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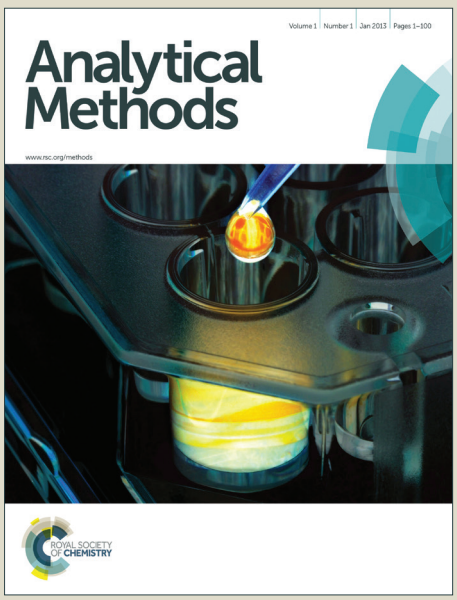

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Monoclonal antibody-based cross-reactive sandwich ELISA for the detection of Salmonella spp. in milk samples

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An immunogen consisting of Salmonella lipopolysaccharide and bovine serum albumin was prepared by periodate oxidation. Mice sera cross-reacted with strains of the genus Salmonella. Monoclonal antibodies (mAbs) against Salmonella lipopolysaccharide core structure were obtained after cell fusion. Based on mAb 6E8, a cross-reactive sandwich enzyme-linked immunosorbent assay was developed. The detection limit of different strains in the genus Salmonella ranged from 1.56×10^6 – 1.25×10^7 colony-forming units/ml. No cross-reactivity was observed with other bacterial strains tested, including *Cronobacter sakazakii*, *Escherichia coli* O6, *E. coli* strain O157:H7, *Campylobacter jejuni* and *Listeria monocytogenes*. Samples of bovine milk spiked with 1 colony-forming unit/ml Salmonella spp. were analysed following enrichment for 24 h.

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Introduction

Salmonella is an important foodborne pathogen worldwide. The presence of Salmonella spp. in foods, e.g. poultry meat, eggs, unpasteurized bovine milk and vegetables, is a significant public health threat[1-3]. Symptoms of salmonellosis include diarrhoea, fever and abdominal cramps[4]. Two species, six subspecies and more than 2500 strains of Salmonella have been identified. Most Salmonella strains are pathogenic to humans[5-8]; however, *S. enterica* serovar Typhimurium and *S. enterica* serotype Enteritidis are commonly involved in salmonellosis[9-12].

Methods for the detection of Salmonella include culture-based methods, enzyme-linked immunosorbent assay (ELISA), polymerase chain reaction (PCR) and biosensors. Culture-based methods, which are considered the gold standard, are laborious and time-consuming[13]. PCR is both accurate and rapid; however, it requires trained personnel[14]. By contrast, ELISA is fast, accurate and user-friendly. Sandwich ELISA has been used in the detection of tumour markers, allergens and pathogens[15-21].

Monoclonal antibodies (mAbs) against Salmonella spp. are of interest because of their wide applications in immunosensors and immunomagnetic separation (IMS) of targets before PCR[16, 22-24]. Tsang and co-authors developed a genus-specific mAb against Salmonella and reported that its epitope was the lipopolysaccharide (LPS) core structure[25, 26]. Antibodies against LPS O-specific chains rather than the LPS core structure, however, tend to be produced with Salmonella bacteria as immunogens. Similar results have been reported with LPS-coated *S. typhimurium* as immunogen; the selected mAb and the sandwich ELISA were very specific to *S. typhimurium*[27]. The LPS core structure is non-repetitive and is buried by the long O-specific chain on the outer side.

In this study, we prepared an effective immunogen for the development of LPS core structure mAbs by conjugating *S. typhimurium* LPS to bovine serum albumin (BSA). The immunogenicity of LPS-BSA conjugates with different degrees of LPS oxidation was evaluated. With the optimal immunogen, mice mAbs specific to the Salmonella LPS core structure were produced by cell fusion technology and a sandwich ELISA for Salmonella spp. was developed, which was evaluated by the analysis of bovine milk samples.

