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Comparison of multiplex polymerase chain reaction and immunoassay to detect *Salmonella* spp., *S*. Typhimurium, and *S*. Enteritidis in Thai chicken meat

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ABSTRACT: The multiplex PCR (mPCR), enzyme-linked immunosorbent assay (ELISA) and immunomagnetic separation enzyme-linked immunosorbent assay (IMS-ELISA) were developed for the detection of *Salmonella* spp. and two specific serovars *S*. Typhimurium and *S*. Enteritidis. The detection limits of the mPCR without the pre-enriched step was at 10^5 cfu/ml, whereas developed immunoassays demonstrated positive results at 10^5, 10^6, and 10^7 cfu/ml by ELISA and at 10^4, 10^4, and 10^6 cfu/ml by IMS-ELISA for detecting *Salmonella* spp., *S*. Typhimurium, and *S*. Enteritidis, respectively. The mPCR did not produce any nonspecific amplified products when tested against other related species of bacteria. In this study, the developed IMS-ELISA gave more sensitivity but less specificity than the mPCR assay. The methods are useful for the rapid detection of salmonellae in naturally infected chicken meat in Thailand.

KEYWORDS: enzyme-linked immunosorbent assay (ELISA), immunomagnetic separation (IMS), food safety

INTRODUCTION

The importance of *Salmonella* spp. as a leading cause of foodborne diseases continues to be emphasized by national epidemiological reports from all over the world. Transmission to humans is mainly foodborne and results from eating undercooked meat, milk, eggs, or from cross-contamination of other foods which are eaten without cooking [1,2].

More than 2500 serovars of *Salmonella enterica* have been identified; most have been described as the cause of human infections, especially *S. enterica* serovars Typhimurium and Enteritidis, which according to a WHO surveillance report have been the serovars most frequently isolated from cases of human food poisoning. Asian, American, European, and African health agencies have notified similar increases in such illnesses related to the consumption of eggs and poultry [3–8].

Over the past decade, a number of studies have reported an increasing incidence of non-typhoid *Salmonella* bacteraemia [9,10]. Food safety in the exporting food industries needs a rapid method for detecting the contamination of *Salmonella*. Several countries, such as the European Union, USA, Japan, Korea, and Singapore, exercise strictly control and specifically forbid the contamination of *S*. Typhimurium and/or *S*. Enteritidis in food samples [11]. It is necessary for chicken exporting countries like Thailand to follow those requirements for chicken meat products.

Salmonella infection and spread can occur at any point during chicken production. Control measurements are needed to be enforced at each point of chicken meat and egg production. A number of rapid methods for the detection of *Salmonella* in foods has been developed and reported, including electrical techniques, nucleic acid analysis, and immunoassay [12–16]. These would reduce the time for testing by a few days. Molecular techniques such as polymerase chain reaction (PCR) can be used to solve that type of problems and increase sensitivity and specificity of pathogen detection. The simultaneous detection of several pathogens with a multiplex PCR (mPCR) approach would be relatively rapid and cost effective [17–23]. The enzyme-linked immunosorbent assay (ELISA) is the most prevalent antibody assay format used to detect pathogen food and it is still a favourable

Table 1 List of target and non-target bacterial species used for determining the specificity of developed mPCR and immunoassay kits for *Salmonella* spp., *S*. Typhimurium, and *S*. Enteritidis.

