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Detection of *Salmonella* Contamination in Food Samples by Dot-ELISA, DNA Amplification and Bacterial Culture

P. Tapchaisri[1], P. Wangroongsarb[1], W. Panbangred[2], T. Kalambaheti[1], M. Chongsa-nguan[1], P. Srimanote[1], H. Kurazono[3], H. Hayashi[3] and W. Chaicumpa[1]

SUMMARY **A dot-blot enzyme-linked immunosorbent assay (dot-ELISA) employing a genus *Salmonella* specific monoclonal antibody (MAb) was used for detection of the bacteria in food samples in comparison with the conventional culture method and the DNA amplification. Among the 200 chicken and pork samples (100 each) tested, 9% and 33%, 7% and 20% and 7 and 23% were positive for salmonellae by the dot-ELISA, the culture method and the DNA amplification, respectively. Statistical analyses revealed that the sensitivity, specificity, efficacy, and positive and negative predictive values of the detection of *Salmonella* in the food samples by dot-ELISA compared with the culture method were 93.33%, 91.76%, 92%, 66.66% and 98.73%, respectively. Comparison of the DNA amplification and the culture method revealed the sensitivity, specificity, efficacy, and positive and negative predictive values of 100%, 91.58%, 92%, 65.21% and 100%, respectively, The dot-ELISA and the DNA amplification results were in a better agreement when the two assays were compared. The sensitivity, specificity, efficacy, positive and negative predictive values of the dot-ELISA compared to the DNA amplification were 91.3%, 100%, 98%, 100% and 97.5%, respectively. From this study, the dot-ELISA is rapid, simple, sensitive, specific at low cost with limited amount of infectious waste to be disposed and offers another advantage in that it detects only the smooth LPS of *Salmonella* which implies the possible presence of the virulent organisms.**

Conventional culture has been used routinely for detecting *Salmonella* contamination of foods. The process starts with pre-enrichment of the food sample in a nutritious, non-selective medium which would resuscitate the injured or stressed bacteria. The pre-enriched sample is then transferred to a selective enrichment medium which allows the *Salmonella* and biochemically related bacteria to grow while others, *e.g.* normal flora, are suppressed. Culture from this selective enrichment is usually plated onto selective, differential agar where different bacteria are recognized on the basis of different colony appearances. The colonies with characteristics of *Salmonella* are subsequently analyzed using a battery of biochemical tests. Serological grouping and typing are then performed accordingly. This labor-intensive and time-consuming procedure (4 to 5 days) is inadequate for making timely assessments on the microbiological safety of foods.[1] Several commercially available media, devices, methods and test kits for rapid detection and identification of *Salmonella* in foods have been developed. These include simple miniaturized biochemical assays, physicochemical tests that measure bacterial metabolites, automated instrument diagnostic systems, nucleic acid-based and antibody-based methods.[2-6] While the miniaturized system has greatly reduced the labor, media and time

From the [1]Department of Microbiology and Immunology, Faculty of Tropical Medicine, [2]Department of Biotechnology, Faculty of Science, Mahidol University, Thailand, and [3]Department of Microbiology, the University of Tsukuba, Japan.
Correpondence: P. Tapchaisri