Materials and methods

Salmonella strains and growth conditions

Salmonella enterica serovar Agona (*S. agona*, CICC 21586, serotype O: 4[B]), *S. enterica* serovar Typhimurium (*S. typhimurium*, ATCC 13311, serotype O: 4[B]), *S. enterica* serovar Thompson (*S. thompson*, CICC 21480, serotype O: 7 [C_1]), *S. enterica* serovar Blockley (*S. blockley*, CICC 21489, serotype O: 8 [C_2]), *S. enterica* serovar Kentucky (*S. kentucky*, CICC 21488, serotype O: 8 [C_3]), *S. enterica* serovar Dublin (*S. dublin*, CICC 21497, serotype O: 9 [D_1]), *S. enterica* serovar Anatum (*S. anatum*, CICC 21498, serotype O: 3,10 [E_1]), *Escherichia coli* O157:H7 (*E. coli* O157:H7, CICC 21530), *Staphylococcus aureus* (ATCC 29213), *Listeria monocytogenes* (ATCC 19111) and *Cronobacter sakazakii* (ATCC 29544) were obtained from the Center of Industrial Culture Collection (CICC, Beijing, China). *S. enterica* serovar Typhi (*S. typhi*, CMCC 50071, serotype O: 9 [D_1]), *S. enterica* serovar Paratyphi A (*S. paratyphi A*, CMCC 50093, serotype O: 2 [A]) and *S. enterica* serovar Paratyphi B (*S. paratyphi B*, CMCC 50094, serotype O: 4 [B]) were obtained from the National Center for Medical Culture Collections (CMCC, China). *S. enterica* serovar Arizona (*S. arizona*, ATCC 13314, serogroup IIIa), *E. coli* O6 (ATCC 25922),

S. enterica serovar Enteritidis (*S. enteritidis*, ATCC 13076, serotype O: 9 [D_1]), *S. typhimurium* (ATCC 14028) and *Campylobacter jejuni* (ATCC 49443) were kindly donated by the Hunan Entry-Exit Inspection and Quarantine Bureau, Hunan Province, China. All bacteria were cultured overnight at 37ºC in Brain-Heart Infusion (BHI) broth (Oxoid, Basingstoke, UK).

Synthesis and characterization of LPS-BSA conjugates

Smooth-type LPS from *S. typhimurium* (Sigma, L6511) was conjugated to BSA by periodate oxidation[28]. Briefly, 10 mg of smooth-type LPS was dissolved in 1 ml of ultrapure water. Sodium periodate (150±l ml of 10 mg/ml in ultra-pure water) was added to the LPS solution and allowed to react for 30 min at room temperature. The oxidized LPS solution was added to 10 mg of BSA dissolved in 1 ml of 0.05 M carbonate buffer, pH 9.6, and allowed to react for 12–24 h at room temperature. Then, $NaBH_4$ (200 µl of 5 mg/ml) was added and allowed to react for 2 h at 4ºC. Lastly, the LPS-BSA conjugate was dialysed against 0.01 M phosphate-buffer saline (PBS), pH 7.3. Different LPS/$NaIO_4$ ratios (1.7:1, 5.1:1 and 15.3:1, w/w), were evaluated for optimal conjugation and immune response. The LPS/BSA ratio was 1:1 (w/w) for all conjugates. Before immunization, the conjugates were analysed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE); the upper and lower gel were 5% and 10% (w/v) polyacrylamide, respectively.

Immunization, cell fusion, selection, and characterization of mAbs against Salmonella spp.

The LPS-BSA conjugate was used as an immunogen for the synthesis of LPS mAbs in mice. Briefly, the LPS-BSA conjugate was emulsified with Freund's adjuvant and

100 μg was injected subcutaneously into 6–8 weeks old BALB/c mice. The same mice were injected again at 3 weeks (100 μg) and at 6 weeks (50 μg) later. At 7 days after the last immunization, samples of mouse serum were collected and analysed by indirect ELISA. The coating concentrations of Salmonella spp. and LPS were 5×10^7 colony-forming units (CFU)/ml and 0.3 μg/ml, respectively. The mouse with the highest titer and greatest cross-reaction with Salmonella spp. was sacrificed, spleen cells were collected and fused with Sp2/0 myeloma cells. The fused cells were selected against *S.* serotype Paratyphi A, *S. typhimurium*, *S. thompson*, *S. enteritidis*, *S. anatum*, and *S. arizona* by indirect ELISA using LPS and Ra LPS (Sigma, SL1181). Positive cells with a high level of affinity and homogeneous cross-reactivity were sub-cloned three times by limiting dilution. The cells were injected into the abdominal cavity of mice to produce mAbs. Isotypes of mAbs were identified with an antibody isotyping kit (Envirologix, Portland, ME); the titer and cross-reactivity with Salmonella, LPS, Ra LPS and other bacteria were determined by indirect ELISA.

