Magnetic relaxation switch immunosensor for the rapid detection of the foodborne pathogen *Salmonella enterica* in milk samplesSongbai Wang^a, Yan Zhang^a, Wenting An^a, Yanli Wei^a, Nan Liu^{b,*}, YipingChen^{b,*}, Shaomin Shuang^{a,*}

a Department of Chemistry and Chemical Engineering, Research Center of Environmental Science and Engineering, Shanxi University, Taiyuan 030006, People's Republic of China

^b *Tianjin Key Laboratory of Risk Assessment and Control Technology for Environment and Food Safety, Tianjin Institute of Health and Environmental Medicine, Tianjin 300050, People's Republic of China*

Corresponding Author. Tel: +86 2284655060, +86 2284655060, +86 3517018842.

E-mail: LNQ555@126.com, cyp89@126.com, smsluang@sxu.edu.cn

Abstract

We present a rapid and sensitive magnetic relaxation switch (MRS) immunosensor based on superparamagnetic nanoparticles (SMNs) for detecting the foodborne pathogen *Salmonella enterica* in milk samples. The MRS immunosensor employed target-induced aggregation (or disaggregation) of SMNs in immunoreaction, which could modulate the spin–spin relaxation time of the neighboring water molecule. Results showed that the linear range of the MRS immunosensor for detecting *S. enterica* was 5×10^3 – 10^6 CFU/mL, and that the relative standard deviation was below 13.5%. The detection limit of the sensor was 10^3 CFU/mL, which was 20 times lower than that of enzyme-linked immunosorbent assay (ELISA, 2×10^4 CFU/mL). The analysis time of the sensor (30 min) was also shorter than that of ELISA (2–4 h). This sensor was successfully applied to detect *S. enterica* in 20 real milk samples, and its accuracy was better than that of ELISA. Therefore, the MRS immunosensor provides a promising platform for the determination of foodborne pathogenic bacteria because of its high sensitivity, simplicity, rapidness, and low cost.

Keywords: Magnetic relaxation switch; Superparamagnetic nanoparticle; *Salmonella enterica*

1. Introduction

Salmonella enterica is a common foodborne pathogen that causes food poisoning worldwide (Cavallaro et al., 2011). *Salmonella* is a leading cause of gastroenteritis in the United States, and the incidence of this disease has slightly changed in the past decade (Matyas et al., 2010). In another report, an estimate of 1 million people suffered from non-typhoidal *Salmonella* infections; 19,000 hospitalized; and 370 died each year in the United States (Scallan et al., 2011). Invasive strains of *Salmonellae* have emerged as a prominent cause of bloodstream infection in African adults and children, with an associated fatality of 20–25% (Feasey, Dougan, Kingsley, Heyderman, & Gordon, 2012).

The development of a point-of-care testing method for detecting *S. enterica* in food samples is highly important to prevent the outbreak of diseases. Many methods have been developed to detect *S. enterica*; these methods include polymerase chain reaction (PCR) (Hein, Flekna, Krassnig, & Wagner, 2006; Perelle et al., 2004; Riyaz-Ul-Hassan, Verma, & Qazi, 2013), enzyme-linked immunosorbent assay (ELISA) (Durant, Young, Nisbet, Stanker, & Ricke, 1997; Galikowska et al., 2011; Mansfield & Forsythe, 2000), gold lateral flow strip (GLFS) (Urata, Iwata, Noda, Murakami, & Kuroda, 2009), and surface plasmon resonance (SPR) (Son, Kim, Kothapalli, Morgan, & Ess, 2007). PCR is highly sensitive, but it requires precise and expensive instruments. ELISA has been widely used to detect pathogens because of its specificity, sensitivity, and low cost. However, ELISA is usually labor intensive and time consuming because it is a heterogeneous mode that needs numerous washing steps. GLFS has been extensively used in pathogen detection because of its simplicity, speediness, and low cost. However, the sensitivity of GLFS is inferior to that of ELISA, which limits its further application. SPR is a label-free biosensor for detecting

pathogens, but its application is hindered by its poor sensitivity and high cost. Therefore, developing an accurate, simple, rapid, sensitive, and robust method to detect *S. enterica* in food samples is important.