Bacterial strains	mPCR			ELISA			IMS-ELISA		
	Salmonella spp. (526 bp)	*S*. Typhimurium (620 bp)	*S*. Enteritidis (316 bp)	*Salmonella* spp.	*S*. Typhimurium	*S*. Enteritidis	*Salmonella* spp.	*S*. Typhimurium	*S*. Enteritidis
S. Typhimurium ATCC 13311	+	+	−	+	+	−	+	+	−
S. Typhimurium ATCC 14028	+	+	−	+	+	−	+	+	−
S. Typhimurium DMST 8536	+	+	−	+	+	−	+	+	−
S. Typhimurium DLDT 20713	+	+	−	+	+	−	+	+	−
S. Enteritidis ATCC 13076	+	−	+	+	−	+	+	−	+
S. Enteritidis DMST 8536	+	−	+	+	−	+	+	−	+
S. Enteritidis DLDT 24046	+	−	+	+	−	+	+	−	+
S. Enteritidis DLDT 24504	+	−	+	+	−	+	+	−	+
S. Anatum DLDT 24434	+	−	−	+	−	−	+	−	−
S. Anatum DLDT 24439	+	−	−	+	−	−	+	−	−
S. Blockley DMST 10639	+	−	−	+	−	−	+	−	−
S. Blockley DLDT 8937	+	−	−	+	−	−	+	−	−
S. Blockley DLDT 24442	+	−	−	+	−	−	+	−	−
S. Brunei DLDT 14794	+	−	−	+	−	−	+	−	−
S. Derby DLDT 24669	+	−	−	+	−	−	+	−	−
S. Hadar DLDT 24426	+	−	−	+	−	−	+	−	−
S. Hvittingfoss DLDT 1494	+	−	−	+	−	−	+	−	−
S. Lexington DLDT 12294	+	−	−	+	−	−	+	−	−
S. London DLDT 12494	+	−	−	+	−	−	+	−	−
S. Muenchen DLDT 24441	+	−	−	+	−	−	+	−	−
S. Panama DLDT 24437	+	−	−	+	−	−	+	−	−
S. Panama DLDT 24439	+	−	−	+	−	−	+	−	−
S. Paratyphi B Var Java DLDT 24305	+	−	−	+	−	−	+	−	−
S. Paratyphi B Var Java DLDT 24309	+	−	−	+	−	−	+	−	−
S. Paratyphi B Var Java DLDT 24311	+	−	−	+	−	−	+	−	−
S. Reading DLDT 14322	+	−	−	+	−	−	+	−	−
S. Saintpaul DLDT 23899	+	−	−	+	−	−	+	−	−
S. Saintpaul DLDT 23900	+	−	−	+	−	−	+	−	−
S. Stanley DLDT 24674	+	−	−	+	−	−	+	−	−
S. Typhi DMST 16122	+	−	−	+	+	+	+	+	+
S. Virchow DLDT 14596	+	−	−	+	−	−	+	−	−
S. Virchow DLDT 24428	+	−	−	+	−	−	+	−	−
S. Weltevreden DMST 10637	+	−	−	+	−	−	+	−	−
S. Weltevreden DLDT 8937	+	−	−	+	−	−	+	−	−
S. Weltevreden DLDT 14932	+	−	−	+	−	−	+	−	−
Campylobacter coli ATTC 43485	−	−	−	−	−	−	−	−	−
C. jejuni ATCC 33291	−	−	−	−	−	−	−	−	−
Citrobacter freundii ATTC 8090	−	−	−	−	−	−	−	−	−
Enterobacter aerogenes ATCC 13048	−	−	−	−	−	−	−	−	−
Enterococcus faecalis ATCC 29218	−	−	−	−	−	−	−	−	−
Escherichia coli ATCC 25922	−	−	−	−	−	−	−	−	−
E. coli ATCC 35218	−	−	−	−	−	−	−	−	−
Pseudomonas aeruginosa ATCC 27853	−	−	−	−	−	−	−	−	−
Staphylococcus aureus ATTC 25923	−	−	−	−	−	−	−	−	−

technique of choice for practical use in most companies. The ELISA combined with immuno-magnetic separation (IMS), which uses magnetic beads conjugated with anti-*Salmonella* antibodies on their surface[24,25]. The bead, when mixed with *Salmonella*-contaminated sample, will bind the organism on their surface via antibodies to eliminate the inhibitory effect of potential competition with other organisms. The *Salmonella*-bead complex can be pulled out of solution by a magnet which would concentrate the organisms left in the solution for further identification by immunoassay[26–28].

In the present work, we reported the development of mPCR system, ELISA and IMS-ELISA and compared the sensitivity and specificity of these methods for the detection of *Salmonella* spp., *S*. Typhimurium and *S*. Enteritidis. The developed assays were also applied to detect these bacteria in raw chicken obtained from fresh markets and exporting companies in Thailand.

MATERIALS AND METHODS

Bacterial strains

All reference micro-organisms listed in Table 1 were obtained from the American Type Culture Collection (ATCC), the Culture Collection for Medical Micro-organism, Department of Medical Sciences Thailand (DMST) and the fresh isolates from the Veterinary Public Health Laboratory, Department of Livestock Development Thailand (DLDT). The micro-organisms were identified by conventional methods

Table 2 PCR primers for *Salmonella* spp., *S*. Typhimurium, and *S*. Enteritidis in multiplex assay.

Species amplified	Target gene	Primer name	Sequence (5′ to 3′)	Size of PCR amplicon (bp)
S. Typhimurium	*FliC*[2]	Typ04	ACT GGT AAA GAT GGC T	620
		Fli15	CGG TGT TGC CCA GGT TGG TAA T	
S. Enteritidis	Insert Element (IE)[5]	IE1L	AGT GCC ATA CTT TTA ATG AC	316
		IE1R	ACT ATG TCG ATA CGG TGG G	
Salmonella spp.	*fimY*[28]	*fimY1*	GAG TTA CTG AAC CAA CAG CT	526
		fimY2	GCC GGT AAA CTA CAC GAT GA	

and serotyping based on the antigenic structure of both somatic or cell wall (O) antigens and flagellar (H) antigens. Those 44 reference strains were 4 strains of *Salmonella* Typhimurium, 4 strains of *S*. Enteritidis, 27 strains of other *Salmonella* spp. and 9 of non-*Salmonella* spp. The micro-organisms were grown following standard methods for *Salmonella* and Enterobacteriaceae and identified by conventional microbiological methods as described in Bacteriological Analytical Manual (BAM) for *Salmonella* detection[7].

