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Monoclonal Antibody-Based Dot-Blot ELISA for the Detection of *Salmonella* in Foods

Wanpen Chaicumpa, Warunee Ngren-ngarmlert, Thareerat Kalambaheti, Yuwaporn Ruangkunaporn Manas Chongsa-nguan, Pramuan Tapchaisri, Varunee Desakorn[1], and Orasa Suthienkul[2]

SUMMARY **Monoclonal antibody (MAb) produced to polysaccharides in the LPS molecule of salmonellae was used in a dot-blot ELISA for detecting *Salmonella* in 873 food samples, ie 100 fresh chicken, 261 frozen chicken, 78 pork, 84 beef, 100 hen eggs, 100 duck eggs, 50 sea-mussels, 50 shrimps and 50 squids in comparison with the conventional culture method. *Salmonella* culture from foods involved the following steps: pre-enrichment, enrichment in selective medium, isolation on selective and indicator media, followed by biochemical and serological identification of appropriate colonies, respectively. The whole culture procedure took 5 days. Food samples from the selective enrichment medium were also subjected to the MAb-based dot-blot ELISA. The whole procedure of dot-blot ELISA took less than 2 hours.**

Among 873 food samples, salmonellae could be recovered from 7.4% of the samples by the bacterial isolation method (16% of fresh chicken, 8.8% of frozen chicken, 24.4% of pork, 3.6% of beef and 2% each of hen eggs and duck eggs, respectively). *Salmonella derby* were predominant among pork samples while *S.paratyphi* B biovar java predominated in chicken. The MAb-based dot-blot ELISA were positive in 19.5% of the food samples, ie 30% of fresh chicken, 27.6% of frozen chicken, 34.6% of pork, 21.4% of beef, 20% of shrimp,16% of sea-mussels, 2% of hen eggs and 4% of duck eggs. The sensitivity and specificity of the MAb-based dot-blot ELISA compared to the bacterial culture method were 81.5% and 85%, respectively. The discrepancy of the data between the culture method and the dot-blot ELISA might be due to the fact that the culture method could detect only living cells at numbers that gave at least one isolated colony on the selective/differential plate while the dot-blot ELISA detects any form of *Salmonella* antigen.

The monoclonal antibody-based dot-blot ELISA offers several advantages over the conventional bacterial culture method when it is used for screening of *Salmonella* contamination in foods, especially export foods. These include rapidity, cost-effectiveness and simplicity (the dot-blot ELISA does not need highly trained personel or equipment, in contrast to the culture method). The test can be performed in field conditions and the result can be read visually. It also offers multisample analysis at one time which renders more samples of food for screening possible, thus false negative results are fewer which, in turn, assures the quality of the export food in a cost-saving, short time frame.

Salmonellosis is one of the most important public and animal health problems, causing morbidity and mortality of humans and animals throughout the world. It is also a communicable disease readily transmissible from animals to man either directly or through contaminated products of animal origin.[1] Because of a steady and significant increase in salmonellosis and a high economic loss,[2] food industries, governments and consumers have been interested in decreasing the incidence of the infection. Therefore detection of *Salmonella* contamination in foods and feeds becomes critical to controlling this disease.

The conventional method of detecting salmonellae in foods is by culture, which depends on selective media and biochemical reactions to separate *Salmonella* strains from other enteric bacteria.[3] Although it is still the most reliable method, culture is expensive, laborious and time consuming.[1,4] The whole process, which involves pre-enrichment, selective enrichment, selective plating and biochemical tests requires at least 5 days, during which export food companies incur additional costs as a result of the prolonged storage of the food.

Attempts have been made to develop simple, accurate, rapid and inexpensive methods to detect salmonellae in foods and feeds. Among them, the most favorable method is enzyme immunoassay because it is sensitive and rapid, permits multisample analysis at one time, and requires only a minimum of equipment.[5] However, the assay gives a high percentage of false positive reactions due to the non-specific (cross-reactive) nature of the polyclonal antibodies used.[6] Mono-

From the Department of Microbiology and Immunology, [1]Department of Clinical Tropical Medicine, Faculty of Tropical Medicine, and [2]Department of Microbiology, Faculty of Public Health, Mahidol University, Bangkok, Thailand.