Development of a cross-reactive sandwich ELISA for Salmonella spp.

The mAbs were conjugated to horseradish peroxidase (HRP) and subjected to pairwise sandwich ELISA[29]. Sandwich ELISA was operated as below. 96-well microplate was added with LPS mAb in coating buffer (100μL/well) and incubated at 4°C overnight. After incubation, the plate was washed three times with washing buffer and blocked with blocking buffer (220μL/well) for 2 h at 37 °C to avoid non-specific binding. After another around of washing, 100 μL of sample was added to each well, and the microplate was sealed and incubated for 1 h at 37 °C. Afterward, the plate was added with 100 μL of HRP-labeled anti-LPS mAb and incubated for another 1 h at 37 °C. After the washing step, 100 μL of TMB substrate solution was

added to each well, and was allowed to react at 37 °C for 15 min in the dark. The reaction was stopped by adding 2 M sulfuric acid (50 μL/well), and the absorbance was measured at 450 nm with a microplate reader. Standard curves of pairs with high positive/negative ratios (P/N) were compared using *S. enteritidis* as the standard for its significance in both food hygiene and clinical research. The pair with the highest level of sensitivity was selected for the development of the sandwich ELISA. Cross-reactivity with Salmonella spp. (*S. agona, S. typhimurium, S. thompson, S. blockley, S. kentucky, S. dublin, S. anatum, S. typhi, S. enteritidis, S. paratyphi A, S. paratyphi B,* and *S. arizona*) and other non-Salmonella strains (*E. coli* O6*, E. coli* O157:H7, *S. aureus, L. monocytogenes*, *C. sakazakii* and *C. jejuni*) was determined. Specifically, Salmonella spp. were diluted to 1×10^8, 5×10^7, 2.5×10^7, 1.25×10^7, 6.3×10^6, 3.2×10^6, 1.6×10^6 and 8×10^5 CFU/ml; non-Salmonella strains were diluted to 1×10^8 CFU/ml in PBS and subjected to the sandwich ELISA. The determination limit was defined as the bacterial concentration with an absorbance (450 nm) 2.1-fold greater compared to the blank.

Milk samples

Bovine milk purchased from a local market was first analysed by plate counting and PCR. Specifically, 10 ml of each milk sample was mixed with 90 ml of buffered peptone water in an Erlenmeyer flask and incubated at 37ºC for 24 h at 120 rpm[30]. After non-selective enrichment for 24 h, 1 ml of each culture was analysed by plate counting and PCR. To evaluate the sandwich ELISA, bovine milk samples free from Salmonella were spiked with 1 CFU/ml of *S. paratyphi A* (serogroup A)*, S. typhimurium* (serogroup B)*, S. thompson* (serogroup C1), *S. enteritidis* (serogroup D1), *S. anatum* (serogroup E1), and *S. arizona* (serogroup IIIa). A non-spiked milk sample

was used as the negative control. Cultures (100 µl) from each sample were collected every 8, 12, and 24 h and subjected to the sandwich ELISA without dilution. Results of the sandwich ELISA was confirmed with plate counting method.

Results and Discussion

Characterization of LPS-BSA conjugates

The LPS-BSA conjugates with different LPS/$NaIO_4$ ratios were analysed by SDS-PAGE. The LPS was partially conjugated to BSA for each of the three conjugates (Figure 1A). Conjugation of LPS to BSA increased, however, when the LPS/$NaIO_4$ ratio was 5.1:1; lower LPS/$NaIO_4$ ratios did not increase the conjugation of LPS to BSA, most likely because LPS was oxidized poorly when higher LPS/$NaIO_4$ ratios (15.3:1) were used. Additionally, the aldehyde group formed on LPS was limiting for conjugation. In contrast, the aldehyde group formed on LPS was optimal for conjugation at lower LPS/$NaIO_4$ ratios. Subsequent characterization of serum samples (Figure 1B) revealed the immune response to Salmonella spp. and Ra LPS with an LPS/$NaIO_4$ ratio of 5.1:1 was significantly stronger compared to a ratio of 1.7:1. This result suggested LPS is over-oxidized with an LPS/$NaIO_4$ ratio of 1.7:1. The optimal LPS-BSA conjugate was obtained with an LPS/$NaIO_4$ ratio of 5.1:1 (w/w) and an LPS/BSA ratio of 1:1 (w/w).