Magnetic relaxation switch (MRS) assay based on superparamagnetic nanoparticles (SMNs) has been the focus of increasing attention in biochemical analysis because of its sensitivity, rapidness, and simplicity (Perez, Josephson, O'Loughlin, Hogemann, & Weissleder, 2002; Perez, Simeone, Saeki, Josephson, & Weissleder, 2003; Peterson, Castro, Lee, & Weissleder, 2012). In MRS assay, the SMN–antibody conjugate (SMN–Ab) can switch their dispersed and aggregated state in the presence of the target. This state of change can modulate the spin–spin relaxation time (T_2) of the neighboring water molecule. The change in T_2 (ΔT_2) positively correlates with the amount of the target in the sample (Perez et al., 2002; Perez et al., 2003). Thus, ΔT_2 can be used as a detection signal in MRS immunosensors. Compared with conventional methods, MRS assay is independent of light and is thus suitable to detect epinephelos in biological or food samples without the need for complex pretreatment (Min et al., 2012; Tassa, Shaw, & Weissleder, 2011). It has been used as a powerful tool to identify and quantify a wide range of targets, including circulating tumor cells (Haun, Devaraj, Hilderbrand, Lee, & Weissleder, 2010; Lee, Sun, Ham, & Weissleder, 2008; Ullal et al., 2011), pathogenic bacteria (Chen et al., 2013; Lee, Yoon, & Weissleder, 2009; Liong, Fernandez-Suarez, et al., 2011), viruses (Koh, Hong, Weissleder, & Josephson, 2008), biomarkers (Ling, Pong, Vassiliou, Huang, & Cima, 2011; Liu et al., 2012), DNA (Liong, Tassa, Shaw, Lee, & Weissleder, 2011), and small molecules (Ma et al., 2009; Xu et al., 2012).

In this work, we present a simple, rapid, sensitive, and homogeneous MRS immunosensor for the one-step determination of *S. enterica* in milk samples (Fig.1).

The sizes and concentrations of the SMN–Ab were optimized to improve the property of the MRS immunosensor. The efficiency of the MRS immunosensor for *S. enterica* detection was compared with that of ELISA. To the best of our knowledge, this is a first report on application of MRS immunosensor for detecting *Salmonella enterica* from foods.

2. Materials and Methods

2.1 Apparatus

A 1.5 T nuclear magnetic resonance (NMR) spectrometer for the measurement of T_2 was provided by Shanghai Shinning Globe Science and Education Equipment Co., Ltd. (Shanghai, China). An orbital shaker (IKA Inc., Germany) was employed to mix the samples and SMN solution. A SuperMag SeparatorTM was purchased from [Ocean NanoTech \(USA\)](#). A microplate reader (Tecan Safire2, Switzerland) was used to measure the absorbance at 450 nm in ELISA.

2.2 Chemicals

SMNs (i.e., carboxyl group-functionalized magnetic nanoparticles) 20, 30, and 50 nm in diameter were purchased from [Ocean NanoTech \(USA\)](#). Bovine serum albumin (BSA), 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC), and hydroxy-2, 5-dioxopyrrolidine-3-sulfonicacid sodium salt (sulfo-NHS) were purchased from Sigma–Aldrich (USA). *S. enterica* monoclonal antibody (Ab1, 1.5 mg/mL) was purchased from Genway (San Diego, California, USA). Horseradish peroxidase (HRP)-labeled secondary antibody was purchased from Jackson Immuno Research Inc. (USA). The 96-well cell culture [plate](#) and 96-well ELISA [plate](#) were supplied by [Costar[®] \(Coring Inc., NY, USA\)](#). Common reagents and solvents were of analytical grade and purchased from Beijing Chemical Reagents Co. (Beijing, China).