Correspondence : Wanpen Chaicumpa, Department of Microbiology & Immunology, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand.

clonal antibody (MAb) specific to salmonellae is likely to solve this problem. Recently, Chaicumpa *et al.*[7] produced a MAb (102 B_2) which is specific to polysaccharides of salmonellae. This MAb reacted with the lipopolysaccharides (LPS) of all *Salmonella* strains tested, but not with antigens prepared from other bacteria.

In the present study, we report the use of the MAb 102 B_2 to develop a dot-blot ELISA for the detection of *Salmonella* antigen in foods. The results so-obtained were compared with those from the conventional bacterial culture method.

MATERIALS AND METHODS

Bacterial strains and antigen preparations

Salmonellae and other bacteria used in this study are listed in Table 1. They were from stock cultures held in the Department of Microbiology and Immunology, Faculty of Tropical Medicine, Mahidol University, Bangkok and the World Health Organization National *Salmonella* and *Shigella* Center, National Institute of Health, Ministry of Public Health, Thailand.

Whole cell lysates (Ly) were prepared from each bacterial strain by suspending the log-phase culture in distilled water to an optical density (OD) of 2.0 at 540 nm and subjecting the preparation twice to ultrasonication at 20 kHz for 5 minutes each. Dry weights of the preparations were then determined. These lysates were used for checking cross-reactivity of MAb 102 B_2 and for determining specificity of the MAb-based dot-blot ELISA.

Lipopolysaccharide (LPS) was extracted from acetone dried cells of *S.typhi* strain O901 by the hot phenol-water method of Westphal and Jann.[8] The single extracted LPS was re-extracted two more times to yield double-extracted and triple-extracted LPS, respectively. Quantitative and qualitative determinations of proteins were performed using the method of Lowry *et al.*[9] and by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) with Coomassie brilliant blue staining.[10]

Monoclonal antibody

A monoclonal antibody (MAb) specific for lipopolysaccharide of salmonellae was obtained from *in vitro* culture of hybrid clone 102 B_2.[7] The ELISA titre of the monoclonal antibody preparation was determined by indirect ELISA; one indirect ELISA unit was the smallest amount of the antibody which gave a positive indirect ELISA reaction.[7] Antigenic specificity of the MAb was redetermined against *S.typhi* Ly using SDS-PAGE and Western blot analysis.

Food specimens

Eight hundred and seventy-three food specimens were used in this study. These included 100 fresh chickens, 261 frozen chickens, 84 beef specimens, 78 pork specimens, 50 shrimps, 50 squids, 50 sea-mussels, 100 hen eggs and 100 duck eggs from various markets in Bangkok. The specimens were transported to the laboratory in clean plastic bags under chilled conditions as fast as possible in order to avoid spoilage or excessive proliferation of other competitive bacteria. From each food sample (except eggs), a 10 g portion was placed in an Erlenmeyer flask containing 90 ml of trypticase soy broth (TSB) supplemented with 0.05% yeast extract (TSBY). The remaining portions of food samples were kept at -70°C and some of them which their counterparts were negative for salmonellae by culture were used in the determinations of the lowest limit of the dot-blot ELISA (see below). The food preparation in TSBY was shaken vigorously for 30-60 seconds and subjected to a conventional culture method. Shell of egg was rinsed with 100 ml of TSBY in clean plastic bag, the rinsing fluid was transfered to a sterile flask and also subjected to the culture procedure.

Bacterial culture method

The procedure for culturing salmonellae described by Andrews *et al.*[4] was followed. Each food specimen prepared as above in TSBY was incubated at 37°C for 16 hours. One ml of of each culture was inoculated into 9 ml of selenite-cystine (SC) broth, a selective enrichment broth, and incubated at 42°C for 16 hours. MacConkey, brilliant green sulfadiazine and bismuth sulfite agars were used for selective plating which were incubated overnight at 37°C. *Salmonella*-like colonies were transferred to tubes of lysine iron and triple sugar iron agars and incubated at 37°C overnight. Isolates positive as *Salmonella* in lysine iron and triple sugar iron agar tests were further characterized by biochemical tests which included ornithine decarboxylase, motility, indole, urease and citrate tests. The typical *Salmonella* isolates were also tested by slide agglutination with *Salmonella* polyvalent O antiserum. Isolates confirmed by the definition of genus *Salmonella* were sent to the WHO National *Salmonella* and *Shigella* Center at the National Institute of Health, Thailand for serotyping.