used formerly for the classic biochemical testing of the bacteria, the process requires pure culture isolate and costly disposable biochemical kits. Most miniaturized tests show incomplete (90 to 99%) accuracy when compared with the standard culture methods.[7] Although the automated systems for specific detection of *Salmonella* growth and metabolism in foods are as efficient as the conventional culture methods, they still require about 24 hour-pre-enrichment prior to transferation of the culture aliquots into expensive, disposable electrode cells in an expensive machine that contain a specially formulated medium.[6,8] Various nucleic acid-based tests have been developed for *Salmonella* detection including the DNA probes and DNA amplification[9-13] Although the DNA hybridization assays may have several advantages, their specificity depends much on that of the probe. The techniques are laborious and thus require skilled personnel to perform the tests. Most of all, it may pose some health hazard and disposal problems when radioisotopes are used for labeling the probes. The detection threshold of DNA hybridization is estimated at about 10^4 to 10^5 bacteria (about 10^7 cells/ml);[9] thus pre-enrichment of the food sample for about 18 to 48 hours is always required before the DNA-based procedure can be applied.[1] The classic versions of DNA hybridization, namely, the dot-blot and colony hybridization themselves may take several days for completion. Both versions involve immobilization of the target DNA (crude, purified DNA or lysed bacteria) on the nitrocellulose membrane, denatured by alkaline treatment and immobilized the single-stranded DNA by baking or microwave treatment. The DNA on the membrane is then probed. For the radioisotope labeled probe, exposure of the membrane to an X-ray film or autoradiography for many days is usually necessary before the result can be visualized. Several attempts have been made to fasten up this hybridization step. One approach is the use of chemiluminescent-solution hybridization of which the entire procedure could be completed in about 30 minutes after picking the bacterial colony from the agar plate. However, expensive luminometer is needed for quantitation of the chemiluminescence.[14,15] DNA amplification or the polymerase chain reaction (PCR) has been carried out for the detection of several food-borne pathogens including *Salmonella.*[13,16-19] Foods, however, not only containing too few target bacteria but often contain also inhibitor(s) of the PCR; thus the PCR procedure is usually started from pre-enrichment and selective enrichment of the *Salmonella* in the food sample. This process increases the number of the target bacteria and also dilutes away the PCR inhibitor(s). The enriched culture is subjected to the repeated cycles of the PCR amplification steps, and electrophoresis and staining of the amplicon. The DNA amplification is very sensitive and can detect picograms of the target DNA. The procedure, however, requires high skill for primer design and expensive reagents and equipment. The PCR is not suitable for routine work but rather used as a standard test with which the other developing tests like the antibody-based tests should be compared for specificity and sensitivity.

Several antibody-based tests have been developed for the detection of *Salmonella* in foods, *e.g.* the immunofluorescence assay,[20-22] the latex agglutination,[23-25] and the enzyme immunoassay.[26-28] Among them the most favorable method is the enzyme immunoassay. This is because of the rapidity, sensitivity, relative stability of the reagents and a minimum, if at all, equipment requirement.[29-31] However, the assay gives a high percentage of false positive reactions due to the non-specific (cross-reactive) nature of the polyclonal antibodies used.[32] Monoclonal antibody (MAb) specific to the *Salmonella* is likely to solve this problem.

In 1988, we produced a hybridoma (clone 102B2) secreting specific monoclonal antibodies (MAb) which gave pan-reactions to the whole cell lysates of all salmonellae tested and not to any other bacteria.[33] This MAb was used in a dot-ELISA for detection of *Salmonella* in various kinds foods, *i.e.* pork, chicken, beef, eggs, etc. in comparison with the conventional bacterial culture method. The food samples were pre-enriched and selectively enriched before aliquots were subjected to ELISA and the remaining portions were used to the completion of bacterial isolation. It was concluded that the method was simple, rapid, inexpensive and highly sensitive in comparison with the standard bacterial culture method.[34] However, there was a discrepancy of the results obtained from the dot-ELISA and the culture method, *i.e.* a higher number of samples tested positive by the former than the latter. It was speculated that this difference might be due to the fact that the culture method could detect only living organisms readily resuscitated by pre-enrichment and successfully grown

in the selective medium and on the differential media, while the dot-ELISA could detect any form of the organism, *i.e.* living, injured/stressed cells (which may regain full virulence if consumed by the host), dead cells, or even released antigen. In order to ratify this notion, the experiments were carried out to compare *Salmonella* detection in food samples using three methods, namely the monoclonal antibody-based dot-ELISA, the culture method and DNA amplification.

MATERIALS AND METHODS

Bacterial strains and antigen preparations

Salmonellae and other bacteria used in the study are listed in Table 1. They were from the stock cultures maintained by the Department of Microbiology and Immunology, Faculty of Tropical Medicine, Mahidol University, Bangkok and The WHO National Salmonella and Shigella Center, Ministry of Public Health, Nonthaburi province, Thailand.

