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SUMMARY

The Path Stik Rapid *Salmonella* Test has been developed by Lumac bv, for the rapid detection of *Salmonella* from foods. The Path Stik assay is based on "dipstick" technology using immunochromatographic techniques which allow detection of *Salmonella* from an enriched sample in 10 minutes. The total test time for the Path Stik test is approximately 48h.

In this study the Path Stik Rapid *Salmonella* Test was evaluated with respect to its sensitivity and ability to detect *Salmonella* spp. from a range of inoculated and naturally contaminated foods. All food samples were tested in parallel with a method based on the BS 5763 Part 4: 1993 (ISO 6579) method.

The sensitivity of the Path Stik was dependent upon the serotype used. Minimum detection levels ranged from 3.0×10^5 cfu/ml to 2.0×10^7 cfu/ml for the 10 *Salmonella* serotypes tested. For the inoculated food tested, *Salmonella* was detected in 84% of the samples using the Path Stik. However, 4% of the positive detections could not be confirmed. When the inoculated samples were tested using the ISO method, *Salmonella* was isolated from 98% of the samples.

For naturally contaminated samples, the evaluation was done in two parts. In part 1 of the trial, the Path Stik was used to test the sample post-enrichment derived from the RV selective broth. A total of 166 food samples thought to be naturally contaminated were tested using the Path Stik and ISO protocols. Of these samples 16% gave positive detections when tested using the Path Stik, although 5% could not be confirmed. In 0.6% of the samples, the Path Stik gave a false negative result, *Salmonella* being isolated from the post-enrichment which tested negative using the device. When tested in parallel using the ISO method, *Salmonella* was isolated from 17% of the samples.

In Part 2 of the evaluation, the Path Stik protocol was modified. Two post-enrichments per sample, derived from the RV and SC selective broths, were tested using the Path Stik. The use of two post-enrichments per sample increased the number of *Salmonella* detections in the 40 chicken samples tested using the Path Stik. Using this, 50% of the samples were found to be *Salmonella* positive when tested, 20% more than when one (the RV) post-enrichment was tested. Twenty per cent of the positive detections, however, could not be confirmed. When tested in parallel with the ISO method, *Salmonella* was isolated from 35% of the samples. The presence of *Proteus* spp. in the majority of naturally contaminated samples was found to make isolation and confirmation of

Salmonella difficult, and resulted in a high number of presumptive false positive results from confirmation procedures of both the Path Stik and ISO methods. The use of Rambach Agar increased the number of confirmations by both detection methods due to the ability of the agar to differentiate between members of the Enterobacteriaceae.

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INTRODUCTION

Salmonella is an important foodborne pathogen which is still considered to be one of the main causes of gastroenteritis in the world. National data show wide variations in the incidence of human salmonellosis with a trend towards marked increases in the total number of cases (D'Aoust, 1989). Increased microbiological surveillance of foods by the food industry and by government regulating authorities has led to increased testing of food pathogens such as *Salmonella.*

Current conventional methods for the detection of *Salmonella* in foods involve laborious multistep cultural processes that can take up to four days for a presumptive identification to be obtained. Within the food industry there is an increasing requirement for the rapid detection of this pathogen and a number of methods have been developed to accelerate its detection. One of the more widely used methods is that based on enzyme immunoassay techniques, the enzyme linked immunosorbant assay (ELISA). A number of ELISA based tests kits are commercially available for rapid screening of *Salmonella* from foods, the majority of which are designed in multisample microtitre plate format. ELISAs offer the advantage of being sensitive, specific and reducing the testing time to within 48h for a presumptive result.

Although rapid methods are an attractive alternative to conventional detection methods, there are still some areas in the procedures that could be improved. With microtitre plate ELISAs, for example, the total assay time is 2.5h due to several incubation periods; there are laborious washing steps and multiple assays have to be performed at the same time due to the microtitre plate design.

Lumac b.v. have developed the Path Stik Rapid *Salmonella* Test, which has been designed to overcome some of the limitations of such methods. The Path Stik assay is based on dipstick technology using immunochromatographic techniques.

The Path Stik dipstick contains a wick and two test areas, C and T. Test area T, consists of a pad impregnated with a conjugate that forms a visible line in the presence of the required level of *Salmonella* antigen. Test area 'C' is a control in which a line always forms to indicate that the test has worked properly.

The detection is initiated by dipping the bottom part of the dipstick into an enriched test sample by approximately 0.5cm. *Salmonella* specific antibody, bound to coloured latex particles, is transported in the membrane of the dipstick in the presence of the test sample and across the reagent pad. If *Salmonella* antigens are present in the sample, a

pink-purple line appears in the test area due to the target antigen forming a sandwich with the latex-labelled and immobilised antibodies on the reagent pad. Excess latex labelled antibody is immobilised further up the test strip in test area C to provide an internal control.

The Path Stik, therefore, offers the advantage of presumptive results within 10 minutes after sample enrichment, with no washing or long, multiple incubation steps.

In this study the Path Stik Rapid *Salmonella* test was evaluated with respect to its sensitivity and ability to detect *Salmonella* spp. from a range of inoculated and naturally contaminated foods. All food samples were tested in parallel with a method based on the BS 5763 Part 4 (ISO 6579) method (Anon, 1993).

MATERIALS AND METHODS

Microorganisms

The organisms used in this study were obtained from the CCFRA Culture Collection and are listed in Table 1.

Foods

All foods were purchased from local retail outlets or obtained from CCFRA stock of naturally contaminated material and were stored at 4°C prior to testing. Powdered and dried materials were stored at ambient temperature pending analysis.

Test Procedures

(i) Conventional Protocol (ISO 6579)

The enrichment procedure based on ISO 6579 (Anon, 1993) was followed (Fig. 1). Samples (25g) were pre-enriched in 225ml Buffered Peptone Water (BPW, Oxoid) and incubated at 37°C for 16-20h. After incubation, 0.1ml and 10ml of pre-enrichment broth were transferred into 10ml Rappaport Vassiliadis (RV, Oxoid) and 90ml Selenite Cystine (SC, Oxoid) respectively. RV broth was incubated at 42°C for 18-24h and SC broth was incubated at 37°C for 18-24h. After 24h (and 48h if the plate was negative from the 24h SC broth) incubation, both selective enrichment broths were streaked onto Xylose Lysine Desoxycholate Agar (XLD, Oxoid) and Brilliant Green Agar (BGA, Oxoid) and incubated at 37°C for 24h. Typical colonies were confirmed biochemically using Vitek GNI cards (bioMérieux).

(ii) Path Stick Protocol

Part 1

The enrichment procedure detailed in ISO 6579 was followed. After the 18-24h incubation of RV at 42°C, 1ml of the selective enrichment was transferred to 10ml BPW and incubated at 37°C for 6-8h. The BPW post-enrichment was subsequently tested with the Path Stik device. The bottom part of the Path Stik dipstick was submerged into the BPW until the liquid front became visible in the test window (approx. 3 sec). The stick was then removed from the medium, placed on a piece of tissue paper and the test strip was read after exactly 10 min. Confirmation of the Path Stik result was carried out by plating the BPW post-enrichment onto XLD and BGA. The plates were incubated at 37°C for 24h and typical colonies were biochemically confirmed using Vitek GNI cards.

Part 2

The enrichment procedure based on ISO 6579 was followed. After 18-24h incubation of the RV and SC, 1ml of the selective broths were individually transferred to 10ml BPW and incubated at 37°C for 6-8h. The two post-enrichments were subsequently tested with the Path Stik. Confirmation of the Path Stik results were carried out by plating the two BPW post-enrichments onto XLD and BGA. The plates were incubated at 37°C for 24h and typical colonies confirmed using Vitek GNI cards.

In some cases, where *Proteus* was suspected as having masked the presence of *Salmonella*, additional confirmation procedures were used. The organisms were confirmed from a Path Stik positive sample by subculture of a sweep of growth, isolated from the BGA or XLD plate. The BPW was incubated at 37°C for 24h and retested using Path Stik. The sub-cultured post-enrichment was plated on XLD and BGA, incubated at 37°C for 24h and typical colonies confirmed using Vitek GNI Cards. Alternatively, where false negative results by Path Stik and ISO methods were suspected due to *Proteus* swarming, a sweep of the selective plates or the Path Stik post enrichment was plated onto Rambach Agar (RA, Merck). The RA was incubated at 37°C for 24h and typical colonies confirmed using Vitek GNI cards.

Sensitivity

Ten serotypes of *Salmonella* were inoculated into BPW (10ml) and incubated at 37°C for 24h (Table 1). The enrichment broths were then serially diluted in BPW to levels between 10^2 and 10^7 cells/ml (as determined by plate counts on Nutrient Agar (NA, Oxoid)) and all the dilutions tested using the Path Stik.