SDS-PAGE and Western blot analysis

The SDS-PAGE and Western blot analysis were carried out as previously described.[11] Whole cell lysate of *S.typhi* was separated in a vertical slab gel apparatus (Bio-Rad Laboratories, USA) according to the system of Laemmli.[10] A 4% acrylamide stacking gel and 10% acrylamide separating gel were used in the process. Western blotting was performed by transblotting the

SDS-PAGE separated antigens from the gel to the nitrocellulose membrane (NCM).[12] The unoccupied sites on the NCM were blocked by soaking in a solution of 2% BSA and 0.2% gelatin at 26°C for 1 hr. After washing thoroughly, the NCM was put in a solution of peroxidase labelled rabbit anti-human immunoglobulins (Dakopatt, Denmark) at a dilution of 1:500 in PBS, pH 7.4 containing 1% BSA and 1% gelatin for 30 minutes at 26°C. The NCM was washed 4 times with PBS containing 0.05% Tween-20 (PBST). Finally it was washed in phosphate buffer, pH 7.6 before placing the membrane into a substrate solution containing 0.2 g of 2, 6-dichlorophenol nitrophenol (Sigma Chemical Co., USA) and 0.01% H_2O_2 in 100 ml phosphate buffer, pH 7.6 until the brown bands appeared, washed thoroughly with distilled water, then air-dried.

MAb-based dot-blot ELISA

Aliquots of food specimens in Eppendorf tubes after the selective enrichment were boiled in a boiling water bath for 15 minutes in order to destroy the endogenous alkaline phosphatase enzyme in the food preparations. The samples were then subjected to the dot-blot ELISA as previously described.[13] Three microlitres of specimens were applied in duplicate to two separate strips of NCM with 0.45 μm pore size (Schleicher and Schuel, Germany). Positive (3 μl of *S.typhi* LPS at 1 mg/ml) and negative (3 μl of distilled water; DW or SC) controls were also dotted onto the strips. The blotted NCM strips were air-dried for about 10 min. The empty sites on the NCM were blocked by placing the strips into a solution of 5% bovine serum albumin in Tris-bufferred saline (TBS), pH 7.5 at room temperature for 20 minutes on a rocking platform. The strips were washed thoroughly with TBS containing 0.05% Tween-20 (TBST). One strip of the NCM was submerged in MAb 102 B_2 solution (640 indirect ELISA units/ml) while another strip was placed in RPMI 1640 medium supplemented with 10% fetal bovine serum (to serve as negative control) for 20 minutes at room temperature. Both strips were washed with TBST and placed in a solution of rabbit anti-mouse immunoglobulin-alkaline phosphatase conjugate (Dakopatt, Denmark) (dilution 1:2,000 in TBST) at room temperature for 20 minutes. After washing as above, the strips were placed in a substrate solution (5 mg of nitroblue tetrazolium and 2.5 mg of 5-bromo-4-chloro-3-indolyl phosphate in 20 ml of 0.1 M Tris-HCl containing 0.1 M NaCl and 50 mM $MgCl_2$). The enzyme-substrate reaction was allowed to take place in the dark for 20 minutes at room temperature. The reaction was stopped by rinsing the membranes with distilled water and air-dried. The positive reaction appeared as a purplish-blue spot on the dot-blotted membrane which could be clearly distinguished from the brown spot of the non-specific reaction and the clear area of the negative reaction.

Analytical specificity of the dot-blot ELISA was determined by using whole cell lysates of all strains of *Salmonella* and other bacteria listed in Table 1 instead of food specimens in the dot-blot ELISA. This was done to demonstrate that the MAb 102 B_2 reacted specifically with *Salmonella* spp. In the test, each whole cell lysate was diluted with distilled water to 1 mg/ml and 3 μl was dotted to the NCM strip.