Whole cell lysates (Ly) were prepared from each strain of bacteria by suspending the cells of the log-phase broth culture in distilled water to an optical density (OD) of 2.0 at 540 nm and subjecting the preparation twice to the ultrasonicator at 20 kHz for 5 minutes each. Dry weight of each preparation was then determine. The lysates were used for checking cross-reactivity of the monoclonal antibody secreted by the hybridoma clone 102B2.[33,34]

Lipopolysaccharide (LPS) was extracted from *Salmonella typhi* strain O901 by the hot phenol-water method of Westphal and Jann.[35]

Monoclonal antibody

A monoclonal antibody (MAb) specific for core polysaccharides in the LPS molecules of the genus *Salmonella* was obtained from an *in vitro* culture of the hybridoma clone 102B2.[33,34] This hybridoma was derived from cell fusion between the spleen cells of a mouse immunized with *Salmonella typhi* strain O901 Barber antigen and Sp2/0 myeloma cells. The MAb secreted by this clone reacted with the fast-moving, non-protein component in the Barber antigen which is located at the position of the core polysaccharides.[33,34,36] The MAb reacted with the Ly antigen prepared from all salmonellae tested but did not cross-react with the antigens of other organisms. The indirect ELISA titer of the monoclonal antibody was determined as previously described.[33,34] One ELISA unit was the smallest amount of the MAb which gave positive indirect ELISA reaction. Antigenic specificity of the MAb was also re-determined using the indirect ELISA against the Ly of the homologous bacteria as well as a panel of the heterologous Ly prepared from the bacteria listed in the Table 1.

Food samples

One hundred pork and 100

Table 1 The bacterial strains used for preparing homologous and heterologous lysates

Salmonellae		
Salmonella aberdeen	*S. enteritidis*	*S. paratyphi* A
S. agona	*S. emek*	*S. paratyphi* B
S. amsterdam	*Salmonella* group E4	*S. paratyphi* C
S. anatum	*S. hadar*	*S. paratyphi* B biovar java
S. bangkok	*S. kentucky*	*S. poona*
S. blockley	*S. lexington*	*S. senftenberg*
S. brunei	*S. montevideo*	*S. typhi* stain O901
S. chicago	*S. orion*	*S. typhimurium*
S. choleraesuis	*S. oslo*	*S. virchow*
S. dublin	*S. panama*	*S. weltevreden*
Other Enterobacteriaceae		
Citrobacter diversus	*Klebsiella pneumoniae*	*Serratia marcescens*
C. freundii	*Morganella morganii*	*Shigella dysenteriae*
Enterobacter aerogenes	*Proteus mirabilis*	*S. boydii*
E. cloacae	*P. rettgeri*	*S. flexneri*
Escherichia coli	*P. stuartii*	*S. sonnei*
Edwardsiella tarda	*P. vulgaris*	*Yersinia enterocolitica*
Hafnia alvei		
Pseudomonadaceae		
Aermonas hydrophila	*Plesiomonas* spp.	*Pseudomonas aeruginosa*
Vibrionaceae		
Vibrio alginolyticus	*V. cholerae* El Tor Inaba	*V. vumissii*
V. anguillarum	*V. cholerae* El Tor Ogawa	*V. parahaemolyticus*
V. cholerae classical Inaba	*V. fluvialis*	

chicken samples were bought in 100 gram portions from local venders of various fresh markets in Bangkok. Food samples were collected individually in clean plastic bags and transported in an ice-bath to the laboratory within 1-2 hours in order to avoid spoilage or excessive proliferation of other competitive bacteria.

Bacterial culture method

The conventional procedure described by Andrews *et al.*[37] was used for *Salmonella* culture of the foods. Ten grams of the 100 gram sample was pre-enriched in 90 ml of trypticase soy broth containing 1% yeast extract (TSBY) in a 37°C shaking incubator for 18 hours (the 90 gram portions were kept frozen at -20°C). One milliliter of each culture was inoculated into 9 ml of tetrathionate broth and/or selenite cystine broth and reincubated. The culture was then plated onto the selective differential media, namely the MacConkey, brilliant green sulfadiazine and bismuth sulfite agar plates. *Salmonella*-like colonies were picked and transferred to tubes of lysine iron agar (LIA) and triple sugar iron (TSI) agar. The bacteria with *Salmonella* characteristics were then subjected to other biochemical assays which included ornithine decarboxylase, motility, indole, urease and citrate tests. The *Salmonella* isolates were also confirmed by slide agglutination with polyvalent O antiserum. All *Salmonella* isolates were further sent to the WHO National Salmonella and Shigella Center for serotyping.