Inoculated Foods

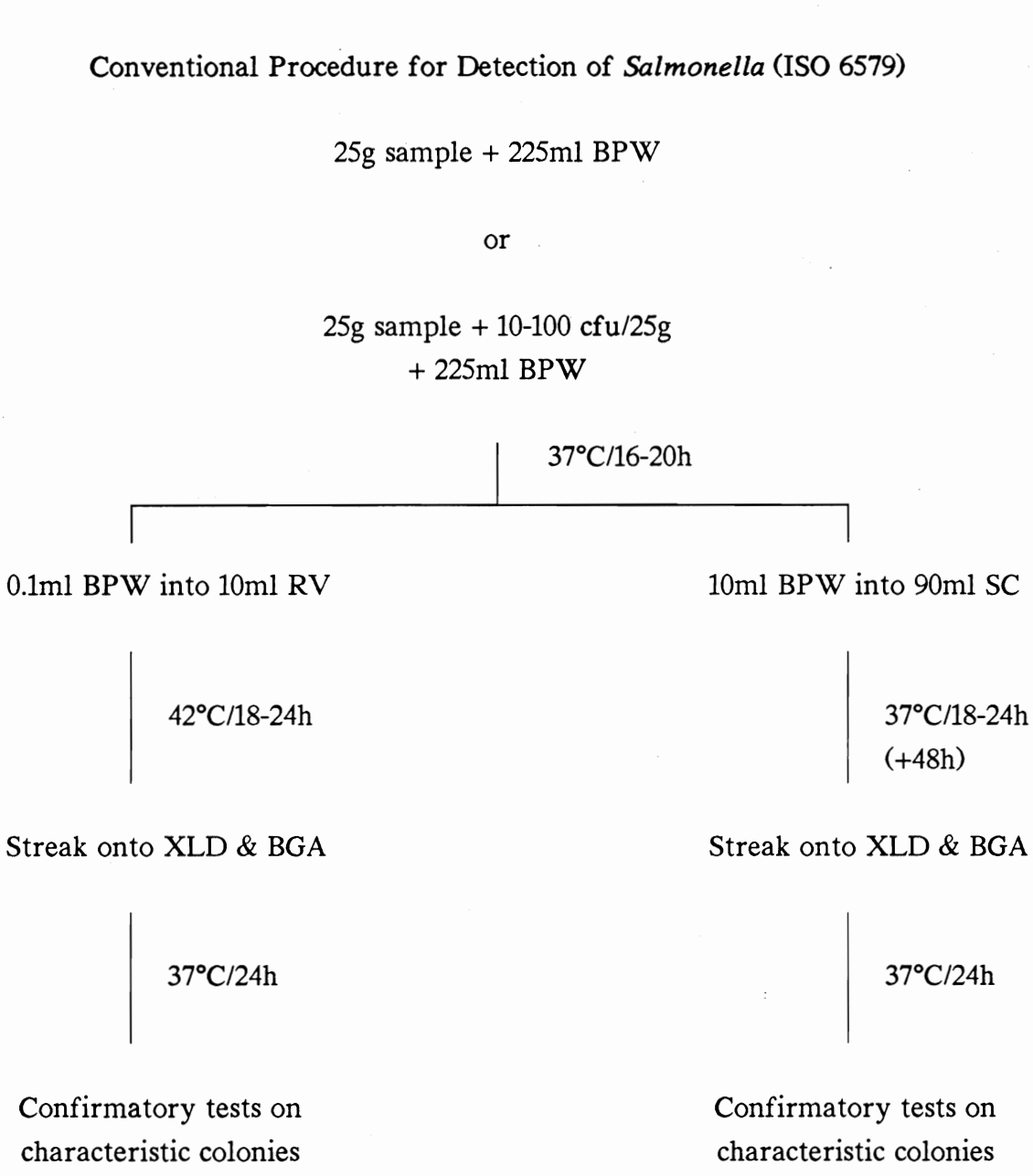
Forty seven samples from seven food groups (dairy, chocolate, an egg product, meat and poultry, herbs and spices, a vegetable product and a composite ready meal) were enriched using the ISO 6579 and Path Stik procedure (Figs. 1 and 2). Foods were inoculated with 10-100 cells of *Salmonella* (or a competitive microorganism) per 25g sample as confirmed by plate counts of the inoculum on NA. Organisms were inoculated into foods similar to those from which they were originally isolated. The serotype used with each food type is shown in Table 2. All the samples were tested using the Path Stik and by the ISO 6579 method.

Naturally Contaminated Foods

Foods thought to be naturally contaminated with *Salmonella* were tested using the Path Stik in parallel with the conventional ISO 6579 procedure. A total of 166 samples, listed in Table 3, were set up according to the enrichment procedures detailed in Figs. 1 and 2. Forty chicken samples listed in Table 4 were set up according to the enrichment procedures detailed in Figs. 1 and 3.

FIGURE 1

Conventional Procedure for Detection of *Salmonella* (ISO 6579)



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

FIGURE 2

Path Stik Procedure 1 for Detection of *Salmonella*

25g sample + 225ml BPW

or

25g sample + 10-100 cfu/25g
+ 225ml BPW

| 37°C/16-20h

0.1ml BPW into 10ml RV

| 42°C/18-24h

1ml RV into 10ml BPW

| 37°C/6-8h

Path Stik — Streak onto XLD and BGA

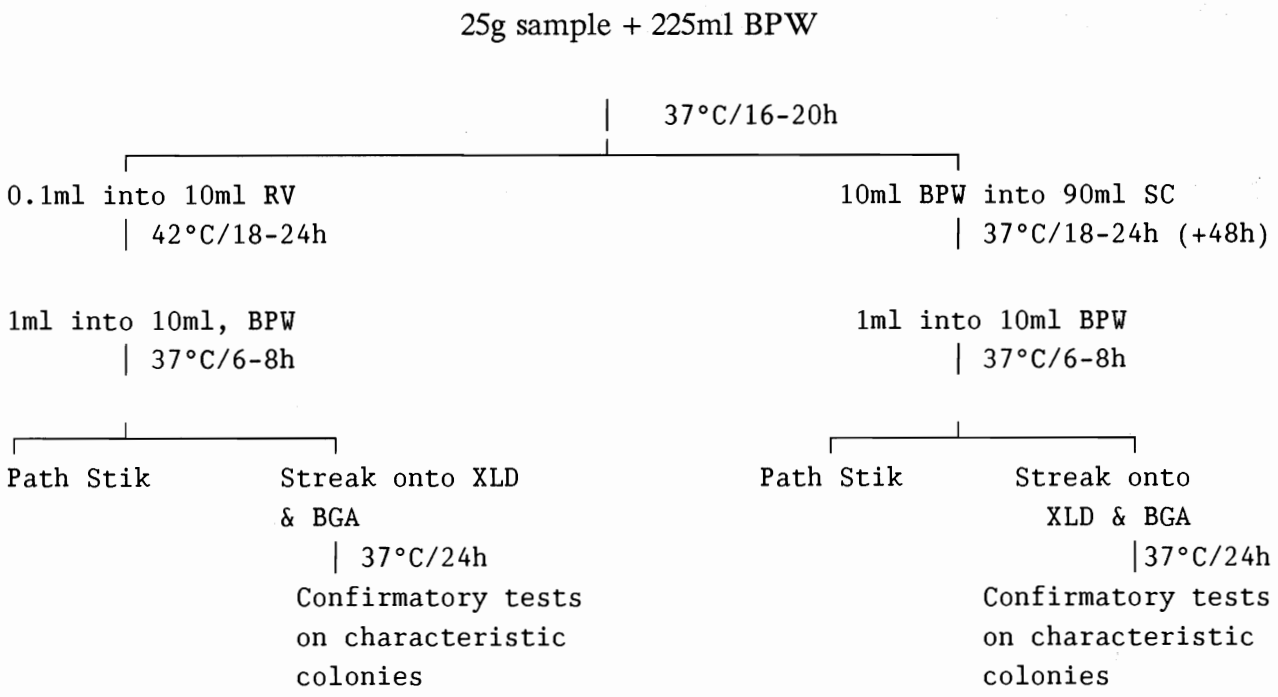
| 37°C/24h

Confirmatory tests on characteristic colonies

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

FIGURE 3

Path Stik Procedure 2 for Detection of *Salmonella*



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

RESULTS AND DISCUSSION

Sensitivity

The sensitivity of the *Salmonella* Path Stik was determined by testing all *Salmonella* cell concentrations from 10^2-10^7 cfu/ml. The minimum detection levels for the ten *Salmonella* strains tested ranged from 3.0×10^5 cfu/ml to 2.0×10^7 cfu/ml with the line in the test area of the Path Stik being generally fainter when used with the cell concentrations below 10^7 cfu/ml (Table 1). The minimum detection levels appeared to be dependent on the serotype used, presumably due to a difference in the amount of antigen expressed by different salmonellae. Generally, the sensitivity of the Path Stik was comparable to sensitivities reported for other commercial rapid methods such as the ELISA (Betts, 1992).

Inoculated Foods

The results from the Path Stik used with foods inoculated with *Salmonella* are shown in Table 2. The results from the Path Stik are directly comparable to the ISO 6579 conventional procedure since the RV selective enrichment used in both methods originated from the same test sample. To confirm the results from the Path Stik, the BPW post-enrichment was used as well as the RV from the ISO method for each sample.