The lowest limit of the dot-blot ELISA was also determined by using various concentrations of *S.typhi* LPS and whole cells of *S. typhi* in normal saline solution or in TSBY containing another 10 g portion of culture negative food specimen in the dot-blot ELISA. The LPS was varied from 1,000 ng/μl to 0.12 ng/μl while the *S.typhi* whole cells was varied from 10^7 cells/μl to 1 cell/μl. One microlitre of each concentration was dotted onto the NCM strip instead of 3 μl. The smallest amount of *S.typhi* LPS and lowest number of *S.typhi* cells which gave positive dot-blot ELISA reactions were regarded as the lowest limits of the assay.

Statistical methods

The method of Galen[14] was used for calculating the diagnostic specificity and sensitivity of the dot-blot ELISA in comparison to the conventional bacterial culture method. The diagnostic specificity is defined as the probability that the MAb-based dot-blot ELISA is negative when no salmonellae could be cultured from the specimen while the diagnostic sensitivity refers to the probability that the ELISA is positive when the culture is positive. Both values could be calculated and expressed in percentages as follows :

$$\text{Diagnostic specificity} = \frac{\text{true negative} \times 100}{\text{true negative} + \text{false positive}}$$

$$\text{Diagnostic sensitivity} = \frac{\text{true positive} \times 100}{\text{true positive} + \text{false negative}}$$

When true negative = number of food samples which was negative by both methods (culture and ELISA)

True positive = number of samples which were positive by both methods

False positive = number of ELISA positive, culture negative samples

False negative = number of ELISA negative, culture positive samples

Table 1. Bacterial strains used in this study

Salmonellae	
Salmonella aberden	*Salmonella lexington*
Salmonella agona	*Salmonella montevideo*
Salmonella amsterdam	*Salmonella orion*
Salmonella anatum	*Salmonella oslo*
Salmonella bangkok	*Salmonella panama*
Salmonella blockley	*Salmonella paratyphi* A
Salmonella brunei	*Salmonella paratyphi* B
Salmonella chicago	*Salmonella paratyphi* C
Salmonella choleraesuis	*Salmonella paratyphi* B biovar java
Salmonella dublin	*Salmonella poona*
Salmonella enteritidis	*Salmonella senftenberg*
Salmonella emek	*Salmonella typhi* strain 0901
Salmonella group E_4	*Salmonella typhimurium*
Salmonella hardar	*Salmonella virchow*
Salmonella kentucky	*Salmonella weltevreden*
Other Enterobacteriaceae	
Citrobacter diversus	*Klebsiella pneumoniae*
Citrobacter freudii	*Morganella morganii*
Enterobacter aerogenes	*Pseudomonas aeruginosa*
Enterobacter cloacae	*Proteus mirabilis*
Escherichia coli	*Proteus rettgeri*
Edwardsiella tarda	*Proteus stuartii*
Hafnia alvei	*Proteus vulgaris*
Serratia marcescens	*Shigella flexneri*
Shigella boydii	*Shigella sonnei*
Shigella dysenteriae	*Yersinia enterocolitica*
Pseudomonadaceae	
Aeromonas hydrophila	*Plesiomonas* spp.
Pseudomonas spp.	
Vibrionaceae	
Vibrio alginolyticus	*Vibrio cholerae* El Tor Ogawa
Vibrio alginosum	*Vibrio fluvialis*
Vibrio anguillarum	*Vibrio furnissii*
Vibrio cholerae Classical Inaba	*Vibrio parahaemolyticus*
Vibrio cholerae El Tor Inaba	

McNemar Chi-square test was used for testing whether a group of samples assayed twice by two procedures (culture *vs* dot-blot ELISA) gave any significant difference in the results.