Polymerase chain reaction primers

PCR primers were synthesizes at the Bioservice Unit (BSU), the National Science and Technology Development Agency, Bangkok. The three pairs of oligonucleotide primers, ranging from 18- to 24-mers, were selected from either published sequences[29–31]. Their corresponding gene targets and sizes of expected amplification products are shown in Table 2.

DNA template for mPCR optimization

Total genomic DNA from all Salmonellae and other bacterial strains listed was purified as described by Ausubel et al[32] and used for mPCR optimization. Briefly, cells were suspended in 567 µl of TE (10 mM Tris-HCl pH 8.0, 1 mM EDTA) buffer with 30 µl of 10% (w/v) sodium dodecyl sulphate and 3 µl of 20 mg/ml proteinase K (Sigma, USA) and were lysed for 1 h at 37 °C. Then, 100 µl of 5 M NaCl and 80 µl of cetyltrimethylammonium bromide (CTAB)-NaCl were added and the solution was incubated for 10 min at 65 °C. DNA was extracted with phenol-chloroform-isoamyl alcohol followed by chloroform-isoamyl alcohol. The concentration of purified DNA was then determined by Lambda II spectrophotometer (Milton Roy Spectronic 300, USA) at a wavelength of 260 nm.

Culture preparation for immunoassay and mPCR

One millilitre of overnight pure bacterial culture was centrifuged at 8000*g* for 5 min (Hettich, MIKRO 22R, Denmark). The supernatant was removed and the packed cells were re-suspended with 1 ml of 0.01 M PBS pH 7.4. The sample was boiled for 10 min, then immediately cooled on ice for 15 min. For immunoassay, the heat lysed bacteria was reported to contain the target antigen and can be used to perform immunoassay. For mPCR detection, the heat lysed bacteria was further centrifuged at 6000*g* for 5 min to remove cell debris. The lysed supernatant was collected and 5 µl was used as the template[33].

Detection of microorganism by polymerase chain reaction

The amplification reaction was performed in a volume of 25 µl containing 5 µl of DNA template from 10^8 cfu/ml of cell lysate as a DNA template. This sample was added to a mixture consisting of 1X PCR buffer (10 mM TrisHCl, pH 8.8; 50 mM KCl, 0.1% Triton X-100); 2 mM $MgCl_2$; 150 µM each dNTP; 0.2 U of *Taq* DNA polymerase (Promega, USA); 0.40 µM each of Typ04, Fli15 primers 0.40 µM each of IE1L, IE1R primers and 0.40 µM each of *fimY1*, *fimY2* primers. The PCR reaction was performed in a GeneAmp PCR System 240 Thermal Cycle (Perkin Elmer Cetus, USA). PCR products were analysed on a 1% agarose gel with 0.5× TBE (44.5 mM Tris base, 44.5 mM Boric acid and 1 mM EDTA) as the running buffer. A 100 bp standard DNA ladder (Bio-Rad) was included on each gel for base pair-size comparison. Gels were stained with 0.5 µg/ml ethidium bromide and visualized under UV light.

Preparation of immuno-magnetic beads

Each rabbit polyclonal anti-*Salmonella* (US Biological), mouse monoclonal anti *Salmonella* D group (US Biological), and mouse monoclonal anti-*S*. Typhimurium (US Biological) were coated on magnetic beads (Dynabeads M-450 Tosylactivated: 4×10^8 beads/ml) (Dynal). Firstly, beads were washed 1 time with 0.1 M PBS pH 7.4 and, the beads were concentrated using magnetic particle concentra-

tors (MPC) (Dynal) for 2 min. Then, each antibody was diluted with 0.1 M PBS pH 7.4 to the appropriate concentration. Each diluted antibody was added to magnetic beads and incubated at 4 °C overnight with gentle rotation. The non-specific binding sites were blocked by BSA at the final concentration 1% (w/v) and incubated at 4 °C overnight with gentle rotation. After this process, the beads were concentrated by MPC and washed twice with 0.1% BSA/0.01 M PBS pH 7.4. Then, the tosyl group was blocked by adding 0.1% (w/v) BSA/0.2 M Tris pH 8.5 and incubated at room temperature overnight. The coated beads were concentrated by MPC and washed once with 0.1% BSA/0.01 M PBS pH 7.4 and stored in 0.1% (w/v) BSA/PBS pH 7.4 at 4 °C.