Forty seven samples from nine food groups (dairy, egg, an egg product, chocolate, meat, poultry, herbs and spices, a vegetable product and a composite ready meal) were tested.

Dairy

The inoculated dairy products tested were cheese, cream dessert and skimmed milk powder. All of the salmonellae inoculated into the foods were detected by both the Path Stik and the conventional ISO procedure. *Salmonella agona* and *S.heidelberg* inoculated into cheese and the H_2S negative *S.livingstone* inoculated into skimmed milk powder, however, gave a faint line on the test area. The positive detections from the Path Stik were confirmed from the sample BPW post-enrichments.

Egg and Egg Product

Salmonella was detected using the Path Stik with the two egg samples inoculated with *S.uganda* and *S.indiana* and two mayonnaise samples inoculated with *S.bariellY* and *S.enteritidis.* The positive detections were confirmed from the sample post-enrichments used to inoculate the Path Stik devices. The organism was also isolated from the samples

TABLE 1

Sensitivity of the Path Stik *Salmonella* Test

Serotype (CRA Code)	Inoculum Level (cfu/ml)	Path Stik
S.enteritidis (1951)	6.3×10^7	+
	6.3×10^6	+ (f)
	6.3×10^5	-
	6.3×10^4	-
	6.3×10^3	-
	6.3×10^2	-
S.typhimurium (1006)	3.5×10^7	+
	3.5×10^6	+ (f)
	3.5×10^5	-
	3.5×10^4	-
	3.5×10^3	-
	3.5×10^2	-
S.virchow (1424)	2.0×10^7	+
	2.0×10^6	-
	2.0×10^5	-
	2.0×10^4	-
	2.0×10^3	-
	2.0×10^2	-
S.newport (1043)	2.8×10^7	+
	2.8×10^6	+ (f)
	2.8×10^5	-
	2.8×10^4	-
	2.8×10^3	-
	2.8×10^2	-
S.agona (1054)	3.1×10^7	+
	3.1×10^6	+ (f)
	3.1×10^5	-
	3.1×10^4	-
	3.1×10^3	-
	3.1×10^2	-
S.infantis (1038)	3.4×10^7	+
	3.4×10^6	+ (f)
	3.4×10^5	-
	3.4×10^4	-
	3.4×10^3	-
	3.4×10^2	-
S.bredeney (1075)	3.0×10^7	+
	3.0×10^6	+ (f)
	3.0×10^5	+ (f)
	3.0×10^4	-
	3.0×10^3	-
	3.0×10^2	-

TABLE 1 Continued ...

Serotype (CRA Code)	Inoculum Level (cfu/ml)	Path Stik
S.heidelberg (1028)	3.5×10^7	+ (f)
	3.5×10^6	+ (f)
	3.5×10^5	-
	3.5×10^4	-
	3.5×10^3	-
	3.5×10^2	-
S.montevideo (1033)	1.7×10^7	+
	1.7×10^6	+ (f)
	1.7×10^5	-
	1.7×10^4	-
	1.7×10^3	-
	1.7×10^2	-
S.hadar (1019)	3.0×10^7	+
	3.0×10^6	+
	3.0×10^5	-
	3.0×10^4	-
	3.0×10^3	-
	3.0×10^2	-

(f) = faint line

following enrichment using the ISO procedure.

Chocolate

Salmonella lexington inoculated into chocolate was detected by the Path Stik following enrichment. The result was confirmed from the sample post-enrichment. The organism was also isolated using the ISO method.

Meat

In the majority of the meats inoculated with serotypes of *Salmonella*, the Path Stik and ISO method were also comparable in terms of *Salmonella* detection. The two *Salmonella* serotypes individually inoculated into raw beef were both detected by the Path Stik and ISO methods. *Salmonella* was isolated from the Path Stik post-enrichment confirming the Path Stik detections. A presumptive false positive occurred using the ISO method in the uninoculated beef control due to *Morganella morganii* after 24h enrichment. (A presumptive false positive by cultural methods refers to a non *Salmonella* organism producing a colony with the appearance of a typical *Salmonella* on a selective agar).

When pork was inoculated with *S.senftenberg* and *S.brandenburg*, *Salmonella* was detected using the Path Stik protocol and the ISO method. The organism was also detected, however, in the control sample. Thus the pork was naturally contaminated with *Salmonella*, and so the inoculated pork tests were repeated using a different sample.

With the second set of samples inoculated with *S.senftenberg* and *S.brandenberg*, *Salmonella* was detected by both methods in the inoculated samples only. The Path Stik results were confirmed from the BPW post-enrichment. *Salmonella virchow* inoculated again into pork was also detected using the Path Stik and ISO method and the results confirmed.

Salmonella java and *S.typhimurium* inoculated into minced beef were both detected using the Path Stik and the ISO method. In an uninoculated minced beef sample, a presumptive false positive occurred from the 24h SC broth of the conventional procedure. This organism was subsequently identified as *Citrobacter freundii.*

Salmonella dublin inoculated into raw minced beef at a level of 55 cfu/25g was not detected by the Path Stik. The organism was isolated, however, when set up by the conventional method but from the 48h SC broth only. The fact that *Salmonella* was not

detected from the selective enrichment broths after 24h indicates that the organism was at too low a level to be detected, or absent in the case of the RV broth. The non-detection of *Salmonella* after 24h enrichment would explain why the Path Stik did not detect the organism, despite post-enrichment of the sample. *Salmonella* was not isolated from the post-enrichment, confirming the negative detection. It has been reported that certain serotypes, especially *S.dublin*, are inhibited in media containing dyes (Fricker and Girdwood 1984). The selective broths, particularly RV, would appear to be unfavourable growth media for *S.dublin*. The possibility that malachite green in RV could inhibit *S.dublin* was suggested by Kalapothaki *et al* (1986).

Salmonella kentucky inoculated into salami was not detected by the Path Stik, following enrichment. The organism was not isolated from the post-enrichment used to inoculate the Path Stik; however, *Salmonella* was isolated from the 24h RV enrichment from the ISO procedure. The fact the *Salmonella* was present in the RV indicates that the post-enrichment was not sufficiently inoculated. When inoculated at a higher level (65 cfu/25g), *S.kentucky* was detected by both methods and the Path Stik result confirmed. *Salmonella montevideo* was detected by both methods when inoculated into Salami at 65 cfu/25g. The organism was confirmed from the Path Stik post-enrichment. *Hafnia alvei* gave a presumptive false positive from SC broth after 24h from a control sample.

Poultry

Some discrepancies between Path Stik and the conventional procedure were noted. Raw chicken inoculated with *S.pullorum* at 5 cfu/25g was not detected by the Path Stik; however, the organism was isolated by the ISO method from the 24h RV and SC selective enrichment broths. *Salmonella* was not isolated from the Path Stik post-enrichment and thus the negative detection was likely to have been due to inadequate cell transfer from the RV to BPW, and not a false negative detection by the Path Stik.

Salmonella was detected by both methods when *S.pullorum* was inoculated into chicken at a higher level of 22 cfu/g and the Path Stik positive detection was confirmed from the post-enrichment. A presumptive false positive occurred from the control chicken samples due to *Proteus* growth.

Salmonella albany, also inoculated into raw chicken, gave a negative detection when tested using the Path Stik. The organism was isolated by the conventional ISO method after 24h selective enrichment. When the Path Stik post-enrichment was plated, *Salmonella* was confirmed as being present and thus a false negative result occurred with the device. When the chicken, inoculated with *S.albany*, was repeated using a higher

inoculum level of 62 cfu/25g, the organism was subsequently detected by both methods and confirmed as being present in the Path Stik post-enrichment.

Salmonella gallinarum inoculated into cooked turkey at 40 cfu/25g was not detected by either method following enrichment and was not isolated from the Path Stik post-enrichment. Thus the *S.gallinarum* failed to grow during enrichment. A presumptive false positive occurred after 48h SC enrichment due to *Hafnia alvei.* When repeated at a higher inoculation level of 68 cfu/25g, *S.gallinarum* was detected by the Path Stik and by the ISO method. The organism was isolated from the Path Stik post-enrichment, confirming the positive detection.

Herbs and Spices

The Path Stik gave a good correlation with the ISO conventional method in the detection of *Salmonella* from herbs and spices. *Salmonella derby* and *S.schwarzengrund,* inoculated into black pepper, both gave a positive detection with the Path Stik, but the reaction line was faint. *Salmonella* was confirmed from both the post-enrichments used to inoculate the Path Stik. The presence of *Salmonella* in these samples was also detected when tested with the ISO method. When *S.hadar* was inoculated into black pepper and enriched in a 1:10 and a 1:100 dilution, *Salmonella* was detected by the Path Stik in both samples. The organism was isolated from the post-enrichment, confirming the result. *Salmonella* was isolated when the two samples were tested using the ISO method.