Monoclonal antibody-based dot-ELISA

A one ml aliquot of each culture in selenite cystine broth was boiled for 20 minutes in order to eliminate the background non-specific reaction and disinfection and this was used in the dot-ELISA. Each sample was duplicately applied onto two different nitrocellulose strips cut from a nitrocellulose (NC) membrane (0.45 μm pore size; Schleicher and Schuell, Germany). Positive and negative controls, namely, the LPS of *S. typhi* and fresh selenite cystine broth, respectively, were included on each NC strip. The strips were air-dried for about 5 minutes and the unoccupied sites of the membranes were blocked by incubating the strips with a blocking solution containing 1% BSA and 3% skim milk in Tris buffered saline, pH 7.5 (TBS) at 26°C for 20 min on a rocking platform. The NC strips were washed three times, three minutes each, with TBS containing 0.05% Tween-20 (TBST). One NC strip, *i.e.* the test strip (TS) was submerged into a MAb 102B2 solution (640 indirect ELISA units/ml) while the second strip was put in fresh RPMI1640 medium and this served as a control strip (CS) for 20 minutes. Both strips were washed with the TBST as above and placed in a solution of rabbit anti-mouse immunoglobulin-alkaline phosphatase conjugate (Dakopatt, Denmark) diluted 1:1,000 in TBST for 20 minutes. The strips were washed three times with the TBST and finally washed with 0.15 M Tris buffer, pH 9.6 before they were put in a substrate solution (5-bromo-4-chloro-3-indolyl phosphate [BCIP] and nitroblue tetrasolium [NBT]). The enzyme-substrate reaction was allowed to take place for 20 minutes at 26°C in the dark. The reaction was stopped by rinsing the strips with distilled water, then they were air-dried. Positive results were interpreted by observing the tested specimens on the TS in comparison with the results on the CS. The positive spots showed purplish-blue color and could be clearly distinguished from the negative counterparts on the CS which might showed other non-specific color, *e.g.* red or brown or may be clear area. The positive (LPS) and negative (selenite cystine broth) controls must showed acceptable appearances in each test.

PCR primers

The DNA Primers (PDU1 and PDU3) used in this study were highly specific for all isolates of *Salmonella* tested and the amplicon size was 833 base pairs (Fig. 1). The nucleotide sequences of the primers will be published elsewhere. The final concentration of the primers in each polymerase chain reaction (PCR) was 1.0 μM.

Preparations of crude and purified bacterial DNA

Purified *Salmonella* DNA and DNA of other gram-negative bacteria were prepared and used as positive and negative controls, respectively, in the DNA amplification experiments. These DNA were prepared by the method of Silhavy *et al.*[38] Five to ten colonies of the bacteria were inoculated into a brain heart infusion broth and incubated in a 37°C shaking incubator for 12 to 16 hours. The bacterial cells were harvested by centrifugation the culture at 12,000 x *g* for 10 minutes. The pellet was resuspended in 250 μl of triple-distilled water (TDW), and heated at 100°C for 30 minutes. The proteins were removed by extraction with an equal volume of phenol-chloroform (1:1 v/v) three