Detection of microorganism by IMS-ELISA and ELISA

One millilitre of sample was added to 10 µl of immuno-magnetic beads and incubated for 1 h at 37 °C with gentle rotation. MPC was used to separate the complex of coated beads and *Salmonella* from the rest of sample and washed 3 times with 0.1% BSA/PBS pH 7.4. The secondary antibody labelled with horseradish peroxidase (rabbit polyclonal anti-*Salmonella* spp.: Biodesign) was diluted with 0.01 M PBS pH 7.4 and incubated at room temperature with gentle rotation for 1 h. Then, *o*-phenylenediamine; OPD (Dako) was added and incubated at room temperature for 5–30 min until yellow colour was observed. Then, 100 µl 1 N H_2SO_4 was added to stop the colour reaction between the enzyme and the substrate. The absorbance was measured at the wavelength of 450 nm using ELISA plate reader (Molecular Devices, Thermomax). The ELISA method was also performed the same as IMS-ELISA, only without using immuno-magnetic beads to remove the excess of solution.

Determination the specificity of developed mPCR, ELISA and IMS-ELISA

The overnight cultures of various reference strains of *Salmonella* and other bacterial control strains were adjusted to a cell density of 10^8 cfu/ml (OD 600 nm was 0.131 for *Salmonella*) (Pharmacia Biotech, Novaspec II, Sweden). The samples were prepared as described above and were tested by developed mPCR, ELISA, and IMS-ELISA.

Determination the detection limit of developed mPCR, ELISA and IMS-ELISA

The suspension of each strain of *Salmonella* grown overnight at 37 °C in buffered peptone water (BPW) (Difco, USA) was adjusted to the cell density of 10^8 cfu/ml. Each sample was 10-fold diluted to a cell density of 10^8 to 1 cfu/ml. The samples prepared as described above were tested by using developed mPCR, ELISA and IMS-ELISA to determine the sensitivity of each technique.

Detection by immunoassay after enrichment step

To observe the earliest incubation time to detect microorganisms present initially at 1 cfu/ml using the developed immunoassay kits, each sample containing 1 cfu/ml of reference strain of *S*. Typhimurium and *S*. Enteritidis, was incubated in BPW at 37 °C. The samples were prepared as described above and were tested by using developed mPCR and immunoassay at 0, 4, 6, and 8 h.

Determination the contamination in chicken samples

The 44 chicken meat samples obtaining from industrial companies (24 samples) and fresh markets (20 samples) in Thailand were surveyed for *Salmonella* contamination. Two samples from raw meat previously identified by microbiological methods free from *Salmonella* spp. contamination were also used as control. They were spiked with a mixture of *S*. Typhimurium and *S*. Enteritidis at 10^2 cfu/ml before the enrichment step as positive controls for the detection of the targeted micro-organism in meat samples. The identification was performed according to the standard protocol for *Salmonella* detection in meat recommended by the Bacteriological Analytical Manual[7] with some modifications. Briefly, all of the samples were treated as follows: a 25 g of sample was chopped and homogenized with 100 ml of BPW in a stomacher (Stomacher 400, UK) for 30 s and incubated at 37 °C overnight. The samples were prepared by lysing cells according to the previous methods for mPCR and immunoassay. All of the samples were paralleled identified with a commercial ELISA diagnostic kit (TECRA *Salmonella* Visual Immunoassay; 3 M Microbiology, Australia) which was the reference method given in the U.S. Food and Drug Administration's Bacteriological Analytical Manual (BAM 7th Ed.).

RESULTS

Specificity

The developed mPCR, ELISA and IMS-ELISA provided specific detection for *Salmonella* spp., and serovars *S*. Typhimurium and *S*. Enteritidis. The PCR products were obtained clearly distinguished by agarose gel electrophoresis (Fig. 1).