Salmonella bredeney, S.mbandaka and *S.aztec* inoculated into oregano were enriched in dilutions between 1:10 to 1:1000 in BPW. This was due to the potential antimicrobial properties of the herb. Oregano is recognized as being particularly inhibitory and dilutions beyond their toxic levels are often carried out. All three serotypes were detected by the Path Stik and using the ISO method from all the enrichment dilutions and the Path Stik results confirmed. The conventional method gave a presumptive false positive after 24h SC enrichment of the control sample due to growth of *Leclercia adecarboxylata.*

Vegetables

Salmonella saint-paul, inoculated into prepacked vegetables at 50cfu/25g, was not detected by the Path Stik following enrichment and it was not isolated from the post-enrichment used to inoculate the device. The organism was, however, detected by the ISO method after 24h selective enrichment. Thus, despite *Salmonella* being confirmed from RV enrichment, *S.saint-paul* was not isolated from the Path Stik post-enrichment after plating. It was not a failure of the Path Stik to detect *Salmonella* but a failure of the

enrichment system. *Salmonella infantis* inoculated into vegetables at 64 cfu/25g was detected by the Path Stik and confirmed from the post-enrichment and the ISO method.

Composite Foods

In the lasagne ready meal inoculated with *S.braenderup* and *S.agona*, *Salmonella* was detected by the Path Stik and the detections confirmed from the post-enrichments. The organism was isolated from the samples by the ISO method.

When *S.panama* was inoculated into lasagne at 35 cfu/25g, *Salmonella* was not detected as being present in the sample when tested using the Path Stik. The organism, however, was isolated from the Path Stik post-enrichment and from the 24h selective enrichments confirming that *Salmonella* was present in the inoculated lasagne. Thus, a false negative result occurred with the Path Stik. When the sample was repeated at a higher inoculation level of 63 cfu/25g, *Salmonella* was detected by the Path Stik and confirmed from the post-enrichment and by the ISO method.

Background Organisms

The exclusivity of the Path Stik was demonstrated by inoculation of six non-*Salmonella* foods with six organisms that had the potential to grow up in the enrichment system. The organisms selected are recognised as frequently causing presumptive false positive results by traditional cultural methods.

The Path Stik did not cross-react with any of the negative controls tested. Two presumptive false positive isolations occurred by the ISO method. *Hafnia alveii* inoculated into lasagne ready meal gave colonies typical of *Salmonella* after 24h enrichment. *Citrobacter freundii, Kluyvera* sp. and an *Enterobacter* sp. were isolated after 24h enrichment of the vegetables inoculated with *Pseudomonas aeruginosa.*

In summary, for inoculated foods, the Path Stik was shown to give similar results to the ISO method in terms of *Salmonella* detection from foods. All 22 salmonellae inoculated into the dairy, egg, egg product, chocolate and herbs and spices were detected by the Path Stik. The organism was isolated from the Path Stik post-enrichment and by the ISO method.

The Path Stik detected *Salmonella* in twelve of the fourteen meat samples tested using the Path Stik protocol and the organism was confirmed as being present in the Path Stik post-enrichments. When the samples were tested using the ISO method, *Salmonella* was isolated from all fourteen meat samples. One of the non-detections by the Path Stik

TABLE 2

Detection of *Salmonella* from Inoculated Foods

Food Sample	Serotype (CRA Code)	Inoculum Level (cfu/ 25g)	Path Stik	Path Stik Confirmation	Conventional Procedure (ISO 6579)
DAIRY					
Cheese	*S.agona* (1433)	13	+(f)	+	+
Cheese	*S.kottbus* (4179)	24	+	+	+
Cheese	Control	0	-	-	-
Cheese	*S.heidelberg* (1028)	3	+(f)	+	+
Cheese	Control	0	-	-	-
Cream Dessert	*S.anatum* (1060)	58	+	+	+
Cream Dessert	*S.stanley* (1056)	53	+	+	+
Cream Dessert	Control	0	-	-	-
Skimmed Milk	*S.newbrunswick* (1399)	40	+	+	+
Skimmed Milk	*S.livingstone* (1963)	34	+(f)	+	+
Skimmed Milk	Control	0	-	-	-
Skimmed Milk	*S.newport* (1043)	48	+	+	+
Skimmed Milk	Control	0	-	-	-
EGG					
Egg	*S.uganda* (5109)	51	+	+	+
Egg	*S.indiana* (1934)	17	+	+	+
Egg	Control	0	_	-	-
EGG PRODUCT					
Mayonnaise	*S.bareilly* (1291)	73	+	+	+
Mayonnaise	Control	0	-	-	-
Mayonnaise	*S.enteritidis* (1951)	11	+	+	+
Mayonnaise	Control	0	-	-	-
CHOCOLATE					
Chocolate	*S.lexington* (5110)	12	+	+	+
Chocolate	Control	0	-	-	-
MEAT					
Beef	*S.blockley* (1087)	75	+	+	+
Beef	*S.thompson* (3506)	83	+	+	+
Beef	Control	0	-	-	F+
Pork (a)	*S.senftenberg* (1573)	25	+	+	+
Pork (a)	*S.brandenburg* (1072)	55	+	+	+
Pork	Control	0	+	+	+
Pork	*S.virchow* (1424)	69	+	+	+
Pork	Control	0	-	-	-
Pork (b)	*S.senftenberg* (1573)	24	+	+	+
Pork (b)	*S.brandenburg* (1072)	59	+	+	+
Pork	Control	0	-	-	-

TABLE 2 Continued

Food Sample	Serotype (CRA Code)	Inoculum Level (cfu/ 25g)	Path Stik	Path Stik Confirmation	Conventional Procedure (ISO 6579)
Minced Beef	*S.java* (1378)	72	+	+	+
Minced Beef	*S.dublin* (1356)	55	-	-	+(48)
Minced Beef	Control	0	-	-	-
Minced Beef	*S.typhimurium* (1006)	71	+	+	+
Minced Beef	Control	0	-	-	F+
Salami	*S.kentucky* (1382)	23	-	-	+
Salami	Control	0	-	F+	F+
Salami	*S.montevideo* (1033)	65	+	+	+
Salami	*S.kentucky* (1382)	63	+	+	+
Salami	Control	0	-	-	-
POULTRY					
Chicken (a)	*S.pullorum* (1956)	5	-	-	+
Chicken (a)	*S.albany* (1275)	16	-	+	+
Chicken	Control	0	-	F+	F+
Chicken (b)	*S.pullorum* (1956)	22	+	+	+
Chicken (b)	*S.albany* (1275)	62	+	+	+
Chicken	Control	0	-	-	-
Cooked Turkey(a)	*S.gallinarum* (1579)	40	-	-	F+
Cooked Turkey	Control	0	-	-	-
Cooked Turkey(b)	*S.gallinarum* (1579)	68	+	+	+
Cooked Turkey	Control	0	-	-	-
HERBS AND SPICES					
Black Pepper	*S.derby* (1352)	60	+(f)	+	+
Black Pepper	*S.schwarzengrund*(1408)	34	+(f)	+	+
Black Pepper	Control	0	-	-	-
Black Pepper	*S.hadar* (1019)	66	+	+	+
Black Pepper(1:100)	*S.hadar* (1019)	67	+	+	+
Black Pepper	Control	0	-	-	F+
Oregano	*S.bredeney* (1075)	96	+	+	+
Oregano	Control	0	-	-	-
Oregano (1:100)	*S.mbandaka* (1935)	83	+	+	+
Oregano (1:100)	*S.aztec* (1287)	63	+	+	+
Oregano (1:100)	Control	0	-	-	F+
Oregano (1:1000)	*S.mbandaka* (1935)	33	+	+	+
Oregano (1:1000)	*S.aztec* (1287)	65	+	+	+
Oregano (1:1000)	Control	0	-	-	-

TABLE 2 continued

Food Sample	Serotype (CRA Code)	Inoculum Level (cfu/25g)	Path Stik	Path Stik Confirmation	Conventional Procedure (ISO 6579)
VEGETABLES					
Vegetable	*S.saint-paul* (1092)	50	-	-	+
Vegetable	Control	0	-	-	-
Vegetable	*S.infantis* (1038)	64	+	+	+
Vegetable	Control	0	-	-	-
COMPOSITE FOODS					
Lasagne	*S.braenderup* (1097)	99	+	+	+
Lasagne	*S.panama* (1403)	35	-	+	+
Lasagne	Control	0	-	-	-
Lasagne	*S.agona* (1054)	85	+	+	+
Lasagne	*S.panama* (1403)	63	+	+	+
Lasagne	Control	0	-	-	-
BACKGROUND ORGANISMS					
Egg	*Citrobacter freundii* (3984)	53	-	-	-
Salami	*Escherichia coli* (4739)		-	-	F+
Lasagne	*Hafnia alvei* (4007)	31	-	F+	-
Cooked Turkey	*Proteus vulgaris* (4003)	11	-	-	-
Vegetables	*Pseudomonas aeruginosa* (4639)	24	-	F+	-
Pork	*Serratia fonticola* (4613)	11	-	-	-
Total			41	43	47

(f) = faint line

(48) = *Salmonella* isolated from 48h Selenite Cystine only

1:100/1:1000 = sample dilutions in pre-enrichment broth (otherwise 1:10)

F+ = presumptive false positive

was due to the fact that the organism was only detected in the ISO 48h SC broth. In the other additional positive sample tested using the ISO method, *Salmonella* was isolated from the 24h RV enrichment broth but not from the subsequent RV post-enrichment. Thus the negative detection was not due to a failure of the Path Stik but due to a failure in the enrichment system.

