10 BPW	25	+	+
			25	+	+
			0	-	-
Skimmed milk powder	*S. saint-paul* CRA 1093	1:10 BPW	19	+	+
			19	+	+
			0	-	-
		1:10 BGW	19	+	+
			19	+	+
			0	-	+
	S. infantis CRA 1035	1:10 BPW	18	+	+
			18	+	+
			0	-	-
		1:10 BGW	18	+	+
			18	+	+
			0	-	-
Infant milk powder	*S. ealing* CRA 1362	1:10 BPW	12	+	+
			12	+	+
			0	-	-
		1:10 BGW	12	+	+
			12	+	+
			0	-	-
Fresh pasteurised milk	*S. brandenberg* CRA 1074	1:10 BPW	17	+	+
			17	+	+
			0	-	-

Table 3.3 continued

Food item	Inoculated Organism	Pre-enrichment medium	Inoculation level (cells/25g food)	Isolation/detection method	
				Confirmed standard cultural	BAX™ system
Fresh pasteurised milk	*S. brandenberg* CRA 1074	1:10 SC	17	+	+
			17	+	+
			0	-	-
		1:10 MK	17	-	-
			17	-	-
			0	-	-
Fresh pasteurised half-fat milk	*S. hadar* CRA 1017	1:10 BPW	11	+	+
			11	+	+
			0	-	-
		1:10 SC	11	+	+
			11	+	+
			0	-	-
		1:10 MK	11	-	-
			11	-	+
			0	-	-
Fresh pasteurised virtually fat-free milk	*S. typhimurium* CRA 1009	1:10 BPW	11	+	+
			11	+	+
			0	-	-
		1:10 SC	11	+	+
			11	+	+
			0	-	-
		1:10 MK	11	-	-
			11	-	-
			0	-	+

Table 3.3 continued

Food item	Inoculated Organism	Pre-enrichment medium	Inoculation level (cells/25g food)	Isolation/detection method	
				Confirmed standard cultural	BAX™ system
UHT single cream	*S. agona* CRA 1433	1:10 BPW	1	+	+
			1	+	+
			0	-	-
		1:10 SC	1	+	+
			1	+	+
			0	-	-
		1:10 MK	1	-	-
			1	-	-
			0	-	-
Fresh pasteurised single cream	*S. typhimurium* CRA 3510	1:10 BPW	1	+	+
			1	+	+
			0	-	-
		1:10 SC	1	-	-
			1	-	-
			0	-	-
		1:10 MK	1	-	-
			1	-	-
			0	-	-
Fresh pasteurised double cream	*S. kedougou* CRA 1966	1:10 BPW	1	+	+
			1	+	+
			0	-	-
		1:10 SC	1	+	+
			1	-	-
			0	-	-
		1:10 MK	1	-	-
			1	-	-
			0	-	-

+, detection of *Salmonella*
-, no detection of *Salmonella*

TABLE 3.4

Summary of results of *Salmonella* detected/isolated from inoculated foods

Result from isolation/ detection method		BAX™	
		+	-
Conventional	+	122	1
	-	1	20

Method agreement $\frac{142}{144}$ x 100 = 98.6%

BAX™ + ve deviations = $\frac{1}{21}$ x 100 = 4.76%

BAX™ - ve deviations = $\frac{1}{123}$ x 100 = 0.81%

TABLE 3.5

Summary of results of *Salmonella* detected/isolated from uninoculated control food samples

Result from isolation/ detection method		BAX™	
		+	-
Conventional	+	1	0
	-	5*	66

Method agreement = $\frac{67}{72}$ x 100 = 93.1%*

BAX™ + ve deviations = $\frac{5}{71}$ x 100 = 7.04%*

BAX™ - ve deviations = $\frac{0}{1}$ x 100 = 0%

* Subsequent analysis of the BHI "grow back" broths of the original food samples proved that *Salmonella* was present in two of the five samples in which BAX™ gave a positive result but conventional analysis gave a negative result. In the other three cases it was not possible to unequivocally prove presence of *Salmonella*.

TABLE 3.6

Isolation/detection of *Salmonella* from potentially naturally contaminated foods

Food Item	Pre-enrichment medium	Isolation/detection method	
		Confirmed standard cultural	BAX™ system
Raw chicken portion	1:10 BPW	- - - - - - - -	- - - - + - - -
Raw chicken quarter	1:10 BPW	- -	- -
Raw chicken breast fillet	1:10 BPW	- -	- -
Raw chicken breast	1:10 BPW	- - - - - -	- - - - - -
Raw chicken	1:10 BPW	- - - - - -	- - - - + -
Raw chicken drumsticks	1:10 BPW	- -	- -
Raw chicken livers	1:10 BPW	- -	- -
Raw turkey steak	1:10 BPW	- -	- -
Raw lean diced turkey thighs	1:10 BPW	- -	- -

Table 3.6 continued

Food Item	Pre-enrichment medium	Isolation/detection method	
		Confirmed standard cultural	BAX™ system
Raw pig's liver	1:10 BPW	-	-
		-	-
		-	-
		-	-
		-	-
		-	-
		-	-
		-	-
		+	+
		+	+
		-	-
		-	+
		-	-
		-	-
		+	+
		+	+
		+	+
		+	+
		-	-
		-	-
		+	+
		+	+
Raw sausages	1:10 BPW	-	-
		-	-
		-	-
		-	-
		-	-
		-	-
		-	-
		-	-
Raw pork sausages	1:10 BPW	-	-
		-	-
Raw white pudding	1:10 BPW	-	-
		-	-
Raw milk	1:10 BPW	-	-
		-	-
		-	-
		-	-
		-	-
		-	-

TABLE 3.7

Summary of results of *Salmonella* detected/isolated from potentially naturally contaminated foods

Result from isolation/detection method		BAX™	
		+	-
Conventional	+	8	0
	-	3	61

Method agreement = $\frac{69}{72}$ x 100 = 95.8%

BAX™ + ve deviations = $\frac{3}{64}$ x 100 = 4.7%

BAX™ - ve deviations = $\frac{0}{8}$ x 100 = 0%

4. CONCLUSIONS

This study has evaluated the performance of the BAX™ system for the detection of *Salmonella* in foods. The technique has been shown to be specific and extremely sensitive when compared with reports on other rapid detection systems. The BAX™ system provided comparable results to conventional cultural analysis for the detection of *Salmonella* in inoculated and naturally contaminated foods (98.6% and 95.8% method agreement respectively). In both cases the BAX™ system yielded more positive results than conventional analysis and given the sensitivity observed with pure cultures, this situation may reflect deficiences in the conventional method and a superior sensitivity of the BAX™ system. Indeed *Salmonella* was shown to be present where there was a BAX™ positive result but a corresponding negative result by conventional analysis (footnote to Table 3.5). Results were obtained just one day after initiating analysis with the BAX™ system. Results were not required to be confirmed due to the use of such a specific genetic-based assay. Difficulties in confirming *Salmonella* characteristic colonies that develop on isolation media are well known. In the current study, great efforts were required to prove, or disprove, that a colony was *Salmonella.* BAX™ negates this requirement. The ease of use, good performance characteristics and speed of analysis make the BAX™ system an attractive approach for the detection of foodborne *Salmonella.*

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