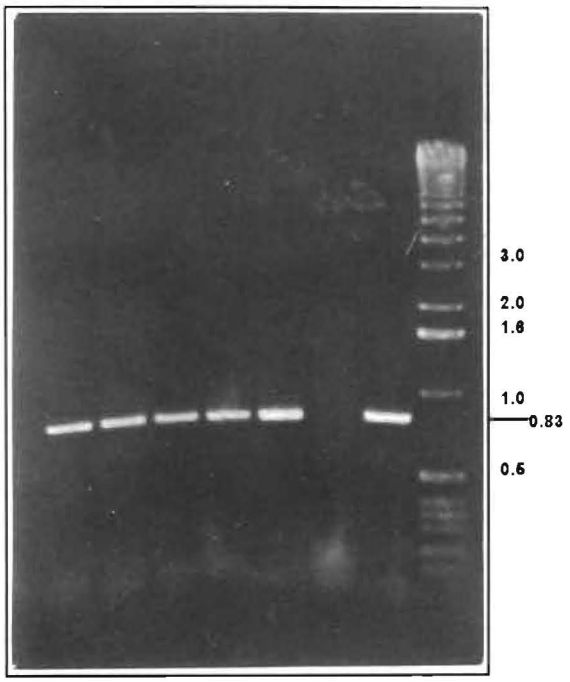

Fig. 1 Specificity of DNA amplification reaction for detection of *Salmonella*. Lanes 1, 2, 3, 4 and 5 are DNA of *S. choleraesuis, S. enteritidis, S. panama, S. paratyphi* A, and *S. typhimurium*, respectively. - = negative control and + = positive control (DNA of *S. typhi* strain O901). Lane M = DNA molecular size standards. Numbers at the right indicate molecular sizes in kilobases.

times and equal volume phenol-chloroform-3% isoamyl alcohol (25:25:1) twice. The aqueous phase was transferred to a new tube and 3 M sodium acetate, pH 5.2 and cold absolute ethanol were added in 1/10 volume and 2 volumes, respectively. The preparation was kept at -20°C for 1 hour before centrifugation at 12,000 x *g* for 20 minutes at 4°C. The DNA pellet was washed with 70% ethanol before dissolving in 50 µl of Tris-EDTA buffer, pH 8.0 and kept frozen at -20°C until use. The amount of the extracted DNA was estimated by measuring its optical density (OD) at 260 nm using a UV spectrophotometer (UV-160). The DNA concentration was calculated by assuming that the OD at 260 nm of 1.0 was equivalent to 50 µg/ml.[39] The purified *Salmonella* DNA was also used to determine the sensitivity of the DNA amplification for the detection of the bacteria.

Crude DNA samples were also prepared from *Salmonella* and other gram-negative bacteria. One milliliter of log phase culture of bacteria in brain heart infusion broth was centrifuged at 12,000 x *g* for 5 minutes. The pellet was washed with PBS pH 7.4 before 1 ml of TDW was added. The preparation was boiled for 30 minutes and centrifuged for 1 minute to pellet the cell debris. The supernatant was collected and kept frozen at -20°C. The crude DNA were used to determine specificity of the DNA amplification.

Preparation of food samples for PCR

One milliliter of the tetrathionate or selenite cystine selectively enriched culture of each food sample was used to prepare crude DNA as described above. Five microliters of each sample was used in the PCR. Purified DNA was also prepared from 7.5 ml of each selectively enriched sample of food. The concentration of the extracted DNA was adjusted to 125 ng/µl for PCR.

DNA amplification procedure

The DNA amplification was performed on 50 µl of the reaction mixture containing 200 µM each of dATP, dCTP, dGTP, dTTP, 2.5 units of *Taq* DNA polymerase (PE Applied Biosystem, U.S.A.), 1 µM of each primer, 2 mM $MgCl_2$ and 1 µl of purified bacterial DNA or 5 µl of crude bacterial DNA in PCR buffer (10 mM Tris-HCl, pH 7.3, 50 mM KCl and 0.01% [w/v] gelatin [PE Applied Biosystem]). This reaction mixture was overlaid with 100 µl of mineral oil (Sigma Chemical Company, U.S.A.) and pre-heated at 94°C for 3 minutes before starting the amplification reaction. The amplification reaction was carried out for 30 cycles, each cycle consisted of denaturation at 94°C for 1 minute, annealing at 55°C for 1 minute and extension at 72°C for 1 minute in a thermocycler (PE Applied Biosystem).

Agarose gel electrophoresis and ethidium bromide staining

The PCR amplicon was subjected to 2% agarose gel electrophoresis using the method of Sambrook *et al.*[39] Briefly, weighed agarose powder (Bio-Rad, U.S.A.) was heated to dissolve in a Tris-acetate-EDTA (TAE) buffer and cooled to 50°C before pouring into a tape-sealed gel tray. Appropriate gel comb was inserted into the gel and the preparation was allowed to set at 26°C for 20-30 minute. The amplicon (12 to 20 µl) mixed with one-fifth volume of a loading buffer (0.25% bromphenol blue, 0.25% xylene cyanol FF and 30% glycerol in distilled water) was loaded into a well in the gel. Electrophoresis was carried out at 7 volts/cm in the TAE buffer for 1 to 2 hours or until the dye front reached 75% across the gel. The gel was then removed and stained with 0.5 µg/ml ethidium bromide for 20 to 30 minutes. The DNA band was visualized at 302 nm by a UV transluminator (Fotodyn Incorporated, U.S.A.).