Water was deionized and ultrafiltered using a Milli-Q apparatus. All other chemicals were of analytical reagent grade.

2.3 Microorganism

The microorganisms used in this study, including *Escherichia coli* (ATCC 25922), *S. enterica* (ATCC 14028), *Shigella* spp. (ATCC 12022), *Staphylococcus aureus* (ATCC 27217), and *Spirillum of Cholera* (ATCC 13312), were obtained from the American Type Cell Collection (ATCC). All strains were cultured and maintained in 500 mL of nutrient broth at 28 °C with shaking for 48 h, harvested by centrifugation (5000 rpm, 20 min), and then stored in 50 mL of sterilized phosphate-buffered saline (PBS, 10 mM phosphate and 150 mM NaCl). The number of bacterial colonies in the cultures was counted using the plate count method. The bacterial concentration in these stock solutions was determined in triplicate by enumeration on standard plate count agar after incubation at 37 °C for 24 h. For the next experiment, PBS solution was used to adjust bacterial concentration.

2.4 Preparation of SMN-Ab1

SMN-Ab1 was prepared in accordance with our previous method with some modifications (Chen et al., 2013). In 1.5 mL tube, add 100 µL 0.01 M MES (pH 5.2) to 200 µL SMNs. Upon mixing add 20 µL EDC (10 mg/ml), and 10 µL sulfo-NHS (10 mg/mL). For activation incubate the mixture for 20 min at room temperature (RT), add 500 µL 0.01 M PBS, 0.1 mg Ab1 and mixture was gently stirred to react for 2 h at RT. 200 µL 3% BSA solution was added and stirred gently for additional 30 min. Free/unconjugated Ab1 were removed using SuperMag Separator™. Finally, the SMNs-Ab1 bioconjugate was redissolved in PBS containing 0.1% BSA at 4 °C.

2.5 Procedure of MRS immunosensor

The total viable bacteria (CFU/mL) were determined by the plate count method as

Subsection 2.3 described. And then PBS solution was used to adjust bacterial concentration for MRS experiment. The 100 μL *S. enterica* of varying cell density (10^7 , 10^6 , 10^5 , 5×10^4 , 10^4 , 5×10^3 , 10^3 , 5×10^2 , 10^2 or 0 CFU/mL) was placed in separate wells of 96-well cell culture plate and mixed with 100 μL SMN-Ab1 solution. The mixture was gently shaken in an orbital shaker (37 °C, 150 rpm) for 20 min. Subsequently, 180 μL of the reacted solution was transferred into a 7.5 mm NMR tube to measure T_2 . Each point was assayed thrice ($n = 3$). T_2 was acquired at a temperature of 35 °C using a 1.5 T ^1H -NMR spectrometer at the 59.095 MHz magnetic field and the following parameters: Carr–Purcell–Meiboom–Gill pulse sequence, 2000 echoes, 3 ms echo time, and 4 s repetition time.

2.6 Optimization of immunological reaction

Experimental factors, such as the dilution ratios of SMN–Ab1 and the sizes of SMNs, were optimized to improve the sensitivity and stability of the MRS immunosensor. In this experiment, three dilution ratios of SMN–Ab1 (1:20, 1:50, and 1:100) and three sizes of SMNs (20, 30, and 50 nm) were selected. The following experimental process is the same as that in Subsection 2.5.

2.7 Sensitivity and specificity of the MRS immunosensor

On the basis of the aforementioned optimized concentrations, different concentrations of *S. enterica* (10^7 , 10^6 , 10^5 , 5×10^4 , 10^4 , 5×10^3 , 10^3 , 5×10^2 , 10^2 , and 0 CFU/mL) were used as analytes to obtain optimal sensitivity. The limit of detection (LOD) was determined as the mean background levels plus three times of the standard deviation. Calculations were performed using OriginPro8.0 software. Unknown samples containing *S. enterica* were calculated on the basis of the calibration curve.