In the six inoculated poultry samples tested, *Salmonella* was detected in three of the samples using the Path Stik protocol. The organism was isolated from four of the sample post-enrichments used to inoculate the devices and hence one false negative occurred. When the samples were tested using the ISO method, an additional sample was identified as positive for *Salmonella.* Despite the fact that the organism was isolated from the 24h RV enrichment broth, it was not found to be present in the post-enrichment sub-cultured from the broth. Hence the non-detection of the *Salmonella* by the Path Stik was again due to a failure in the enrichment system.

Salmonella was detected in one of the two inoculated vegetable samples using the Path Stik protocol. The organism was only found to be present in one of the Path Stik post-enrichments, confirming the results. When tested with the ISO method, however, both samples were found to be positive for *Salmonella.* The organism was isolated from the 24h RV enrichments and thus, again, the failure of the Path Stik to detect the organism in the second sample was due to a failure of the enrichment system.

Salmonella was detected in three of the four composite foods inoculated with *Salmonella.* The organism was confirmed as being present in all four of the Path Stik post-enrichments and when tested using the ISO method, and so a false negative detection occurred using the Path Stik.

In total, the Path Stik detected *Salmonella* in 41 of the 47 inoculated samples and in an uninoculated control. 46 of the 47 inoculated samples and the uninoculated control were positive when tested using the ISO method.

The inoculum used contained cells that were presumed to be physiologically fit. Naturally contaminated samples were used to investigate the efficacy of the methods to detect potentially stressed or injured *Salmonella* from foods.

NATURALLY CONTAMINATED FOODS - PART 1

A total of 166 samples from a range of potentially naturally contaminated foods were tested by both the Path Stik and by the ISO conventional method (Table 3).

TABLE 3

The Detection of *Salmonella* from Potentially Naturally Contaminated Foods

Food Sample	Path Stik	Path Stik Confirmation	Conventional Procedure (ISO 6579)
Egg	-	-	-
Egg	+	+	+
Egg	-	-	-
Egg	-	-	-
Egg	-	-	-
Raw Chicken	-	-	F+
Raw Chicken	-	-	F+
Raw Chicken	-	-	F+
Raw Chicken	-	-	-
Raw Chicken	-	-	-
Raw Chicken	-	-	F+
Raw Chicken	-	-	F+
Raw Chicken	+	+	+
Raw Chicken	+	F+	F+
Raw Chicken	-	-	-
Raw Chicken	-	-	F+
Raw Chicken	-	-	+ (48)
Raw Chicken	-	-	+ (48)
Raw Chicken	+	+	+
Raw Chicken	-	-	F+
Raw Chicken	-	-	F+
Raw Chicken	-	-	F+
Raw Chicken	-	-	-
Raw Chicken	-	-	F+
Raw Chicken	-	-	-
Raw Chicken	-	-	-
Raw Chicken	-	-	-
Raw Chicken	-	-	-
Raw Chicken	-	-	-
Raw Chicken	-	-	-
Raw Chicken	+	+	+
Raw Chicken	-	F+	F+
Raw Chicken	-	-	F+
Raw Chicken	+	+	+
Raw Chicken	-	-	+
Raw Chicken	+	+	+
Raw Chicken	+	+	+
Raw Chicken	+	+	+
Raw Chicken	+	+	+
Raw Chicken	-	-	-
Raw Chicken	-	-	-
Raw Chicken	-	-	F+
Raw Chicken	-	-	F+

TABLE 3 CONTINUED ...

Food Sample	Path Stik	Path Stik Confirmation	Conventional Procedure (ISO 6579)
Raw Chicken	-	-	F+
Raw Chicken	-	-	F+
Raw Chicken	+	F+	F+
Raw Chicken	-	-	F+
Raw Chicken	-	-	-
Raw Chicken	-	-	+ (48)
Raw Chicken	-	-	F+
Raw Chicken	-	-	-
Raw Chicken	-	-	-
Raw Chicken	-	-	-
Raw Chicken	-	F+	F+
Raw Chicken	-	-	-
Raw Chicken	-	-	-
Raw Turkey	-	-	-
Raw Minced Turkey	-	-	-
Raw Minced Turkey	-	-	-
Poussin	-	-	F+
Raw Pork	-	-	F+
Raw Pork	-	-	-
Raw Pork	-	-	-
Raw Pork	-	-	-
Raw Pork	-	-	-
Raw Pork	-	-	-
Raw Pork	-	-	-
Raw Pork	-	-	-
Raw Pork	-	F+	F+
Raw Pig Liver	+	+	+
Raw Pig Liver	-	-	+ (48)
Raw Pig Liver	+	F+	F+
Raw Pig Liver	+	+	+
Raw Pig Liver	+	F+	F+
Raw Pig Liver	-	-	-
Raw Pig Liver	-	-	F+
Raw Pig Liver	-	-	-
Raw Pig Liver	-	-	-
Raw Pig Liver	-	-	-
Raw Pig Liver	-	-	-
Raw Pig Liver	-	-	-
Raw Pig Liver	-	-	-
Raw Pig Liver	-	-	F+
Raw Pig Liver	-	-	F+
Raw Pig Liver	-	-	F+
Raw Pig Liver	-	-	F+

TABLE 3 CONTINUED ...

Food Sample	Path Stik	Path Stik Confirmation	Conventional Procedure (ISO 6579)
Raw Minced Pork	-	-	-
Raw Minced Pork	-	-	-
Raw Minced Pork	+	F+	F+
Raw Minced Pork	+	F+	F+
Raw Minced Pork	-	F+	F+
Raw Minced Pork	-	-	-
Raw Minced Pork	+	F+	F+
Raw Minced Pork	-	-	-
Raw Pig Kidney	-	-	F+
Raw Pig Kidney	-	-	F+
Raw Pig Kidney	-	-	-
Raw Pig Heart	-	-	-
Raw Pig Heart	-	-	-
Raw Pig Heart	+ (f)	-	-
Raw Pig Heart	-	-	-
Raw Pig Heart	-	-	-
Raw Pig Heart	+	+	+
Raw Pig Heart	-	-	F+
Raw Pig Heart	-	-	F+
Raw Pig Heart	-	-	-
Raw Lamb Liver	-	-	-
Raw Minced Beef	-	-	-
Raw Minced Beef	-	-	-
Raw Minced Beef	-	-	F+
Raw Minced Beef	-	-	-
Raw Minced Beef	-	-	-
Raw Minced Beef	-	-	-
Raw Sausage	-	-	-
Raw Sausage	-	-	-
Raw Sausage Meat	-	-	-
Raw Sausage Meat	-	-	-
Raw Sausage Meat	-	-	-
Raw Sausage Meat	-	-	-
Raw Sausage Meat	-	-	-
Raw Sausage Meat	-	-	-
Raw Sausage Meat	-	-	-
Raw Sausage Meat	-	-	-
Raw Sausage Meat	-	-	-
Raw Sausage Meat	-	-	-
Raw Sausage Meat	-	-	-
Raw Sausage Meat	-	-	-
Raw Sausage Meat	-	-	-
Cooked Turkey	-	-	F+
Cooked Turkey	-	-	+ (48)
Cooked Turkey	+(p)	+	+
Cooked Turkey	+(p)	+	+
Smoked Turkey	-	-	+

TABLE 3 CONTINUED ...

Food Sample	Path Stik	Path Stik Confirmation	Conventional Procedure (ISO 6579)
Smoked Ham	-	-	-
Crumbed Ham	-	-	-
Crumbed Ham	-	-	-
Honey Roast Ham	-	-	-
Cottage Cheese	-	-	-
Beansprouts	-	-	-
Sesame Seeds	-	-	-
Sesame Seeds	-	-	F+
Sesame Seeds	-	-	-
Sesame Seeds	-	-	-
Sesame Seeds	-	-	-
Cereal Oats	-	-	-
Cereal Grain	-	-	+
Cereal Grain	-	-	-
Milk Powder	-	-	-
Milk Powder	-	-	-
Egg Powder	-	-	-
Whey Powder	-	-	-
Whey Powder	+	+	+
Whey Powder	-	-	-
Whey Powder	+	+	+
Whey Powder	-	-	-
Whey Powder	-	-	-
Whey Powder	-	-	-
Buttermilk Powder	-	-	F+
Buttermilk Powder	+	+	+
Dried Egg Albumen	+	+	+
Dried Egg Albumen	+	+	+
Dried Egg Albumen	-	-	+
Dried Egg Albumen	-	-	-
Dried Egg Albumen	-	+	+
Dried Egg Albumen	-	-	-
Total	27	20	29

(p) = partial line

(f) = faint line

(48) = *Salmonella* isolated from 48h Selenite Cystine only

F+ = presumptive false positive

Of the five egg samples tested, one was positive for *Salmonella* by both the Path Stik and the ISO 6579 method. The presence of *Salmonella* in the post-enrichment used to inoculate the Path Stik was culturally confirmed.