RESULTS

The antigenic specificity of the MAb from clone 102 B_2 was re-determined against SDS-PAGE separated whole cell lysate of *S.typhi* O901 using peroxidase-labelled anti-mouse immunoglobulin and substrate to reveal the Ag-Ab complexes instead of the ^{125}I-labelled anti-mouse immunoglobulin and autoradiography in the Western blot analysis. The enzyme system was found to be rapid, more convenience and more sensitive than the radioisotope system used previously.[7] From Fig. 1 it was found that the MAb,

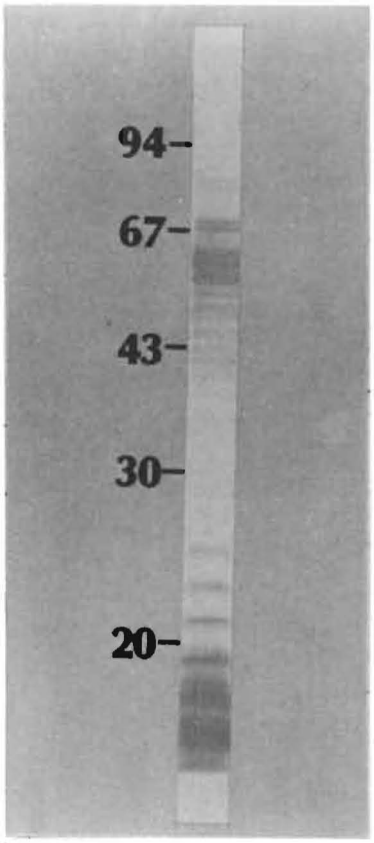

Fig. 1 Western blot pattern of Mab from clone 102 B_2 against SDS-PAGE separated *S.typhi* Ly using peroxidase-labelled rabbit anti-mouse immunoglobulin and substrate to reveal the Ag-Ab complexes. Numbers of left indicate mol. wt. $\times 10^{-3}$ The MAb reacted to core polysaccharides (lower portion) as well as O repeating units of the side chain (upper portion).

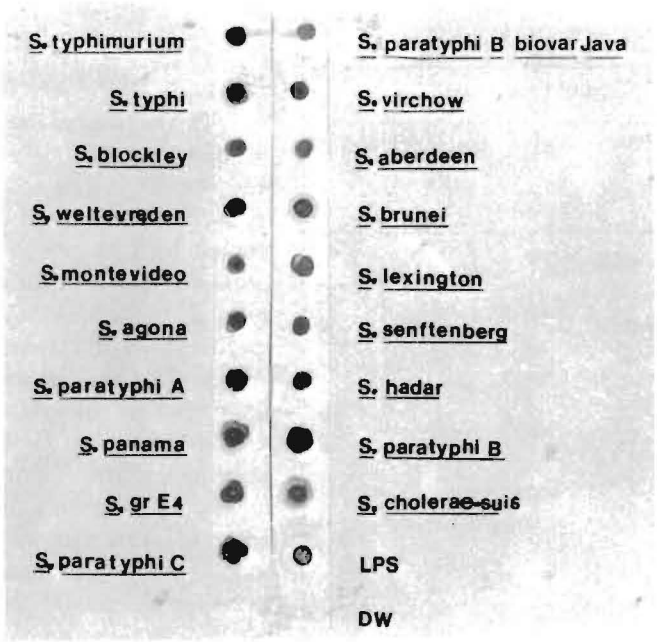

Fig. 2 Representative results of dot-blot ELISA performed on whole cell lysates of various salmonellae
LPS = positive control
DW = negative control

Table 2. Prevalence of salmonellae in foods detected by dot-blot ELISA and culture method.

Type of food	Number of positive specimens/total number examined			
	Dot blot ELISA		Culture method	
Fresh chickens	30/100	(30%)	16/100	(16%)
Frozen chickens	72/261	(27.6%)	23/261	(8.8)
Pork	27/78	(34.6%)	19/78	(24.4%)
Beef	18/84	(21.4%)	3/84	(3.6%)
Shrimp	10/50	(20%)	0/50	(0%)
Sea-mussels	8/50	(16%)	0/50	(0%)
Squid	0/50	(0%)	0/50	(0%)
Hen egg shells	2/100	(2%)	2/100	(2%)
Duck egg shells	4/100	(4%)	2/100	(2%)
Total	171/873	(19.5%)	65/873	(7.4%)

besides reacting with the core polysaccharide portion of the LPS as previously reported,[7] also gave a reaction with a portion of O repeating side chain of the *S.typhi* LPS.[7,15]

Whole cell lysates of all salmonellae tested gave positive dot-blot ELISA seen as purplish-blue spots distinguishable from the colorless area of the negative controls (Fig. 2). The test was negative when whole cell lysates of other bacteria were used. These negative results appeared as brown spots or colorless areas. Thus the analytical specificity of the MAb-based dot-blot ELISA was 100%.