For specificity of MRS immunosensor, five bacterial strains (5×10^4 CFU/mL)

as mentioned as the control group in Subsection 2.3 and were assayed separately. In the control group, 5×10^4 CFU/mL of *S. enterica* was mixed with the unconjugated SMNs.

2.8 Sandwich ELISA

ELISA for *S. enterica* detection was performed in accordance with previous work with some modifications (Abdel-Hamid, Ivnitski, Atanasov, & Wilkins, 1999). A 96-well ELISA plate was coated overnight at 4 °C with 100 µL/well of a capture antibody diluted in 50 mM sodium carbonate–bicarbonate buffer (pH 9.6). The plates were washed thrice with 200 µL of PBST and then blocked with 100 µL of 1% BSA in PBST for 1.5 h at RT. The plates were washed again as mentioned above, added with the pathogens (100 µL/well) at the designated concentration in 1% BSA in PBST, and then incubated for 1 h at RT. Each plate was washed, added with an HRP-labeled secondary antibody for diluting in PBS, and then incubated for 1 h at RT. The washing step was repeated before an HRP substrate (TMB; 100 µL/well, Millipore) was added and then incubated in the dark for 10 min at RT. H₂SO₄ solution (500 mM; 50 µL/well) was used to stop the reaction, and the absorbance at 450 nm was measured using a microplate reader.

2.9 Detection of *S. enterica* in milk samples

Thirty milk samples were selected to evaluate the MRS immunosensor. Samples 1–3, which were purchased from a local supermarket with Tetra-PakTM aseptic packaging, were set as blank controls. Samples 4–10 were spiked with *S. enterica* at 10^2 , 10^3 , 10^4 , 10^5 , 10^6 , 10^7 and 10^8 CFU/mL, respectively. Samples 11–30 were positive samples that were detected by RT-PCR method, which were provided by the Chinese Academy of Inspection and Quarantine (CAIQ). CAIQ is an authoritative institution of inspection and quarantine for food in China. All milk samples were

197 diluted by 10-fold using PBS solution.

198 **3. Results and Discussion**

199 *3.1 Optimization of the MRS immunosensor*

200 In order to increase the sensitivity of the MRS immunosensor, we optimized
201 parameters including (i) dilution ratio of SMN-Ab1, which influences the valency and
202 magnetic susceptibility of the immunosensor, and (ii) size of SMNs, which directly
203 affects the sensitivity.

204 The dilution ratio of the SMN-Ab1 conjugate influences the signal-to-noise ratio
205 and the magnetic susceptibility of the immunosensor. As shown in Fig. 2, the ΔT_2 of
206 the three groups increased when the concentration of *S. enterica* increased. ΔT_2 was
207 the **highest** when the dilution ratio of the SMN-Ab1 conjugate was 1:50. According to
208 Chen et al. (2013), the appropriate concentration of SMN-Ab1 is critical in detecting
209 pathogen. Thus, this dilution ratio (1:50) was selected as the optimized condition for
210 the next step.

211 **Fig. 1.**

212 **Fig. 2.**

213 The second critical factor that we optimized was size of SMNs. In general, large
214 SMNs have strong magnetic susceptibility. Thus, the sensitivity of the MRS
215 immunosensor increases as the size of the employed SMNs increases (Koh, Hong,
216 Weissleder, & Josephson, 2009). Fig. 3 shows that the ΔT_2 of the three groups
217 increased when the concentration of *S. enterica* increased. The ΔT_2 value was the
218 largest and thus the sensitivity of the immunosensor peaked when the size of SMNs
219 was 50 nm. However, the error bar was larger when 50 nm SMNs were used than
220 when the other sizes of SMNs were employed, indicating poor stability of the
221 immunosensor. The suspension stability of the large SMNs was worse than that of the

small SMNs, which affected the accuracy of the MRS immunosensor. To balance sensitivity and stability, 30 nm SMN was selected for the subsequent experiments.