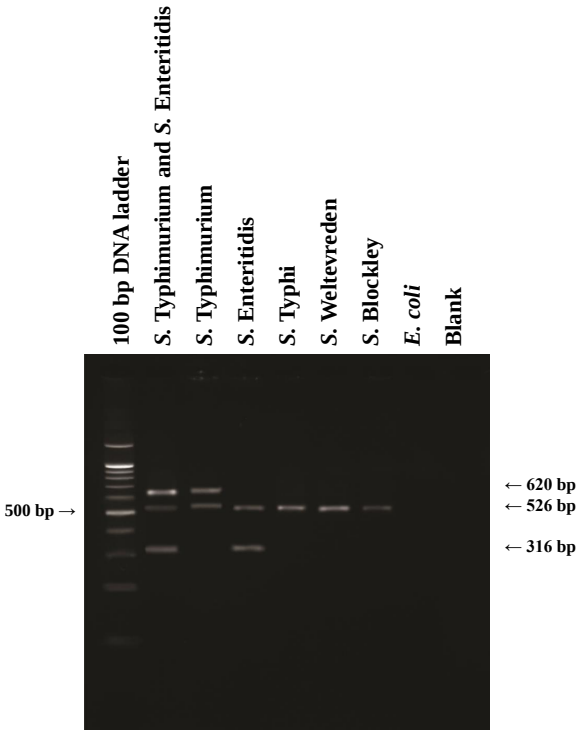

Fig. 1 Specificity of mPCR. PCR reactions were performed with a DNA template from different *Salmonella* serotypes and *E. coli*; each sample contains 10^8 cfu/ml of reference strain microorganism. M: 100 bp DNA ladder; Lane 1: mixed culture of *S*. Typhimurium ATCC13311 and *S*. Enteritidis DMST8536; Lane 2: *S*. Typhimurium ATCC13311; Lane 3: *S*. Enteritidis DMST8536; Lane 4: *S*. Typhi DMST16122; Lane 5: *S*. Weltevreden DMST10637; Lane 6: *S*. Blockley DMST10639; Lane 7: *E. coli* DMST7948 (negative control); and Lane 8: blank.

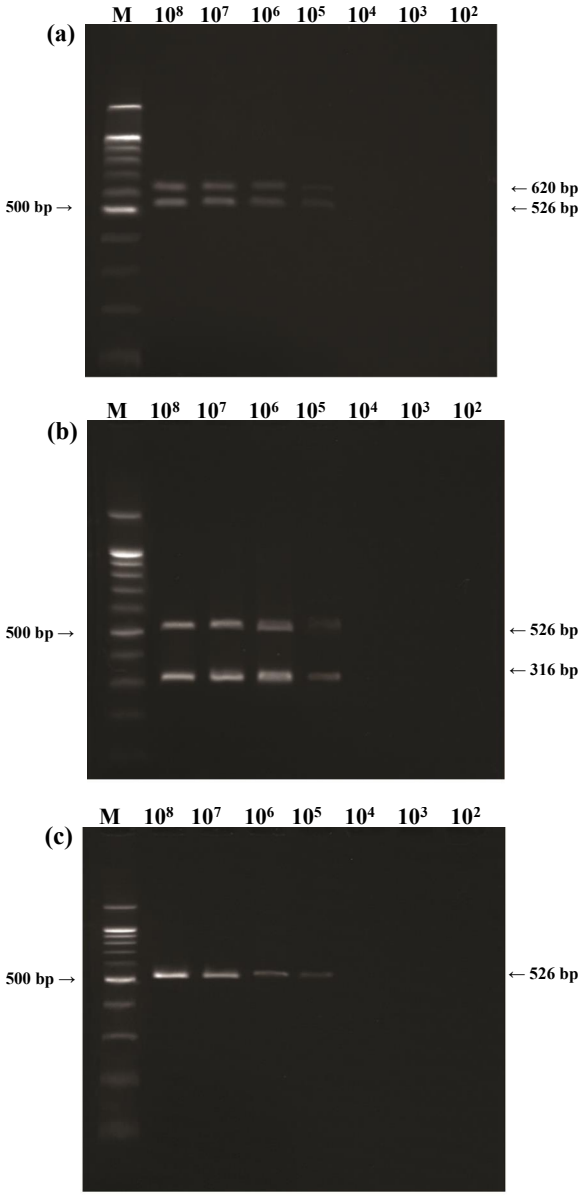

Fig. 2 Sensitivity of each primer pair in mPCR reaction: (a) gel A detected *S*. Typhimurium using Typ04, Fli15 primer, (b) gel B detected *S*. Enteritidis using IE1L, IE1R, and (c) gel C detected *S*. Typhi using *fimY1*, *fimY2*. PCR reactions were performed with each DNA template from serial dilutions at 10^8, 10^7, 10^6,10^5, 10^4, 10^3, and 10^2 cfu/ml without pre-enrichment step.

Detection limit

The detection of the serial dilutions of boiled lysate of *Salmonella* at exact numbers of bacterial cells were performed and subjected to mPCR, ELISA, and IMS-ELISA. The lowest cell density at 10^5 cfu/ml could be detected by the developed mPCR (Fig. 2). Whereas, IMS-ELISA gave the sensitivity at 10^4, 10^4, and 10^7 cfu/ml for detection of *Salmonella* spp., *S*. Typhimurium and *S*. Enteritidis, respectively (Fig. 3) and using ELISA at 10^6 cfu/ml for all *Salmonella* tested.