When the dot-blot ELISA was performed on serially diluted *S.typhi* whole cells and LPS, it was found that the lowest amounts of the bacterial cells and LPS in NSS and in TSBY containing culture negative food sample which gave positive results were the same, ie 100 cells and 1-2 ng, respectively.

Dot-blot ELISA was also performed to determine the sensitivity of the test for detecting salmonellae in the presence of large amounts of heterologous bacteria. Log phase cultures of *Escherichia coli* in TSB containing approximately 10^9 cells/ml was prepared. *S.typhi* were then inoculated into the *E.coli* culture to obtain the concentrations of 10^7, 10^6, 10^5, 10^4, 10^3, 10^2, 10 and 1 cells of *S.typhi* per ml of *E.coli* culture, respectively. The volume of 1 μl from each of the above concentrations was dotted onto the NCM and subjected to the dot-blot ELISA. It was found that a positive result was obtained when *S.typhi* were present at 10^5 cells/ml (100 cells/μl) although with the high density of *E.coli*. Experiments on the experimentally contaminated food samples with various amounts of *E.coli* and *S.typhi* revealed the same lowest limit of the assay.

From 873 food samples, salmonellae were isolated from 65 samples (7.4%) by culture method,

Table 3. Serotypes of salmonellae found in food specimens.

Serotypes	Total of positive samples	Type of food					
		Chicken		Pork	Beef	Egg	Seafood
		fresh	frozen				
S. anatum	7	-	-	6	1	-	-
S. blockley	1	-	1	-	-	-	-
S. derby	11	-	1	10	-	-	-
S. enteritidis	2	1	1	-	-	-	-
S. hadar	3	-	3	-	-	-	-
S. group I	5	1	2	2	-	-	-
S. kentucky	1	-	1	-	-	-	-
S. lexington	4	-	1	2	1	-	-
S. london	2	-	1	-	-	1	-
S. bandaka	1	-	-	-	1	-	-
S. ohio	1	-	-	-	-	1	-
S. paratyphi B biovar java	19	12	6	-	-	1	-
S. senftenberg	5	3	2	-	-	-	-
S. stanley	1	-	1	-	-	-	-
S. typhimurium	1	-	1	-	-	-	-
S. virchow	3	-	2	-	-	1	-
Total	67	17	23	20	3	4	0

Table 4. Results of salmonellae dection in 873 food samples by culture method and the dot-blot ELISA.

Dot-blot ELISA	Culture method		Total
	+	-	
+	53	118	171
-	12	690	702
Total	65	808	873

namely from 16 fresh chickens, 23 frozen chickens, 19 pork samples, 3 beef samples, 2 hen eggs and 2 duck eggs (Table 2). Serotypes of these isolates are shown in Table 3. There were 1 chicken sample and two pork samples where two serotypes of salmonellae were isolated from the same specimen. *S.paratyphi* B biovar java predominated in chicken while *S.derby* and *S.anatum* were found predominantly in pork.

Dot-blot ELISA was positive for 171 of 873 food samples (19.5%). These were 30 fresh chickens, 72 frozen chickens, 27 pork samples, 18 beef samples, 2 hen eggs, 4 duck eggs, 10 shrimps and 8 sea-mussels (Table 2). The overall results of *Salmonella* detection in the 873 food samples by the culture method and by the dot-blot ELISA are given in Table 4. From these data, the diagnostic sensitivity and specificity of the dot-blot ELISA were 81.5% and 85%, respectively. There were 118 dot-blot ELISA positive, bacterial culture negative samples.