Fig. 3.

3.2 Sensitivity of the MRS immunosensor and ELISA

The detection range was determined by measuring ΔT_2 for the samples with different concentrations of *S. enterica*. Results showed that ΔT_2 continuously increased when the *S. enterica* concentration range was 10^3 – 10^7 CFU/mL (Fig. 4A). Thus, the LOD of the MRS immunosensor for *S. enterica* detection was 10^3 CFU/mL. A linear relationship ΔT_2 vs. *S. enterica* concentration was observed in the concentration range of 5×10^3 – 10^6 CFU/mL, and the linear equation was $Y = 10.901X - 28.654$ ($R^2 = 0.9951$) ($X = \lg[C_{S. enterica}]$). However, the linear detection range of ELISA was 10^5 – 10^7 CFU/mL, and the LOD was 2×10^4 CFU/mL, which was 20 times higher than that of the MRS immunosensor (Fig. 4B). The linear equation was $Y = 0.3678X - 1.4509$ ($R^2 = 0.9984$) ($X = \lg[C_{S. enterica}]$).

Fig. 4.

3.3 Specificity of the MRS immunosensor

The target strain *S. enterica* (ATCC 14028) and the other negative control strains (ATCC 12022, ATCC 13312, ATCC 25922, and ATCC 27217) were separately tested by using the proposed immunosensor. ΔT_2 significantly increased upon the addition of *S. enterica*, and ΔT_2 of *S. enterica* was greater than those of the other strain groups (Fig. 5). In the control group, SMN–Ab1 was replaced with SMNs to confirm that ΔT_2 resulted from the specific binding between *S. enterica* and SMN–Ab1 but not from non-specific adsorption. The SMNs did not specifically bind with *S. enterica*, which resulted in minimal ΔT_2 . This result ensures that the aggregation of SMN–Ab1 resulted from the Ag–Ab specific recognition rather than non-specific adsorption.

Fig. 5.

3.4 Milk sample analysis

Outbreaks of *Salmonella*-associated foodborne illnesses following consumption of raw milk and products made from raw milk have been reported in recent years (Jayarao et al., 2006; Reed & Grivetti, 2000). Detecting *Salmonella* in milk samples is necessary for the quality control in milk factory and public food safety (Cusato et al., 2013; Cusato et al., 2014; Dias et al., 2012). To further evaluate the reliability and accuracy of the MRS immunosensor, 30 milk samples were selected to be detected by this sensor. In the spiked milk samples (1–10), the ΔT_2 value was negligible in the blank samples (1–3) but increased with increasing *S. enterica* concentration in samples 4–10 [Fig. 6A (I) and 6B (I)]. These results indicate that the immunosensor can detect *S. enterica* in milk samples. Further, MRS and ELISA were used to detect the positive milk samples (11–30). Seventeen samples were detected to be positive by MRS and fourteen samples positive by ELISA [Fig. 6 A (II) and 6B (II)]. Samples 14, 29, and 30 were positively detected by the MRS immunosensor but not by ELISA.

Fig. 6.

The MRS immunosensor has many advantages over the conventional ELISA. The principle of the MRS immunosensor is based on the state change of SMN, which consequently alters ΔT_2 . Furthermore, biological and food samples have only negligible magnetic susceptibility. Thus, the MRS immunosensor requires minimal sample preparation and can be used to analyze epinephelos and complicated samples within a short analytical time. Moreover, the MRS immunosensor employs a homogeneous immunoassay method. The reactive system is suspended in solution, and this feature of homogeneous assays not only accelerates the Ag–Ab reaction but also avoids multiple washing steps. These characteristics greatly reduce detection

time and largely increase sensitivity and accuracy, making the MRS immunosensor an attractive platform for pathogen detection (Chen et al., 2013).