Detection the contamination in chicken samples

The developed methods were applied to survey the contamination of *Salmonella* spp., *S*. Typhimurium and *S*. Enteritidis in 44 chicken meats sampling from Thai industrial meat products and fresh markets. The mPCR could identify 1 sample as, *S*. Enteritidis, which gave the positive PCR product of *Salmonella* spp. and *S*. Enteritidis. Whereas both developed immunoassay procedures could identify 2 samples contaminated with *Salmonella*. One sample was contaminated with *S*. Enteritidis which was the same

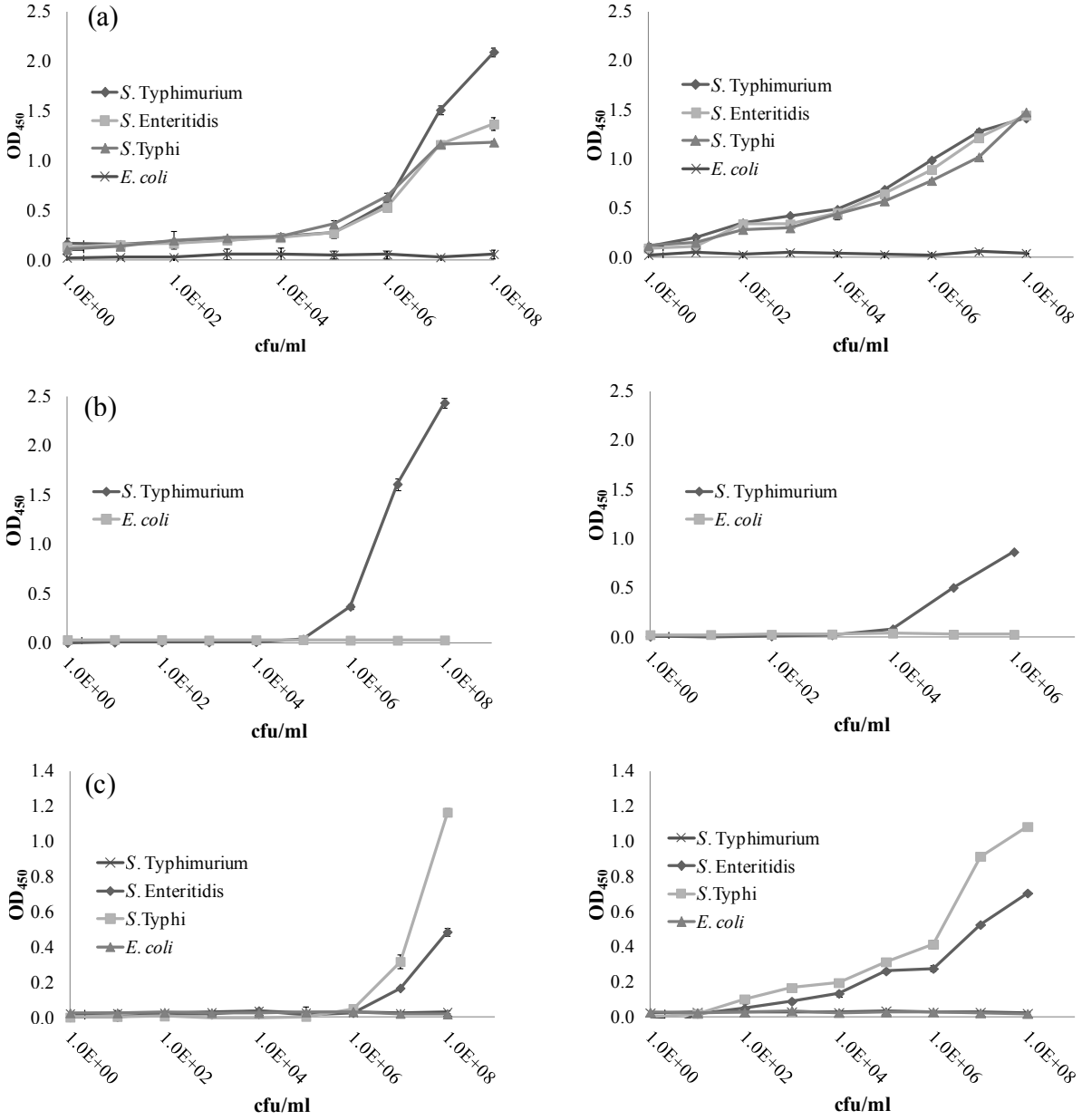

Fig. 3 Sensitivity of the developed immunoassays. Left panels: ELISA method, right panels: IMS-ELISA method. (a) *Salmonella* spp., (b) *S*. Typhimurium, (c) *S*. Enteritidis. All samples were enumerated exactly identified numbers of organisms at various concentrations varying from 1–10^8 cfu/ml and applied to each well at 100 µl.

sample detected by mPCR and other sample was contaminated with *Salmonella* spp. but not serovars *S*. Typhimurium or *S*. Enteritidis. These 2 contaminated samples from 44 samples could be detected by TECRA *Salmonella* Visual Immunoassay, as the same samples as detected by using developed immunoassay. Those two contaminated samples were obtained from fresh markets. Samples from Thai exporting companies were found not contaminated by Salmonellae in our experiment. The results obtained from both methods were compatible.

Detection by immunoassay after enrichment step

The sample initially contained 1 cfu/ml of microorganism were incubated at 37 °C and collected at 0, 4, 6, and 8 h interval. The results showed that the developed immunoassay could begin to identify the positive results at 6 h (Fig. 4).