Statistical analyses

The method of Galen[40] was used for calculating the diagnostic specificity, diagnostic sensitivity, efficacy and predictive values of the tests. Degree of agreement among the tests were determined by calculating the Kappa coefficient (K) values[41] while the Kappa probability (Z)[42] was used to indicate reliability of the results of the *Salmonella* detections by dot-ELISA, PCR and culture method.

RESULTS

Specificity of the MAb 102B2 was assessed by dot-ELISA against the Ly of salmonellae and other bacteria listed in Table 1. It was found that the MAb reacted only to the Ly of *S. typhi* 0901 and 29 other *Salmonella* strains but did not react to the Ly of bacteria of the other genera. The smallest amount of the *S. typhi* LPS which could be detected by the dot-ELISA was 1 ng while the smallest number of the bacteria reacted positively in the test was 100 cells. It was also found that the dot-ELISA was still positive for a 3 µl dot of a mixture of 100 *Salmonella* cells and 10,000 *E. coli* cells.

The specificity of the DNA primers in *Salmonella* detection was evaluated using 1 µl of crude DNA of 29 *Salmonella* and 30 other bacteria as the templates in the PCR. It was found that all salmonellae DNA yielded the expected amplicon whereas the other bacteria were negative. Fig. 1 illustrates the representative results of the PCR performed on some *Salmonella* crude DNA preparations. When the varying amounts of the *Salmonella* whole cells and purified DNA were used as the templates for the PCR, the smallest number of the whole cells and the smallest amount of the pure DNA which could be detected were 5,000 cells and 30 pg, respectively.

When the crude bacterial DNA prepared from the enrichment broth cultures of the food samples tested positive for *Salmonella* by either dot-ELISA or culture or both were used as the templates for PCR it was found that only 7% and 2% of the samples from tetrathionate and selenite cystine broth, respectively, were positive for the *Salmonella* DNA. It was concluded that the crude preparations were not suitable for the DNA amplification and, thus, the pure DNA extracts prepared from the selective enrich-

Table 2 The results of *Salmonella* detection in the 200 chicken and pork samples by culture method and dot-ELISA

Dot-ELISA	Culture method		Total
	Positive	Negative	
Positive	28	14	42
Negative	2	156	158
Total	30	170	200

Diagnostic specificity	= 91.76%
Diagnostic sensitivity	= 93.33%
Efficacy	= 92%
Positive predictive value	= 66.66%
Negative predictive value	= 98.73%
Kappa coefficient calculation	
Observed agreement	= 0.9200
Chance expected agreement	= 0.7030
Kappa coefficient (K)	= 0.7306
Standard error of Kappa	= 0.0692
Kappa probability (Z)	= 10.55
p-value	= < 0.0001

ment broths of the food samples were used as the DNA templates in the subsequent experiments.

The monoclonal antibody-based dot-ELISA was positive for 9/100 (9%) of chicken and 33/100 (33%) of pork samples, while *Salmonella* could be isolated from only 7% and 20% of the samples, respectively. Among these 200 samples, 25 of them were positive by both dot-ELISA and bacterial culture, 17 samples were positive only by the former, 2 samples were positive only by the latter and 156 were negative by both methods. Another 10 gram aliquots of the 17 samples which were dot-ELISA positive, culture negative were retested by both methods and it was found that all of them were still dot-ELISA positive but 3 samples became positive by the culture while the remaining 14 samples were culture negative. The 2 samples which were culture positive, dot-ELISA negative yielded the same results upon retesting. The overall results of the food analysis by the dot-ELISA and the culture method are shown in comparison in Table 2. The prevalence of the *Salmonella* serotypes among the isolates from the food samples are given in Table 3. There was 1 chicken sample that had mixed contamination of *S. hadar* and *S. senftenberg*. *S. derby* and *S. anatum* predominated among the pork samples.