4. Conclusion

An MRS immunosensor was successfully developed to detect *S. enterica* in milk samples. This immunosensor increased sensitivity by 20 times and reduced detection time from 2-4 h to 0.5 h in comparison with the conventional ELISA. The MRS immunosensor based on SMNs is an attractive analytical platform for detecting pathogen in food because of its sensitivity, homogeneity, and rapidness. Combined with microfluidics and signal amplification system, this immunosensor can become an attractive platform for point-of-care testing fields, such as food safety, criminal investigation, clinical diagnosis, and environmental monitoring.

Acknowledgments

The authors gratefully acknowledge the financial supported by the National Natural Science Foundation of China (No. 21305082 and 21347008) and the Capital Health Research and Development of Special (No. 2011-1003-02).

References

- Abdel-Hamid, I., Ivnitski, D., Atanasov, P., & Wilkins, E. (1999). Highly sensitive flow-injection immunoassay system for rapid detection of bacteria. *Analytica Chimica Acta*, 399, 99-108.
- Cavallaro, E., Date, K., Medus, C., Meyer, S., Miller, B., Kim, C., Nowicki, S., Cosgrove, S., Sweat, D., Phan, Q., Flint, J., Daly, E. R., Adams, J., Hyytia-Trees, E., Gerner-Smidt, P., Hoekstra, R. M., Schwensohn, C., Langer, A., Sodha, S. V., Rogers, M. C., Angulo, F. J., Tauxe, R. V., Williams, I. T., Behraves, C. B., & Outbreak, S. T. (2011). Salmonella typhimurium infections associated with

peanut products. *New England Journal of Medicine*, 365, 601-610.

Chen, Y. P., Zou, M. Q., Wang, D. N., Li, Y. L., Xue, Q., Xie, M. X., & Qi, C. (2013). An immunosensor based on magnetic relaxation switch and polystyrene microparticle-induced immune multivalency enrichment system for the detection of *Pantoea stewartii* subsp. *Stewartii*. *Biosensors and Bioelectronics*, 43, 6-11.

Cusato, S., Gameiro, A. H., Corassin, C. H., Sant'Ana, A. S., Cruz, A. G., Faria, J. D. F., & de Oliveira, C. A. F. (2013). Food safety systems in a small dairy factory: Implementation, major challenges, and assessment of systems' Performances. *Foodborne Pathogens and Disease*, 10, 6-12.

Cusato, S., Gameiro, A. H., Sant'Ana, A. S., Corassin, C. H., Cruz, A. G., & de Oliveira, C. A. F. (2014). Assessing the costs involved in the implementation of GMP and HACCP in a small dairy factory. *Quality Assurance and Safety of Crops and Foods*, 6, 135-139.

Dias, M. A. C., Sant'Ana, A. S., Cruz, A. G., Faria, J. D. F., de Oliveira, C. A. F., & Bona, E. (2012). On the implementation of good manufacturing practices in a small processing unity of mozzarella cheese in Brazil. *Food Control*, 24, 199-205.

Durant, J. A., Young, C. R., Nisbet, D. J., Stanker, L. H., & Ricke, S. C. (1997). Detection and quantification of poultry probiotic bacteria in mixed culture using monoclonal antibodies in an enzyme-linked immunosorbent assay. *International Journal of Food Microbiology*, 38, 181-189.

Feasey, N. A., Dougan, G., Kingsley, R. A., Heyderman, R. S., & Gordon, M. A. (2012). Invasive non-typhoidal salmonella disease: An emerging and neglected tropical disease in Africa. *The Lancet*, 379, 2489-2499.

Galikowska, E., Kunikowska, D., Tokarska-Pietrzak, E., Dziadziuszko, H., Los, J. M., Golec, P., Wegrzyn, G., & Los, M. (2011). Specific detection of *Salmonella enterica* and *Escherichia coli* strains by using ELISA with bacteriophages as recognition agents. *European Journal of Clinical Microbiology and Infectious Diseases*, 30, 1067-1073.