Periodate oxidation has been used in the development of polysaccharide-based vaccines for *Haemophilus influenza* and *Neisseria meningitides*[31, 32]. LPS conjugates such as *S. enteritidis* core O polysaccharide-H:g,m flagellin and *S. paratyphi A* O:2-CRM 197 were reported to be effective vaccines for protection of the corresponding strains[33, 34]. Recently, Pakkanen and co-authors developed typhoid vaccines with oral whole-cell *S. typhi* Ty21a and Vi capsular polysaccharides[35, 36]. The developed vaccines elicit cross-reactive immune responses against *S. paratyphi* A, B and *S.*

enteritidis with O-9 and O-12 antigens. However, Cross-protection against other serogroups of salmonella was ether limited or not tested in these studies. Actually, Salmonella LPS conjugates have been seldom studied for the production of cross-reactive antibodies against Salmonella spp. In this study, the prepared LPS conjugate with controlled oxidation induced cross-reactive antibodies against Salmonella spp. in mice, which could contribute to optimal conjugation with little denaturation of the Salmonella core structure.

Monoclonal antibodies against Salmonella

Eleven stable cell lines against Salmonella LPS were obtained and the cross-reactivity of mAbs was characterized with indirect ELISA. Titer of the mAbs against different tested strains and LPS antigens and affinity against *S. enteritidis* was presented in Table 1. Affinity of mAb was characterized by measuring the equilibrium dissociation constant (Kd) of mAb with an ELISA method as reported by Friguet and co-authors [37]. The mAbs cross-reacted to different degrees with *S. paratyphi A, S. typhimurium, S. thompson*, *S. enteritidis*, *S. anatum* and *S. arizona*. Furthermore, there was strong reactivity between LPS and Ra-LPS (having the complete core oligosaccharide but without the O-specific chains) from *S. typhimurium* but not with other Gram-negative bacteria tested, including *E. coli* O6, *E. coli* O157:H7 or *C. sakazakii,* which share common inner core oligosaccharides, but not the LPS outer core structure. This result showed these antibodies recognize outer core structures of Salmonella LPS. The lowest Kd value at pM level was from mAb 8G7, followed by 1C6 and 6E8, which have Kd value at nM level. The lower Kd value of these mAbs means they have higher affinity against salmonella. Interestingly, mAbs with high affinity towards Ra-LPS, such as 8G7, had a stronger cross-reactivity with Salmonella spp., indicating the

degree of cross-reactivity with Salmonella spp. is highly dependent on the reactivity with the LPS outer core structure.

Cross-reactive mAbs of Salmonella spp. have been produced to neutralize endotoxins during infections or serological diagnoses[38, 39]. Franco and co-authors used a mixture of heat-killed *E. coli* O6 and *S. minnesota* R60 as immunogens to develop mAb WN1 222-5, which is cross-reactive with Salmonella spp. and *E. coli*[40]. The epitope of this mAb was the distal part of the inner core region of LPS (Re mutant), which is shared by the family Enterobacteriaceae[41]. The 202D7 mAb with the same epitope and with heat-killed *S. minnesota* 8595 of the Re chemotype as immunogen was reported by Haralambieva[42]. This mAb cross-reacted with *Chlamydia trachomatis* and Salmonella spp. as well as and several S- and R-LPS antigens of other Gram-negative bacteria. Tsang and co-authors used acetone-fixed *S. typhi* 620Ty to develop mAb T6, which cross-reacts specifically with Salmonella spp.[26]. The epitope of this mAb had a complete Ra core structure of Salmonella LPS, which was confirmed by Nnalue, who reported alpha-GlcNAc-1→2-alpha-Glc was a conserved LPS motif of Salmonella spp.[43] In this study, LPS-BSA conjugates were prepared as immunogens; mAbs specific to the Salmonella LPS outer core structure were produced with a high level of affinity and broad cross-reactivity.

Development of a specific sandwich ELISA for Salmonella

Pairwise study of the selected mAbs (Table 2) showed all conjugated LPS mAb-HRP can be paired with the other LPS mAbs; 6E8 HRP paired successfully with itself because abundant LPSs were distributed on the cell surface of Salmonella spp. Higher P/N ratios were observed and confirmed, however, when 2F7HRP and 6E8HRP were paired with 6E8. The pair 6E8–6E8HRP had greater sensitivity and more

homogeneous cross-reactivity with Salmonella spp. compared to the pair 6E8–2F7HRP, probably because 6E8 has a higher titer against Salmonella compared to 2F7.