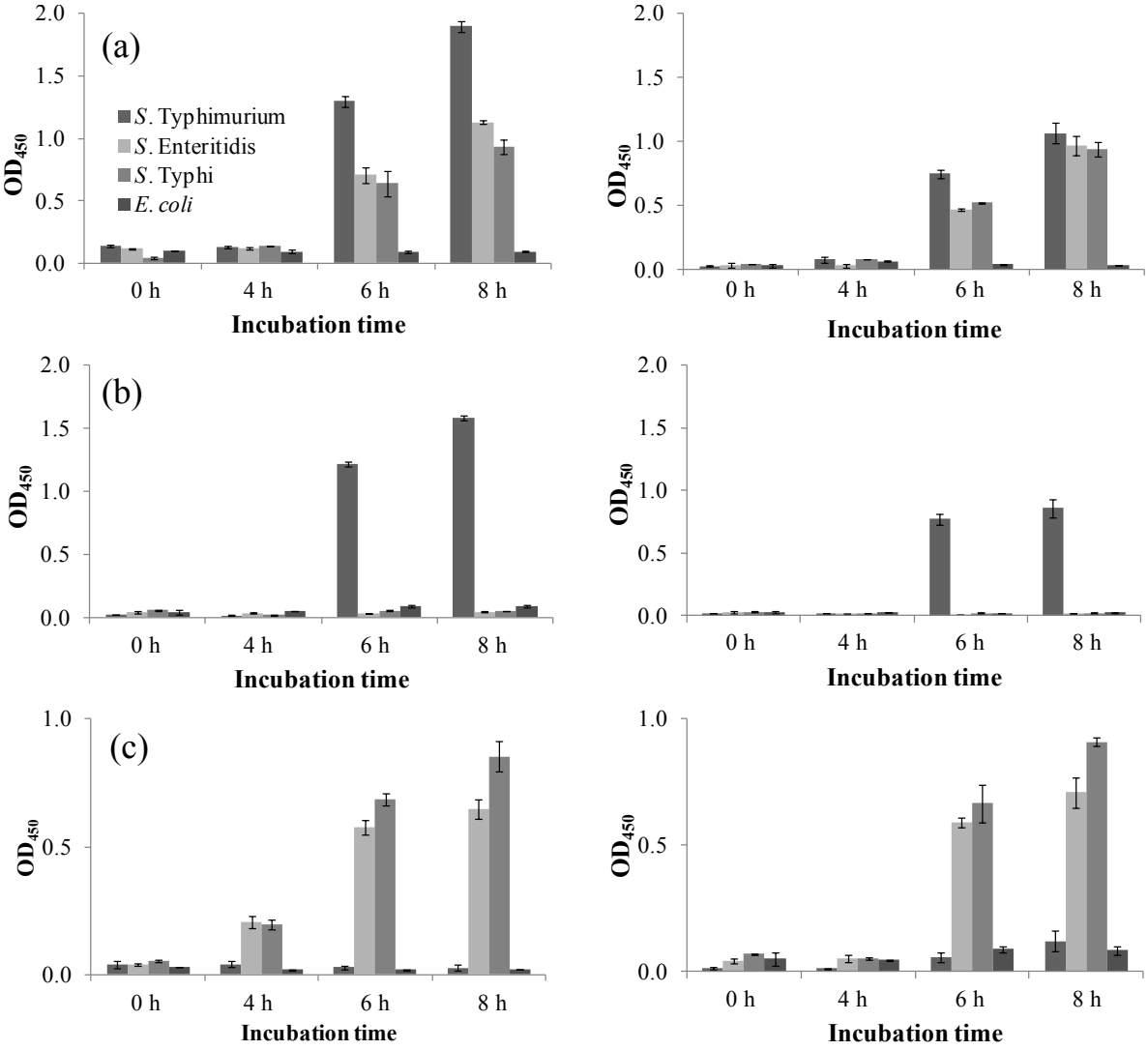

Fig. 4 The detection of *Salmonella* after enrichment of the developed immunoassays. Left panels: ELISA method, right panels: IMS-ELISA method. (a) *Salmonella* spp., (b) *S*. Typhimurium, and (c) *S*. Enteritidis. Sample was inoculated with 1 cfu/ml of *S*. Typhimurium, *S*. Enteritidis, and *S*. Typhi and incubated at 37 °C for 0, 4, 6, and 8 h before testing. Non-inoculated medium was used as a blank and *E. coli* as a negative control.