PCR was positive for the 28 food samples which were culture positive, dot-ELISA positive; it was also positive for the 14 samples of dot-ELISA positive, culture negative and also the 2 samples of culture positive, dot-ELISA negative. There were 2 samples of the 156 dot-ELISA negative, culture negative that were positive by the PCR. Thus, the PCR were positive for 46 samples (10 of the chicken and 36 of the pork samples). Tables 4 and 5 compare the results of the *Salmonella* detection in the food samples between the DNA amplification and the culture method and the DNA amplification and the dot-ELISA, respectively.

The sensitivity, specificity, efficacy, and positive and negative predictive values of the *Salmonella* detection of the food samples by the

Table 3 Serotypes of the *Salmonella* spp. found in pork and chicken samples

Serotypes	Type of food	
	Pork	Chicken
S. agona	2	-
S. anatum	10	-
S. derby	11	-
S. blockley	-	1
S. cerro	-	1
S. hadar	-	2
S. senftenberg	-	2
S. typhimurium	-	1
Total	23	8

Table 4 The results of *Salmonella* detection in chicken and pork samples by culture method and DNA amplification

DNA amplification	Culture method		Total
	Positive	Negative	
Positive	30	16	46
Negative	0	154	154
Total	30	170	200

Diagnostic specificity	= 91.58%
Diagnostic sensitivity	= 100%
Efficacy	= 92%
Positive predictive value	= 65.21%
Negative predictive value	= 100%
Kappa coefficient calculation	
Observed agreement	= 0.9200
Chance expected agreement	= 0.6890
Kappa coefficient (K)	= 0.7427
Standard error of Kappa	= 0.0683
Kappa probability (Z)	= 10.87
p-value	= < 0.0001

Table 5 The results of *Salmonella* detection in chicken and pork samples by DNA amplification and dot-ELISA

Dot-ELISA	DNA amplification		Total
	Positive	Negative	
Positive	42	0	42
Negative	4	154	158
Total	46	154	200

Diagnostic specificity	= 100%
Diagnostic sensitivity	= 91.3%
Efficacy	= 98%
Positive predictive value	= 100%
Negative predictive value	= 97.5%
Kappa coefficient calculation	
Observed agreement	= 0.9800
Chance expected agreement	= 0.6566
Kappa coefficient (K)	= 0.9417
Standard error of Kappa	= 0.0705
Kappa probability (Z)	= 13.34
p-value	= < 0.0001

dot-ELISA compared with the culture method were 93.33% 91.76%, 92%, 66.66% and 98.73%, respectively. The results assayed by the PCR compared to the culture method were 100%, 91.58%, 92%, 65.21% and 100%, respectively. However, the dot-ELISA and the PCR results were in a better agreement than when the results of the two assays were compared with the culture method. The sensitivity, specificity, efficacy, and positive and negative predictive values of the dot-ELISA compared to the PCR were 91.3%, 100%, 98%, 100% and 97.5%, respectively.

DISCUSSION

Our monoclonal antibody-based dot-ELISA detected as low as 100 *Salmonella* cells (10^5 cells/ml) or 1 ng of the LPS. This level of analytical sensitivity of the assay is equal to or better than those of the previous reports, *i.e.* the immunofluorescent technique[21] which also detect the bacteria at 10^5 cells/ml, the ES method[43] and DNA-DNA hybridization assay,[9] both of which could detect 10^7 cells/ml and the enzyme immunoassay[26] which could detect 10^6 cells/ml of the bacteria. This level of sensitivity is less than a minimum infectious dose of *Salmonella* in foods.[44] Besides, the dot-ELISA was not affected by the presence of a large number of other common bacteria like *E. coli* in the sample.