A cross-reactive sandwich ELISA based upon the mAb pair 6E8–6E8 was developed for Salmonella spp. The detection limits (P/N ≥2.1) of the sandwich ELISA for *S. thompson* and *S. enteritidis* were 3.2×10^6 CFU/ml and 6.12×10^6 CFU/ml, the detection range was 3.2×10^6 – 1×10^8 CFU/ml and 6.12×10^6 CFU/ml – 1×10^8 CFU/ml. The standard curves for *S. thompson* and *S. enteritidis* were shown in Figure 2A. Nonoverlapping of the standard curves between different strains of salmonella make this ELISA method more suitable for qualitative detection than quantitative detection of salmonella. Sandwich ELISA cross-reacted broadly with Salmonella spp., including *S. paratyphi A* (detection limit 1.56×10^6 CFU/ml), *S. agona* (3.13×10^6 CFU/ml), *S. typhimurium* (3.13×10^6 CFU/ml), *S. paratyphi B* (6.12×10^6 CFU/ml), *S. blockley* (6.12×10^6 CFU/ml), *S. kentucky* (6.12×10^6 CFU/ml), *S. dublin* (1.25×10^7 CFU/ml), *S. typhi* (1.25×10^7 CFU/ml), *S. anatum* (6.12×10^6 CFU/ml) in *S. enterica* subsp. *enterica* and *S. arizona* (1.25×10^7 CFU/ml) in *S. enterica* subsp. *arizonae*. In addition, no cross-reactivity was observed with *C. sakazakii*, *E. coli* O6, *E. coli* O157:H7, *C. jejuni* or *L. monocytogenes* (Figure 2B). An insignificant cross-reaction with *S. aureus* might reflect the presence of protein A on the cell surface[44].

Several sandwich ELISAs have been developed to detect Salmonella spp. Most of these assays are specific to one serotype or one serogroup of Salmonella[45-47]. Linh Thuoc Tran and co-authors developed an ELISA to detect Salmonella spp. in foods using recombinant H antigen[48]. The method was highly specific in food samples (99% of 60 strains positive); however, the sensitivity was not reported. L. Croci and co-author developed a sandwich ELISA for the detection of Salmonella spp. in meat

using a commercial mAb as the capture antibody and a polyclonal antibody (pAb) as the detection antibody. *Salmonella enteritidis*, *S. derby* and *S. typhimurium* were detected in different food samples (5×10^3 CFU/ml). The sandwich ELISA was electrochemically based, and a pAb with a multi-binding epitope was used. Compared to mAb, however, pAb differs among batches and the quality is not stable. Sandwich ELISA based on T6 mAb specific for the Salmonella Ra core structure is broadly cross-reactive with Salmonella spp.[49] The detection limit and cross-reactivity of the sandwich ELISA we developed are comparable with those of the T6-based sandwich ELISA. The sandwich ELISA was not very sensitive, which might be because the surface availability of the core structure was limited; however, mAbs of high affinity are promising for improving the sensitivity of sandwich ELISA. Our future studies will select mAbs of high affinity against Salmonella LPS outer core structure.

Milk samples

Bovine milk samples were confirmed to be free of Salmonella spp. by plate counting and PCR. Milk samples spiked with 1 CFU/ml Salmonella spp. from different serogroups (A, B, C, D, E and IIIa) were enriched and analysed by the sandwich ELISA we developed. Table 3 shows bovine milk samples with 1 CFU/ml *S. paratyphi* A (serogroup A), *S. typhimurium* (serogroup B), *S. thompson* (serogroup C1), *S. enteritidis* (serogroup D1) and *S. anatum* (serogroup E1) were detected (P/N ≥ 2.1) following enrichment for 12 h, and *S. arizona* (serogroup IIIa) was detected following enrichment for 24 h. These results might reflect a relatively weaker affinity of mAbs against serogroups D, E and IIIa. By contrast, control milk (i.e. not spiked) was negative for Salmonella spp. even after enrichment for 24 h. Results obtained by the developed sandwich ELISA were comparable with that of plate counting method,

which showed that the developed sandwich ELISA was accurate to detect salmonella. In short, these results show that low levels of Salmonella spp. (1 CFU/ml) can be detected in milk samples following enrichment for 24 h by the sandwich ELISA we developed.