DISCUSSION

In this study, the monoclonal antibody specific to core polysaccharides of salmonellae obtained from the hybrid clone 102 B_2 constructed by Chaicumpa *et al.*[7] was employed for detecting salmonella antigen in foods. The antigenic specificity of the monoclonal antibodies (MAb) was re-determined against whole cell lysates of *S.typhi* O901 using an anti-mouse immunoglobulin-enzyme conjugate and substrate system in Western blot analysis. This enzyme system was found to be rapid, more convenient and more sensitive than the ^{125}I-labelled anti-mouse immunoglobulin system used previously.[7] The MAb, basides reacting with the core polysaccharide portion of the LPS as reported by Chaicumpa *et al.*,[7] also gave a reaction with a portion of O repeating side chain of the *S.typhi* LPS.[7,15] The finding suggested that the MAb from the clone 102 B_2 recognized an epitope located at the juxtapose area of the core polysaccharide and the O specific side chain repeating portion. These MAb were also re-assessed for their analytical specificities against various salmonellae and other genera of bacteria by dot-blot ELISA. The monoclonal antibody reacted with the antigens from all *Salmonella* tested but did not cross-react with organisms other than salmonellae (analytical specificity is 100%). These results confirmed the report of Chaicumpa *et al.*[7] in which the monoclonal antibody was assessed against Barber antigen (Ba) of homologous *S.typhi* O901 and whole cell lysates of *S.typhi* from clinical isolates and heterologous organisms by indirect ELISA. The dot-blot ELISA could detect as little as 1-2 ng of purified LPS and approximately 10^5 cells of *S.typhi*/ml or 100 cells per spot. This level of

analytical sensitivity was not interfered with the presence of high number of other bacteria or food material and also as sensitive as that found in the published fluorescent antibody test.[16] The minimum cell concentration for reliable results tested by enrichment serology[2] and DNA-DNA hybridization assays[17] was 10^7 cells/ml and was 10^6 cells/ml by the EIA.[18] One hundred bacterial cells were less than the minimum infectious dose of salmonellae in food vehicles. Thus, the dot-blot ELISA based on the monoclonal antibody is an effective means for the sensitive detection of *Salmonella* antigen in foods.

The percent distribution of salmonellae in foods (meat products and seafoods) detected by the two methods, ie dot-blot ELISA and culture was different. salmonellae could be detected more readily by the dot-blot ELISA (19.5%) than by the culture method (7.4%). McNemar's test was used to assess whether there was any significant difference between the number of disagreement between the culture method and the dot-blot ELISA (false positives and false negatives; Table 4). It was found that the number of false positives (118 samples) was significantly higher than the number of false negatives (12 samples). The reason for the discrepancy might be due to the fact that the culture method could detect only living cells while the dot-blot ELISA detected any form of *Salmonella* antigen. Moreover, positive results by the culture method require that two conditions are met. First, a sufficient number of salmonellae must be present in the selective enrichment step to assure that one or more *Salmonella* cells in aliquots streaked onto the differential plate. Second, the relative proprotion of salmonellae to other organisms that are capable of growing in/on the selective/differential media must be such that at least one isolated colony of *Salmonella* can be obtained. Thus, obtaining a positive result by the culture method is based primarily on the chance of selectivity, while a positive result in the dot-blot ELISA depends more on actual numbers of salmonellae or the amount of their antigen presented. High numbers of competitors might inhibit growth of salmonellae to the level that prevents isolation by the culture method. However, this has been found in our study not to interfere with the dot-blot ELISA.

Deliberate investigations on food samples which gave dot-blot ELISA-positive, culture-negative by reculturing of the remaining food portions (which had been kept at -70°C) by a secondary selective enrichment and replating and by polymerase chain reaction using different specific oligonucleotide primers for salmonellae have revealed that either living salmonellae and/or their specific DNA sequences could be detected in those samples. Thus, the 118 food samples which gave false positive results by the dot-blot ELISA when compared with the culture method in the present report might be the true positive specimens. Details of the methodology and results of the investigations of this issue will be reported elsewhere.

The monoclonal antibody-based dot-blot ELISA offers several advantages over the conventional bacterial culture method when it was used for screening of *Salmonella* contamination in foods, especially export foods. These include rapidity, cost-effectiveness and simplicity (the dot-blot ELISA does not need highly trained personel or equipment; in contrast to the culture method). The test can be performed in field conditions and the result can be read visually. It also offers multisample analysis at one time which renders more samples of food for screening possible, thus false negative results are less which, in turn, assures the quality of the export food in a cost-saving short time frame.

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