Of the 52 raw chicken samples tested by the Path Stik and the ISO method, the presence of *Salmonella* was not detected in 38 of the samples by either method. In addition, the organism was not isolated from the Path Stik post enrichment. Eighteen presumptive false positive detections occurred by the ISO method, mainly due to *Proteus* growth on the selective agars.

In eight of the samples tested, *Salmonella* was detected by both the Path Stik and the ISO method. The Path Stik results were all confirmed from the post-enrichment. With the remaining six raw chicken samples, a number of differences between the two methods occurred.

In two chicken samples, the Path Stik gave a positive detection whilst *Salmonella* was not isolated by the conventional method. In both cases only *Proteus* could be identified from the ISO RV and SC enrichment broths due to the swarming nature of the organism on the selective agars. *Proteus* was also the only organism to be isolated when the two Path Stik post-enrichments were plated, and so the positive detections remained unconfirmed. It is possible that the growth of the *Proteus* on the selective plates masked the presence of *Salmonella*, particularly if it was present at low levels. The *Proteus* isolated from the selective and post-enrichment broths was grown up in BPW and tested with the Path Stik to verify that the Path Stik had not given a false positive result. There was no cross reaction of the Path Stik with *Proteus* and so it is possible that the ISO method gave a false negative result for the two raw chicken samples tested.

In the remaining four raw chicken samples, the Path Stik gave a negative detection whilst the organism was isolated by the ISO method. In three of these samples, *Salmonella* was only detected after 48h incubation of SC broth, indicating that the pathogen was either absent or present at a very low level in the selective broths after 24h enrichment. The organism was not detected in the Path Stik post-enrichment so it would appear that the non detection by Path Stik was due to failure of the enrichment system and not a failure of the test stick to detect the organism.

In the fourth sample, *Salmonella* was isolated from the SC broth after 24h but not from the RV broth. This suggests that the organism was either absent or present at very low levels in the RV selective enrichment broth and thus gave a negative detection when

tested with the Path Stik, even after post-enrichment.

Salmonella was not detected by either method in the other raw poultry samples tested which included minced turkey and poussin. The organism was not isolated from the Path Stik post-enrichments, confirming the results. A presumptive false positive detection occurred from the poussin sample tested by the ISO method due to *Proteus* growth.

The results of the nine raw pork samples by the Path Stik and the ISO protocol did not show a positive detection by either method. The organism was also shown to be absent from the Path Stik post-enrichment. A presumptive false positive, however, occurred from two samples tested by the ISO method.

Of the eighteen raw pig liver samples tested, *Salmonella* was not detected in thirteen of the samples tested using the Path Stik and the organism was not isolated from the post-enrichment. When tested by the ISO method, all thirteen of the samples were negative but five presumptive false positive results occurred due to *Proteus* growth.

In two of the eighteen samples, *Salmonella* was detected by the Path Stik and the result was confirmed from the post enrichment and by the ISO method. With the remaining three samples, discrepancies between the two methods occurred. Two of the samples gave a positive detection when tested with the Path Stik. When the post-enrichment was plated, however, only *Proteus* could be isolated. *Proteus* was also the only organism to be identified when the two samples were tested by the ISO method. Thus, the positive detections by the Path Stik remained unconfirmed. It is possible that *Salmonella* was present in the enrichment but that it was masked by *Proteus* swarming on the selective plates.

Salmonella was detected from the remaining pig liver sample, but by the ISO method only. The organism was only recovered from the 48h SC broth, and so *Salmonella* was not present at detectable levels, or absent, in the broths after 24h. *Salmonella* was not isolated from the Path Stik post-enrichment and hence the Path Stik gave a negative detection.

Five of the eight raw minced pork samples tested with the Path Stik were negative for *Salmonella* and the organism was not detected in the post-enrichment after confirmation. The samples were also negative when tested with the ISO procedure but one of the five samples gave a presumptive false positive result due to *Proteus* growth.

The remaining three raw pork mince samples all gave positive detections by the Path Stik. The organism, however, was not isolated by the ISO method or from the Path Stik

post-enrichment and presumptive false positive results were obtained from both methods due to *Proteus* growth. As seen with raw chicken and pigs' liver, it is possible that *Proteus* masked the presence of *Salmonella* on the plating media. The *Proteus* isolates from the minced pork selective enrichments and Path-Stik post-enrichments were tested with the Path Stik to investigate whether false positive reactions had occurred. The Path Stiks did not give a positive reaction and so it is possible that the ISO method had given false negative results.

Of the three raw pig kidney samples tested, *Salmonella* was not detected by either the Path Stik or ISO methods. In addition, the organism was not isolated from the Path Stik post-enrichment, confirming the result. Two presumptive false positive results occurred from two samples tested by the ISO method due to growth of *Proteus* and *Morganella* spp.

With the nine raw pig hearts tested, *Salmonella* was not detected by the Path Stik in seven of the samples tested. The organism was not isolated from either the Path Stik post-enrichment or by the ISO method, confirming the results. Two presumptive false positives occurred by the ISO method due to *Proteus* and *Citrobacter freundii* growth. In one pig heart sample *Salmonella* was detected by both methods. The organism was also isolated from the Path Stik post-enrichment, confirming the positive detection.

The remaining sample gave a positive result by the Path Stik; however, the reaction line was faint. *Salmonella* was not isolated from the Path Stik post-enrichment or when tested by the ISO method and thus a false positive occurred with the Path Stik.

Salmonella was not detected in the one raw lamb liver sample tested by the Path Stik and the ISO method. The organism was not found to be present in the Path Stik post-enrichment.

With the six raw minced beef samples tested, *Salmonella* was not detected by either the Path Stik or by the ISO method. *Morganella morganii* isolated after SC enrichment gave a presumptive false positives in one sample tested by the ISO method.

Salmonella was also found to be absent in the two raw sausage and twelve raw sausage meat samples tested using the Path Stik and ISO protocols.

In four of the cooked turkey samples, two of the samples were positive for *Salmonella* when tested with the Path Stik, although the reaction line was partial. *Salmonella* was confirmed from the BPW post-enrichment and when tested by the ISO method. The

remaining two cooked turkey samples tested with the Path Stik were negative and *Salmonella* was not found to be present in the post-enrichments used to inoculate the Path Stiks. When tested by the ISO method, however, a presumptive false positive was obtained from one sample; in the second turkey sample, *Salmonella* was isolated but from the 48h SC broth only. It is likely that the organism was either absent or at a very low cell concentration in the RV when subsequently transferred to the post-enrichment broth.

With the remaining cooked meats tested (smoked turkey and ham), *Salmonella* was not detected by either method, with the exception of the smoked turkey sample in which the organism was isolated from 24h SC enrichment by the ISO method only. The organism was not isolated from the RV broth or post-enrichment and consequently the Path Stik was negative. Again the failure of the Path Stik to detect *Salmonella* from the sample was due to a problem with the enrichment procedure.

Salmonella was also shown to be absent in the cottage cheese and beansprouts tested by both methods. *Salmonella* was also not detected from the five sesame seed samples tested by the Path Stik and by the ISO protocol. A presumptive false positive occurred in one sample tested by the ISO method due to *Escherichia hermanii.*

Of the three cereals tested, *Salmonella* was detected in one sample by the ISO method only. The organism was only isolated from the 24h SC enrichment broth and *Salmonella* was not present in the RV broth at a detectable level. Thus, the organism was not detected by the Path Stik when tested with the sample post-enrichment.

With the powder samples tested, *Salmonella* was not isolated by either method from the two milk powder samples or the egg powder. Two positive *Salmonella* samples occurred from the seven whey powders tested by the Path Stik. The organism was isolated from the Path Stik post-enrichment and when tested by the ISO method, confirming the result.

A further *Salmonella* positive sample was obtained from one of two buttermilk powder samples. The organism was detected by the Path Stik from the post-enrichment and by the ISO method. A presumptive false positive occurred in the second sample when tested by the ISO method due to growth of *Escherichia vulneris* from 24h SC enrichment.

With the six dried egg albumen samples tested two were positive for *Salmonella* when tested using the Path Stik procedure and the ISO method. An additional two *Salmonella* positive samples were identified by the ISO method but the organism was not detected in the samples by Path Stik. In one of the two samples, the organism was detected as being

present in the RV selective enrichment but it was not isolated from the Path Stik post enrichment. Consequently the Path Stik gave a negative result. In the second sample *Salmonella* was isolated from the SC broth at 24h but not from the RV broth by the ISO method. Despite not being at detectable levels after selective enrichment, *Salmonella* was identified as being present in the Path Stik post-enrichment after 6h incubation. When tested with the Path Stik, a negative result was obtained. It would appear that although *Salmonella* was present in the post-enrichment it did not grow to levels high enough to give a positive detection by the Path Stik when tested.

In summary, the Path Stik detected 27 *Salmonella* positive samples compared to 29 when tested using the ISO method. Eight of the Path Stik detections could not be confirmed either directly from the post-enrichment or indirectly by the ISO method. In the majority of these cases *Proteus* spp. were the only organism to be identified from the plating media and so the results remained unconfirmed.