Conclusion

In this study, Salmonella LPS complete antigen (LPS-BSA) was prepared with periodate oxidation and used as an immunogen for the production of LPS mAbs in mice. The LPS-BSA conjugate with an LPS/$NaIO_4$ ratio of 5.1:1 (w/w) and an LPS/BSA ratio of 1:1 (w/w) induced antibodies against the Salmonella LPS core structure and cross-reacted with strains of different O antigen groups in the genus Salmonella. Subsequently, mAbs against the Salmonella LPS core structure were obtained following cell fusion and selection. A cross-reactive sandwich ELISA was developed based upon mAb 6E8. The detection limit for different strains in the genus Salmonella ranged from 1.56×10^6 – 1.25×10^7 CFU/ml. No cross-reaction was observed with other bacteria including *C. sakazakii*, *E. coli* O6, *E. coli* O157:H7, *C. jejuni* and *L. monocytogenes*. Salmonella was detected in milk samples spiked with 1 CFU/ml following enrichment for 24 h. The Salmonella LPS complete antigen was effective for the production of cross-reactive antibodies against Salmonella spp. The sandwich ELISA based on mAb 6E8 we developed is promising as a simple and accurate method for the detection of Salmonella spp. in food.

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Captions:

Fig. 1 (A) SDS-PAGE of LPS-BSA conjugates. (B) Cross-reaction of the sera from LPS-conjugates.

Fig. 2 (A) Standard curve of the developed sandwich ELISA for salmonella (*S. Thompson* and *S. enteritidis*). (B) Cross-reactivity of developed sandwich ELISA for salmonella.

Table 1 Cross-reactivity and affinity of the 11 selected Ra LPS monoclonal antibodies.

Table 2 Pairwise study of 10 selected salmonella Ra LPS monoclonal antibodies.

Table 3 Detection of salmonella in spiked milk sample with ELISA (A450nm) and plate counting (1 CFU/mL) after enrichment.

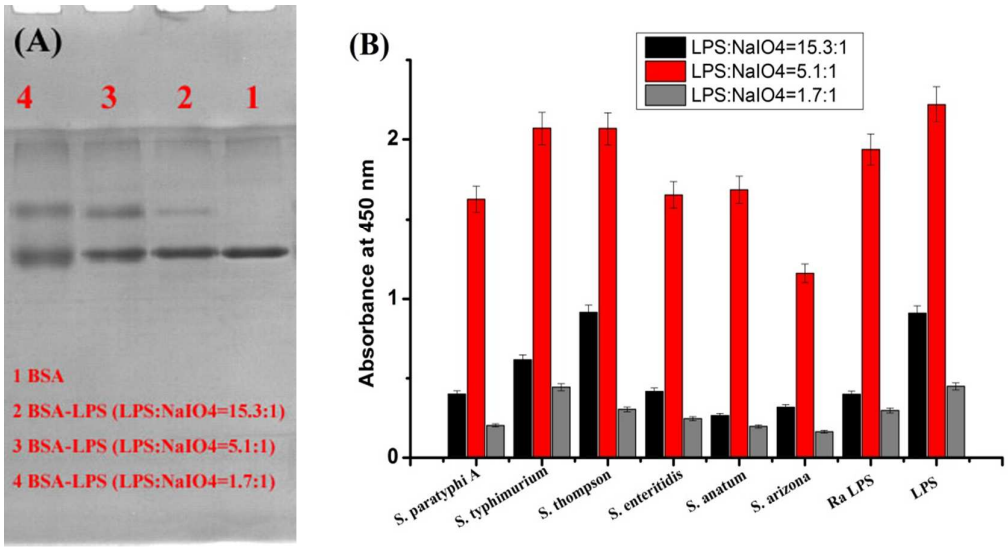

Fig. 1 (A) SDS-PAGE of LPS-BSA conjugates. Line 1: BSA, line 2: BSA-LPS with LPS to $NaIO_4$ ratio of 15.3:1(w/w), line 3: BSA-LPS with LPS to $NaIO_4$ ratio of 5.1:1(w/w), line4: BSA-LPS with LPS to $NaIO_4$ ratio of 1.7:1(w/w). (B) Cross-reaction of the sera from LPS-conjugates. Sera were all dilluted 3000 times with antibody dillution buffer.