It was found in this study that the dot-ELISA had 93.33%, 91.76%, 92%, 66.66% and 98.73% sensitivity, specificity, efficacy, and positive and negative predictive values, respectively, when compared with the bacterial culture method. Analysis of the Kappa coefficience revealed the two methods to have a good degree of agreement beyond chance (K = 0.7306) while the value of Kappa probability (Z) was 10.55 value which was significantly high ($p < 0.0001$) indicating that the observed degree of agreement is reliable[45] These results were similar to those reported previously.[34] There were 2 samples (one each of chicken and pork) which were dot-ELISA negative, culture positive and 14 samples (3 chicken and 11 pork) which were dot-ELISA positive, culture negative. The former discrepancy might be due to the *Salmonella* spp. presented in the 2 samples were rough mutants which lacked the core polysaccharide epitope for the MAb 102B2 thus rendering the dot-ELISA negative while the culture as well as PCR were positive. Unfortunately, the two *Salmonella* isolates were not preserved and attempts to recover the strains from the remaining frozen portions of the samples were not successful; thus analysis of their sugar moieties in the LPS could not be done. The higher positivity of the dot-ELISA than the culture when both methods were used to detect the same food samples reproduced the results of the previous finding.[35] These results were attributable to the fact that the culture method was able to detect only living bacteria which were presented in sufficient number, could be resuscitated by the pre-enrichment and selective enrichment and grew into at least one isolated *Salmonella* colony. Additionally, high amounts of the other bacteria in the food samples might compete with *Salmonella* to a level that prevents growth and isolation by the culture method even though this did not interfere with the dot-ELISA. Thus, the positive results by the culture method were based primarily on the combined 3 successful chances, *i.e.*

pre-enrichment, selective enrichment and selective and differential plating whereas the dot-ELISA depended more on the actual number of the bacteria/amount of the smooth LPS presented in the food samples. Repeated cultures of the 17 originally dot-ELISA positive, culture negative samples yielded 3 additional culture positive samples which supported the validity of the dot-ELISA.

Clarification of the remaining food samples which gave discrepant results by the dot-ELISA and the culture method was carried out using DNA amplification. The DNA primers used in the PCR were specific for *Salmonella* as they amplified only the DNA prepared from the *Salmonella* spp. and not the 30 other bacteria. The amplicon was 833 base pairs and could be clearly distinguishable from the negative result under the UV transluminator. However, the minimum amount of the *Salmonella* DNA which could be detected was 30 pg of purified DNA or the DNA prepared from at least 5,000 cells in tetrathionate broth. Theoretically, this level of sensitivity was rather low since the PCR should detect a single DNA sequence or a single cell.[46] This low level of sensitivity could be due to many factors which might influence the amplification reaction, *e.g.* purities and qualities of the reagents used, the enzyme *Taq* polymerase, the buffer pH, the magnesium concentration *etc.*[39] The DNA amplification did not work well when the crude bacterial lysates from selective enriched food samples were used as the DNA templates; only few samples were positive. The negative results were not due to the system used for amplification, as the positive control using purified DNA as the template gave satisfied results, but rather due to the presence of some inhibitor(s) in the crude DNA preparation. As such, the DNA amplification for the detection of *Salmonella* in this study were carried out using the DNA purified from the selective enrichment cultures of the food samples.

The sensitivity, specificity, efficacy and positive and negative predictive values of the PCR using the bacterial culture as a standard method were 91.58%, 100%, 92%, 65.21%, and 100%, respectively. The discrepancy of the results between the DNA amplification and the culture method, *i.e.* the PCR positive, culture negative samples, were similar to those found between the dot-ELISA and the culture and could be similarly explained by the different detection basis in that the PCR could detect DNA of all forms/origins, namely living, injured/stress, or dead cells while the culture could detect only living, readily grown bacteria which were presented in abundant numbers.

Statistical analysis of the dot-ELISA in comparison with the DNA amplification revealed interesting results. The ELISA had 91.3%, 100%, 98%, 100% and 97.5% sensitivity, specificity, and positive and negative predictive values, respectively, when the PCR was used as a standard technique. These indicate perfect degree of agreement on specificity and the positive predictive value and very high degree (almost perfect) on the sensitivity, efficacy and negative predictive value of the two tests. There were 4 samples which were positive by the PCR but negative by the dot-ELISA. One reason that may be given for this difference is that the dot-ELISA detect only the smooth variants of the bacteria while the PCR would give positive results on DNA of all of the bacterial variants.

From this study, it becomes clear that the food samples which were dot-ELISA positive, culture negative were indeed contaminated with the *Salmonella* organisms as revealed by the presence of the *Salmonella* DNA by the PCR. Beside being rapid, simple, sensitive, specific at low cost with limited amount of infectious waste to be disposed, compared to the other two methods, the ELISA offers another advantage in that it detects only the smooth LPS which implies the possible presence of virulent organisms.

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