DISCUSSION

The aim of the present study was to compare the developed mPCR and immunoassay for the detection of *Salmonella* in chicken meat samples. In recent years, mPCR tests for *Salmonella* have been used to identify bacterial contamination in various samples, such as seafood, meat, fresh vegetables, and poultry, from non-selective enrichment with the limit of detection at 10^3 cfu/ml[14,34]. For the ELISA methods, there were available commercial ELISAs for monitoring the infection status of swine, poultry, meat, and other foods for the presence of *Salmonella* spp.[35], with the detection limit of 10^5–10^6 cfu/ml[36]. Several attempts were made to lower the detection limits for *Salmonella* spp. based on ELISA method. The IMS-ELISA aiming to pre-concentrate cells from mixed cultures was reported to have a sensitivity limit of 10^4–10^5 cfu/ml[37].

The mPCR based assay was a rapid method and compatible with most methods used to ensure the safety of food products. Bacterial contaminations could be detected specifically depending on the selected primers and the process of determination was much shorter than standard microbiological methods. To develop the mPCR detection, the choice of the

primer sets and optimization of the mPCR given suitable product sizes that could be separated by agarose gel electrophoresis, were initially performed. The *Tm* of each set of primers had to be considered in developing the system[38] for optimization of mPCR. In this study, we selected 3 sets of primers resulted in 3 bands after amplification.

The mPCR in the study could differentiate *Salmonella* spp., *S*. Typhimurium and *S*. Enteritidis in a single tube reaction. The specific primers for the detection can be easily modified or designed to obtain specific reactivity to the target DNA from *Salmonella* in the sample. However, the suitable results, sensitivity, and specificity from mPCR have to be optimized and determined. For ELISA and IMS-ELISA methods, most of the assays are simple, easy to perform, and give quick results, but attention should be paid to the test quality concerning the characteristics of antibodies used for detection the target antigen of *Salmonella* in the sample. Since more than 2500 serovars of *Salmonella enterica* have been documented, it is at present too difficult to obtain the antibodies without cross reaction with very closely related serovars, even if monoclonal antibodies are used. The developed ELISA and IMS-ELISA in this study could not differentiate between *S*. Enteritidis and *S*. Typhi. Since, these 2 serovars were categorized in *Salmonella* group D, which had the similar O antigen, acted as the target of the antibody using in the immunoassays. However, most of *S*. Enteritidis is found in contaminated in poultry, then possibly transferring the infection to human, whereas *S*. Typhi is the serovar concerning transferring between human cases. The immunoassays require very highly specific and sensitive antibodies to develop the tests. The production and characterization of such antibodies would require substantial effort.

The developed IMS-ELISA gave the higher sensitivity (10^4cfu/ml) to detect target the microorganism than mPCR (10^5 cfu/ml) (Fig. 2 and Fig. 3). Therefore, the sensitivity limit of the developed IMS-ELISA is comparable with that of the commercially-available immunoassays for *Salmonella* spp. of 10^5–10^7 cfu/ml[37]. The IMS-ELISA method uses a specific antibody coated on paramagnetic bead which help the antibody binding to antigen through the sample and to separate and concentrate the antigen by using the magnet, while in the mPCR method directly detection the DNA from the sample without included the concentration step in the technique.

Two specimens from fresh markets were found to be contaminated with *Salmonella* and *S*. Enteritidis by the developed immunoassays whereas mPCR could also identify one same sample contamination with *S*. Enteritidis but could not detect another one sample contamination. Commercial immunoassay kits control were able to identify the two sample contamination the same as our developed immunoassays, but the commercial kit could determine only as *Salmonella* spp. The specimens from industrial companies, however, were free from *Salmonella* contamination by all assays in the study.

The developed immunoassays can detect the contamination starting at 1 cfu/ml after 6 h enrichment. Generation time of *S*. Typhimurium and *S*. Enteritidis were 16.8 and 17.4 min, respectively, (authors, personal communication). Therefore, after 6 h the organism number from 1 cfu/ml can be increased to the level that can detect by the assays (Fig. 4). Although the detection of bacteria in food by the prior enrichment of samples and increasing the time of analysis, but the enrichment brings benefits such as dilution of inhibitors, differentiation between viable and non-viable cells, and repairing of injured bacterial cells[35,36,39–41]. All processes for the developed mPCR, ELISA, and IMS-ELISA identification including pre-enrichment required only 16–24 h, which was less time-consuming than the microbiology-conventional methods. The conventional method needs at least 5–7 days to identify the serovar of *Salmonella* contamination.

We have compared the developed mPCR and immunoassay methods for determining *Salmonella* contamination in chicken. The data showed that the IMS-ELISA have more sensitivity but less specificity than mPCR assay. All the developed techniques are rapid, simple, specific and sensitive than the conventional methods. *Salmonella* serovars can be detected directly from chicken meat samples in Thailand. These mPCR, ELISA and IMS-ELISA assays would offer an effective alternative to conventional identification and differentiation of the *S*. Typhimurium, *S*. Enteritidis, and *Salmonella* spp. contamination. It would be beneficial to apply in industrial and governmental laboratories for food safety control and for consumer protection.

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