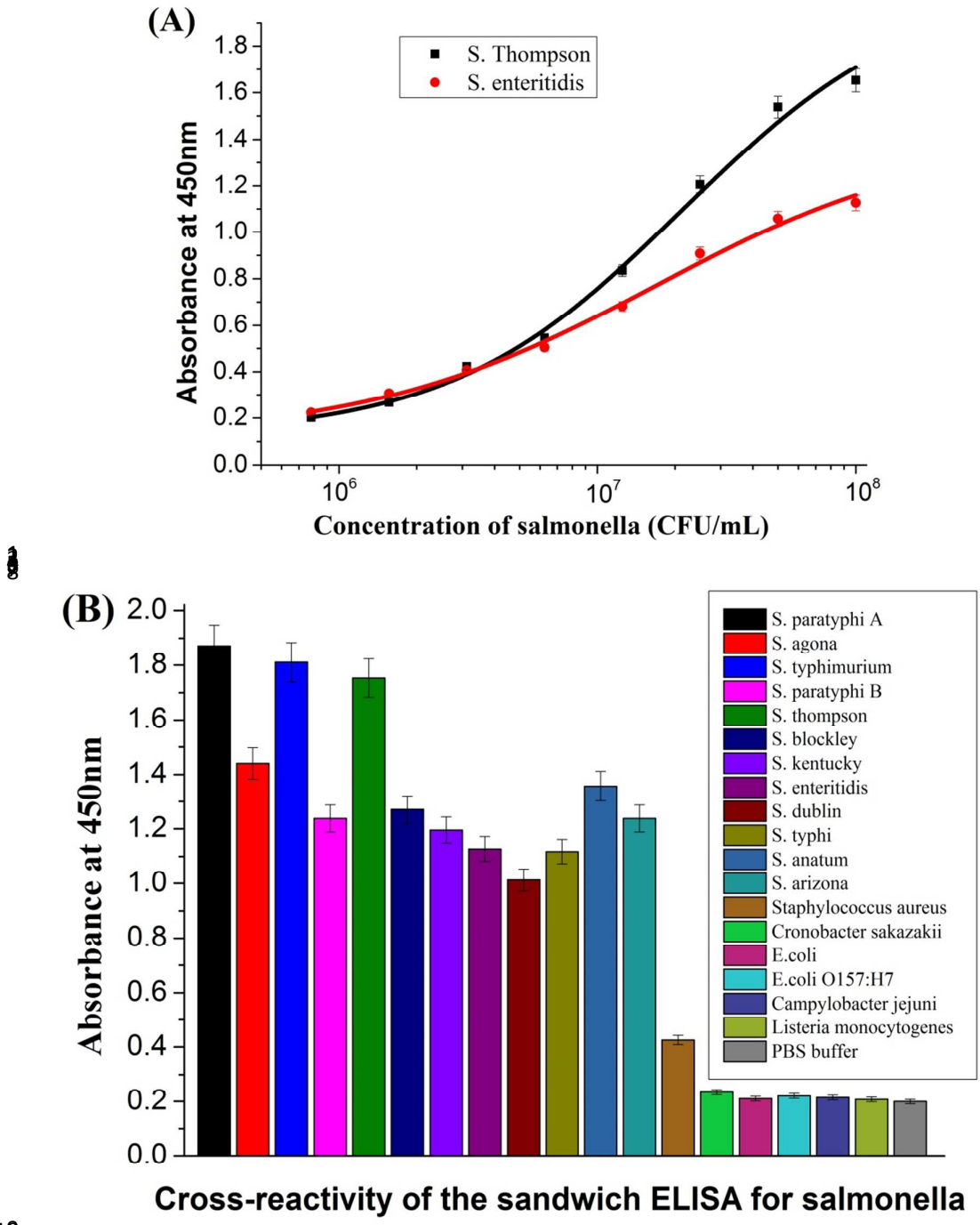

Fig. 2 (A) Standard curve of the developed sandwich ELISA for salmonella (*S. Thompson* and *S. enteritidis*). (B) Cross-reactivity of developed sandwich ELISA for salmonella. All the tested strains were diluted at 10^8 CFU/mL in 0.01M PBS.

Table 1 Cross-reactivity and affinity (Kd) of the 11 selected Ra LPS monoclonal antibodies. Coating concentration of bacteria and LPS was 5 × 10^7CFU/mL and 0.3ug/mL, respectively. 'k' in the table means the dilution fold (1000) of the mAb. Kd means the equilibrium dissociation constant of the mAb against *S. enteritidis*.