In four cases, the ISO method isolated *Salmonella* from the 24h SC broth only and so the Path Stik gave a negative detection since the 24h RV broth was used in the Path Stik protocol. Similarly, in five samples tested by the ISO method, *Salmonella* could only be isolated from the 48h SC broth and not either of the 24h selective broths. Hence the Path Stik did not detect the organism in the sample when tested.

Thus, from the results of the potentially naturally contaminated foods it would appear that the majority of the discrepancies between the Path Stik and the ISO method could be attributed to either overgrowth by competitive microflora on the selective plates from the ISO method or Path Stik confirmations or a failure in the enrichment system from selective enrichment to post enrichment (either *Salmonella* growth only occurred in SC, or after 24h incubation).

Where *Proteus* is present in foods, particularly raw meats, the presence of *Salmonella* may not be detected due to overgrowth and swarming of the organism. To overcome this, a novel plating medium such as Rambach Agar (Merck) or SMID (bioMérieux) could be used to allow differentiation of members of the Enterobacteriaceae.

Generally RV enrichment broth has been found to be superior to most other media in recovering salmonellas from food products and water (Peterz *et al*, 1989). In this evaluation, however, *Salmonella* growth in RV selective enrichment was often found to be slow or absent, leading to a false negative detection by the Path Stik. In the majority of these cases *Salmonella* was isolated from the corresponding SC broth. Selenite cystine broth is known to be less selective than RV as the latter contains malachite green and a

high concentration of magnesium chloride, and is incubated at an elevated incubation temperature to reduce the growth of competitors. However, it has been found that certain *Salmonella* serotypes, especially *S.dublin*, are sometimes inhibited in media containing dyes. The possibility that malachite green in RV could inhibit this serotype was reported by Kalapothaki *et al* (1986). Peterz *et al* (1989) has suggested that certain *Salmonella* serotypes would not be found in food samples that are enriched in certain commercially available RV.

The suggestion that certain *Salmonella* may not be recovered by RV could potentially be resolved by inclusion of the SC selective enrichment in the Path Stik protocol, ie both the RV and SC selective enrichment broths from the ISO protocol going on to post-enrichment for subsequent testing with the Path Stik devices. The post-enrichments derived from the RV and SC selective broths are subsequently referred to as the RV and SC post-enrichments.

NATURALLY CONTAMINATED FOODS - PART 2

In total, 40 chicken samples were tested with the Path Stik protocol as shown in Figure 2 and by the ISO method (Table 4). This procedure was used to show the results using SC and RV media in Path Stik methods.

Eighteen of the chicken samples were negative for *Salmonella* when both RV and SC post-enrichments were tested by Path Stik (samples No.s 4, 6, 11, 14, 16, 18, 19, 20, 21, 22, 25, 28, 29, 31, 36, 37, 39, 40). *Salmonella* was not isolated from the post-enrichments after confirmation tests, confirming the negative Path Stik results. Similarly the organism was not isolated from the 20 chicken samples tested in parallel with the ISO method. In one of the samples tested, however, *Salmonella* was isolated from the 48h RV enrichment broth only. (The ISO 6579 method only states to incubate the RV for 24h and thus the detection was not recorded as a positive detection by the conventional method). Presumptive false positives occurred in 15 of the 18 negative samples tested using the ISO selective broths and in 16 of the 18 Path Stik sample post-enrichments due to *Proteus* growth.

Five of the chicken samples gave positive detections from both SC and RV post-enrichments when tested according to the Path Stik protocol and *Salmonella* was isolated using the ISO method (sample Nos. 1, 2, 5, 8, 32). In one of the five positive samples (No. 1), *Salmonella* was confirmed from both Path Stik post-enrichments used to inoculate the devices. In three samples (Nos. 2, 5, 8) *Salmonella* could only be confirmed from the Path Stik RV post-enrichment and *Proteus* spp. were originally isolated from

the SC post-enrichments. To check to see if false positives had occurred with the enrichments, a sweep of the *Proteus* spp isolated from the three SC post-enrichments were sub-cultured in BPW, incubated at 37°C for 24h and retested using Path Stiks. Two of the Path Stiks again gave positive detections (Nos. 2, 5). When plated, *Salmonella* was confirmed as being present in the two positive sub-cultured post-enrichments. Thus, it is likely that *Proteus* originally masked the presence of *Salmonella* from the SC post-enrichments. The third Path Stik positive detection from the SC post-enrichment remained unconfirmed as *Salmonella* was not isolated (No. 8). When compared to the ISO method in this sample, *Salmonella* was only isolated from the RV selective enrichment after testing. The SC gave a presumptive false positive detection and it is likely that the presence of *Salmonella* in the enrichment was masked by the high level background microflora. In the fifth sample (No. 32) *Salmonella* was only isolated from the SC post-enrichment after it was streaked directly onto Rambach Agar (RA). The organism was not detected from the XLD and BGA plates. The organism could be not isolated from the RV post enrichment.

In three of the remaining 17 chicken samples tested, the Path Stik detected *Salmonella* in both the sample post-enrichments but the organism could not be isolated by the ISO method when tested in parallel (sample Nos. 7, 9, 17). *Proteus* was the only organism to be identified from the ISO selective enrichments. When a sweep of the XLD and BGA plates from sample No. 17 was taken and subcultured on RA, *Salmonella* was isolated after incubation of the RA at 37°C for 24h. This demonstrated that although the organism was present in the sample, the ISO method had failed to detect it. *Salmonella* was isolated from all three of the RV Path Stik post-enrichments confirming the presence of *Salmonella* in the samples. The positive detections from the SC post-enrichment using the Path Stik remained unconfirmed. *Proteus* spp. were the only organism to be isolated due to the swarming nature of the organism.

In two chicken samples (sample Nos. 23, 34) *Salmonella* was not detected in either the SC or RV post-enrichments tested using the Path Stik protocol. The organism was not isolated from the Path Stik post-enrichments, confirming the absence of *Salmonella* in the broths. *Proteus* spp. gave presumptive false positives from the post-enrichments. When the two samples were tested in parallel using the ISO method, *Salmonella* was detected in the 24h selective broths showing that the samples were naturally contaminated. Thus, despite *Salmonella* being isolated from the 24h RV broth it was not found in the RV Path Stik post-enrichment indicating that *Salmonella* was either absent in the post-enrichment or not present at a detectable level. The non-detection of the organism in the two samples using the Path Stik protocol appears to have been due to a failure in the enrichment system.

TABLE 4

The Detection of *Salmonella* from Potentially Naturally Contaminated Chicken

Sample		Path Stik		Path Stik Confirmation		ISO
		SC	RV	SC	RV	
Chicken	1	+	+	+	+	+
Chicken	2	+	+	-[a]	+	+
Chicken	3	+	-	-[a]	FP	FP
Chicken	4	-	-	-[a]	-	-
Chicken	5	+(w)	+	-	+	+
Chicken	6	-	-	-	-	-
Chicken	7	+	+	FP	+	FP
Chicken	8	+	+	FP	+	+
Chicken	9	+	+	FP	+	FP
Chicken	10	+	-	+	FP	+
Chicken	11	-	-	-	FP	FP
Chicken	12	+	-	-	FP	+
Chicken	13	+ (w)	-	+	FP	FP
Chicken	14	-	-	-	FP	FP
Chicken	15	+	-	+	+	+
Chicken	16	-	-	FP	FP	FP
Chicken	17	+(w)	+	FP	+	-[b]
Chicken	18	-	-	FP	FP	FP
Chicken	19	-	-	FP	FP	FP
Chicken	20	-	-	FP	FP	FP
Chicken	21	-	-	-	FP	-[c]
Chicken	22	-	-	FP	FP	FP
Chicken	23	-	-	FP	FP	+
Chicken	24	-	+ (w)	+	+	+
Chicken	25	-	-	FP	FP	FP
Chicken	26	-	+	-	-	+
Chicken	27	-	+ (w)	FP	FP	FP
Chicken	28	-	-	FP	FP	FP
Chicken	29	-	-	FP	FP	FP
Chicken	30	-	+ (w)	FP	FP	FP
Chicken	31	-	-	FP	FP	FP
Chicken	32	+(w)	+	-[d]	FP[b]	+
Chicken	33	+	-	+	-	+
Chicken	34	-	-	FP	FP	+
Chicken	35	+	-	-[d]	FP	+
Chicken	36	-	-	FP	FP	FP
Chicken	37	-	-	FP	FP	FP
Chicken	38	+(w)	-	-[d]	FP	FP
Chicken	39	-	-	FP	FP	FP
Chicken	40	-	-	FP	-	FP
Total		16	12	6	9	14

(w) = weak detection
a = Originally only *Proteus* spp. isolated. *Salmonella* confirmed after sweep of a plate subcultured in BPW and retested.
b = Originally only *Proteus* spp. isolated. *Salmonella* confirmed after sweep of plate onto Rambach Agar.
c = Positive from 48h RV only.
d = *Salmonella* isolated when post-enrichment streaked on Rambach Agar.
FP = Presumptive false positive.