	1C6	2F7	3B9	3F5	4D7	5F11	6E8	7F2	8G3	8G7	10F11
S. paratyphi A(A)	243k	27k	27k	9K	81k	27k	81k	81k	81k	243k	27k
S.typhimurium(B)	243k	81k	27k	27k	243k	27k	243k	243k	243k	243k	27k
S. Thompson(C1)	729k	243k	27k	243k	243k	81k	729k	729k	243k	243k	81k
S. enteritidis(D1)	243k	27k	27k	27k	81k	27k	243k	243k	243k	729k	27k
S. anatum (E1)	81k	27k	27k	27k	81k	27k	81k	81k	243k	243k	27k
S. Arizona	243k	27k	9k	27k	81k	27k	81k	243k	243k	243k	27k
LPS	729k	81k	27k	27k	243k	81k	243k	243k	243k	729k	81k
RaLPS	243k	27k	9k	9k	81k	27k	81k	81k	243k	243k	27k
E. coli	-	-	-	-	-	-	-	-	-	-	-
E. coli O157	-	-	-	-	-	-	-	-	-	-	-
Cronobacter sakazakii	-	-	-	-	-	-	-	-	-	-	-
Kd	1.15 × 10^{-9}	24.0 × 10^{-9}	46.73 × 10^{-9}	11.76 × 10^{-9}	3.38 × 10^{-9}	18.98× 10^{-9}	1.62 × 10^{-9}	2.66 × 10^{-9}	1.72× 10^{-9}	0.89× 10^{-9}	12.05× 10^{-9}

Table 2 Pairwise study of 10 selected salmonella Ra LPS monoclonal antibodies. Concentration of coating antibody and *S. enteritidis* was 4ug/mL and 5×10^7CFU/mL, respectively. The data was the ratio of positive/negative.

	1C6	3B9	3F5	4D7	5F11	6E8	7F2	8G3	8G7	10F11
2F7-HRP	7.6	7.2	5.8	4.9	7.7	**8.1**	6.8	7.3	6.8	6.4
3B9-HRP	4.8	4.7	4.3	3.6	4.2	4.4	3.8	4.7	4.2	4.1
3F5-HRP	3.5	3.5	3.3	3.6	3.4	5.0	3.4	3.6	2.8	3.2
4D7-HRP	4.4	5.2	3.8	4.6	4.5	5.0	4.1	4.5	4.0	4.9
6E8-HRP	7.0	6.6	5.7	6.5	7.2	**8.6**	6.7	5.3	5.6	5.5
7F2-HRP	4.3	4.0	4.0	3.4	3.4	3.9	3.4	3.3	3.1	3.5
8G7-HRP	5.2	5.3	5.5	4.3	5.2	5.2	4.7	5.1	5.1	5.3
10F11-HRP	5.5	5.5	5.0	4.6	4.3	5.7	4.4	4.8	4.7	3.7

Table 3 Detection of salmonella in spiked milk sample with ELISA (A450nm) and plate counting (1 CFU/mL) after enrichment. Data was average of three parallel tests ± SD.

	S. paratyphi A (A)		*S. typhimurium* (B)		*S. Thompson* (C1)		*S. enteritidis* (D1)		*S. anatum* (E1)		*S. Arizona* (IIIa)		control	
	ELISA	Plate counting	ELISA	Plate counting	ELISA	Plate counting	ELISA	Plate counting	ELISA	Plate counting	ELISA	Plate counting	ELISA	Plate counting
8 hours	0.232 ± 0.012	(3.61 ± 0.12) $\times 10^5$	0.244 ± 0.011	(3.10 ± 0.13) $\times 10^5$	0.238 ± 0.010	(3.48 ± 0.16) $\times 10^5$	0.212 ± 0.011	(3.31 ± 0.12) $\times 10^5$	0.202 ± 0.013	(3.42 ± 0.11) $\times 10^5$	0.189 ± 0.014	(3.52 ± 0.13) $\times 10^5$	0.186 ± 0.010	0 ± 0
12 hours	0.602 ± 0.014	(3.32 ± 0.13) $\times 10^6$	0.641 ± 0.018	(6.54 ± 0.11) $\times 10^6$	0.582 ± 0.016	(4.62 ± 0.13) $\times 10^6$	0.421 ± 0.014	(3.56 ± 0.18) $\times 10^6$	0.448 ± 0.015	(5.62 ± 0.15) $\times 10^6$	0.306 ± 0.012	(5.47 ± 0.12) $\times 10^6$	0.191 ± 0.011	0 ± 0
24 hours	2.148 ± 0.121	(5.68 ± 0.11) $\times 10^8$	2.204 ± 0.122	(8.6 ± 0.16) $\times 10^8$	2.046 ± 0.051	(6.68 ± 0.11) $\times 10^8$	1.532 ± 0.031	(5.92 ± 0.12) $\times 10^8$	1.678 ± 0.028	(6.80 ± 0.11) $\times 10^8$	1.307 ± 0.025	(6.67 ± 0.16) $\times 10^8$	0.202 ± 0.012	0 ± 0