In the remaining twelve samples, the testing of the RV and SC post-enrichments with the Path Stik following enrichment did not produce the same results.

In five samples the Path Stik detected *Salmonella* present in the SC post-enrichment, but not in the RV post-enrichment (sample Nos. 10, 12, 15, 33, 35). The organism was confirmed as being present in all the samples when tested in parallel with the ISO method. When the Path Stik SC post-enrichments were plated for confirmation, *Salmonella* was isolated from three of the five broths (Nos. 10, 15, 33). The organism was not isolated from the SC post enrichment from sample No. 12 and so the detection by the Path Stik remained unconfirmed. In the SC post-enrichment from sample No. 35, the organism was only isolated when streaked on RA. *Proteus* was isolated on XLD and BGA. Thus, the Path Stik confirmation protocol failed to detect the presence of *Salmonella* even though it was present. When the RV post-enrichments were plated for confirmation of the Path Stik results *Salmonella* was isolated from sample No. 15. In sample No. 35 *Proteus* was originally isolated from the post-enrichment. The organism was isolated after a sweep of the plates was taken and plated onto RA. Despite *Salmonella* being confirmed from the RV post-enrichment, therefore, the Path Stik had failed to detect it. In the other three RV post-enrichments *Salmonella* was not isolated, confirming the negative results by Path Stik. *Proteus* spp. gave a presumptive false positive from three of the RV broths. *Salmonella* was not isolated from the RV selective enrichment broths from the samples tested with the ISO method and so the medium must have been unfavourable for *Salmonella* growth.

In two of the remaining seven chicken samples, the opposite scenario occurred. From the RV post-enrichments tested with Path Stik, *Salmonella* was detected in both of the samples and using the ISO method (sample Nos. 24, 26). However the organism was not detected as being present in the SC post-enrichments tested. *Salmonella* was confirmed from the RV post-enrichment from sample No. 24, whilst *Proteus* was the only organism to be isolated from the sample No. 26 RV post-enrichment. When the SC post-enrichments were plated *Salmonella* was isolated from sample No. 24. Despite the organism being confirmed as being present in the post-enrichment, the Path Stik did not detect it.

From the remaining five samples, Path Stik detected *Salmonella* in three of the chicken samples tested with the SC post-enrichment (sample Nos. 3, 13, 38). The organism was not detected by the Path Stik in the corresponding RV post-enrichments or when tested with the conventional ISO method only. *Proteus* spp were isolated from these samples when taken on for confirmation. When the SC post-enrichments were plated, *Salmonella* was

isolated from one of samples (No. 13), confirming the Path Stik detection. *Salmonella* was only isolated from the post-enrichments from sample No. 38 after it was streaked directly onto RA. *Proteus* was isolated on XLD and BGA. The organism was isolated from sample No. 3 SC post-enrichment after a sweep of the plates were taken and plated onto RA. Thus, although all three of the Path Stik positive SC post enrichments were confirmed, two of the confirmations were not detected using the original Path Stik confirmation procedure. The ISO method failed to detect the presence of the organism in all three samples presumably due to the growth of *Proteus* which gave presumptive false positive results.

In the two remaining chicken samples, *Salmonella* was detected by the Path Stik in the RV post-enrichment only (sample Nos. 27, 30). The Path Stik gave negative results when tested with the SC post-enrichment and using the ISO method. *Proteus* was the only organism to be isolated, giving presumptive false positive results. When the RV post-enrichments were plated for confirmation, only *Proteus* spp were isolated from both the positive samples and thus the detections could not be confirmed.

In summary, from the results of the 40 chicken samples tested, it can be seen that *Proteus* spp were present in the majority of samples which resulted in a high number of presumptive false positive results. The presence of *Proteus* also made recovery and confirmation of *Salmonella* difficult. The use of Rambach Agar identified five positive samples that were originally negative using traditional plating media due to the ability of the agar to differentiate between members of the Enterobacteriaceae.

The advantage of testing the two post-enrichments with Path Stik was also demonstrated. Of the 20 positive raw chicken samples detected by Path Stik, eight were positive when both the RC and SC post-enrichments were tested. Five of the detections from the SC post-enrichments, however, remained unconfirmed due to growth of *Proteus.* In four of the 20 samples, the Path Stik gave a positive detection from the RV post-enrichment only. Two of the sample post-enrichments were confirmed as *Salmonella* positive whilst in the remaining two only *Proteus* could be isolated. In eight of the samples, the Path Stik only gave a positive detection when tested with the SC sample post-enrichments. Seven of these detections were confirmed.

Thus, by incorporating the SC post-enrichments into the Path Stik protocol, a further eight samples were identified as being positive for *Salmonella.* These would have been missed if the recommended Path Stik enrichment procedure, using one post-enrichment, had been used. In the majority of cases, where one of the sample post-enrichments gave a negative result and the other positive when tested with the Path Stik, *Salmonella* could

not be isolated from the negative broth. Thus, it was not a failure of the Path Stik to detect *Salmonella* but a failure in the enrichment system.

In total, the Path Stik yielded eight more positive detections that the conventional ISO cultural procedure, although two of the detections could not be confirmed. The ISO method, however, isolated *Salmonella* from two samples in which the Path Stik did not detect the organism.

SUMMARY OF RESULTS

Sensitivity

The sensitivity of the Path Stik appeared to be dependent upon the serotype used. Minimum detection levels ranged from 3.0×10^5 cfu/ml to 2.0×10^7 cfu/ml in the 10 *Salmonella* serotypes tested.

Inoculated Foods

A total of 77 samples were tested, of which 47 were inoculated (2 were found to be naturally contaminated). Of the 45 inoculated samples, 38 (84%) were positive by Path Stik and 44 (98%) were confirmed positive by the conventional ISO method. In two (4%) samples which were negative by Path Stik, *Salmonella* could be isolated from the post-enrichment broth.

Naturally Contaminated Foods - Part 1

A total of 166 food samples thought to be naturally contaminated were tested using the Path Stik and ISO methods. Of these samples, 27 (16%) were positive by Path Stik and 29 samples (17%) were positive using the ISO method. The positive samples from both methods are not directly comparable since only 19 samples (11%) were confirmed as being *Salmonella* positive by both methods. In eight (5%) of the 27 positive samples tested using Path Stik, the results could not be confirmed and so false positives occurred. In one sample (0.6%) which was negative when tested with the Path Stik *Salmonella* was isolated from the Path Stik post-enrichment and so a false negative result was obtained. In ten samples (6%), *Salmonella* was isolated by the ISO method only.

Naturally Contaminated Foods - Part 2

A total of 40 chicken samples thought to be naturally contaminated were tested using

Path Stik (with RV & SC post-enrichments) and ISO methods. Using the confirmation procedures stated in the methods section, 20 samples (50%) were positive using Path Stik and 14 samples (35%) were positive by the ISO method. The results are not directly comparable since only eight samples (20%) were confirmed as being *Salmonella* positive by both methods. From the 20 positive Path Stik detections, eight (20%) of the samples could not be confirmed and thus false positive results were obtained. Path Stik gave four confirmed (10%) and four unconfirmed (10%) positive samples which were negative when tested using the ISO method. Path Stik, however, failed to detect *Salmonella* from two chicken samples (5%) which were positive using the ISO method.

When additional confirmation procedures, such as the use of Rambach Agar or results from 48h RV selective enrichments, were used, *Salmonella* was confirmed from more samples tested by both methods.

The number of confirmed positive samples increased from 14 to 16 (40%) using the ISO based method compared to the 20 positive samples (50%) tested using the Path Stik. *Salmonella* was confirmed as being present in 12 of the samples (30%) by both methods. The Path Stik results from four positive detections (10%) could not be confirmed and in one sample (3%) *Salmonella* was isolated from the post-enrichment despite a negative result when tested with the Path Stik. Path Stik detected *Salmonella* from five confirmed (13%) and two unconfirmed (5%) positive samples which were negative when tested using the ISO method. *Salmonella* was isolated, however, from three samples (8%) using the ISO method which were negative using the Path Stik protocol.

OVERALL CONCLUSIONS

1. The use of Path Stik with both RV and SC enrichment increases the number of true positives detected. The use of RV alone will result in some positive samples being missed. The use of two enrichment broths is recommended for use with the Path Stik.

2. In some inoculated and naturally contaminated samples, detection by the conventional method occurred only after 48h incubation of SC selective broths. Such samples would not be detected with the Path Stik.

3. In both the ISO method and the Path Stik confirmations, problems occurred with overgrowth of *Salmonella* by *Proteus* spp. This could result in "apparent" false positive detections by Path Stik (ie true false negatives with the ISO method). The problem could be partially overcome by the use of different selective agars e.g. Rambach agar.

4. The standard Path Stik method (i.e. using RV) gave results that were almost comparable to ISO methods - a few samples were missed due to the factors mentioned above.

5. The modified Path Stik method (using RV and SC and Rambach confirmation) was able to detect more contaminated samples than the ISO method.

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