Haun, J. B., Devaraj, N. K., Hilderbrand, S. A., Lee, H., & Weissleder, R. (2010). Bioorthogonal chemistry amplifies nanoparticle binding and enhances the sensitivity of cell detection. *Nature Nanotechnology*, 5, 660-665.

Hein, I., Flekna, G., Krassnig, M., & Wagner, M. (2006). Real-time PCR for the detection of *Salmonella* spp. in food: An alternative approach to a conventional PCR system suggested by the FOOD-PCR project. *Journal of Microbiological Methods*, 66, 538-547.

Jayarao, B. M., Donaldson, S. C., Straley, B. A., Sawant, A. A., Hegde, N. V., & Brown, J. L. (2006). A survey of foodborne pathogens in bulk tank milk and raw milk consumption among farm families in Pennsylvania. *Journal of Dairy Science*, 89, 2451-2458.

Koh, I., Hong, R., Weissleder, R., & Josephson, L. (2008). Sensitive NMR sensors detect antibodies to influenza. *Angewandte Chemie-International Edition*, 47, 4119-4121.

Koh, I., Hong, R., Weissleder, R., & Josephson, L. (2009). Nanoparticle-target interactions parallel antibody-protein interactions. *Analytical Chemistry*, 81, 3618-3622.

Lee, H., Sun, E., Ham, D., & Weissleder, R. (2008). Chip-NMR biosensor for detection and molecular analysis of cells. *Nature Medicine*, 14, 869-874.

Lee, H., Yoon, T. J., & Weissleder, R. (2009). Ultrasensitive detection of bacteria using core-shell nanoparticles and an NMR-Filter system. *Angewandte Chemie-International Edition*, 48, 5657-5660.

Ling, Y. B., Pong, T., Vassiliou, C. C., Huang, P. L., & Cima, M. J. (2011). Implantable magnetic relaxation sensors measure cumulative exposure to cardiac biomarkers. *Nature Biotechnology*, 29,

273-277.

Liong, M., Fernandez-Suarez, M., Issadore, D., Min, C., Tassa, C., Reiner, T., Fortune, S. M., Toner, M., Lee, H., & Weissleder, R. (2011). Specific pathogen detection using bioorthogonal chemistry and diagnostic magnetic resonance. *Bioconjugate Chemistry*, 22, 2390-2394.

Liong, M., Tassa, C., Shaw, S. Y., Lee, H., & Weissleder, R. (2011). Multiplexed magnetic labeling amplification using oligonucleotide hybridization. *Advanced Materials*, 23, H254-H257.

Liu, D. S., Tangpeerachaikul, A., Selvaraj, R., Taylor, M. T., Fox, J. M., & Ting, A. Y. (2012). Diels-Alder cycloaddition for fluorophore targeting to specific proteins inside living cells. *Journal of the American Chemical Society*, 134, 792-795.

Ma, W., Chen, W., Qiao, R. R., Liu, C. Y., Yang, C. H., Li, Z. K., Xu, D. H., Peng, C. F., Jin, Z. Y., Xu, C. L., Zhu, S. F., & Wang, L. B. (2009). Rapid and sensitive detection of microcystin by immunosensor based on nuclear magnetic resonance. *Biosensors and Bioelectronics*, 25, 240-243.

Mansfield, L. P., & Forsythe, S. J. (2000). The detection of *Salmonella* using a combined immunomagnetic separation and ELISA end-detection procedure. *Letters in Applied Microbiology*, 31, 279-283.

Matyas, B., Cronquist, A., Cartter, M., Tobin-D'Angelo, M., Blythe, D., Smith, K., Lathrop, S., Morse, D., Cieslak, P., Dunn, J., Holt, K. G., Henao, O. L., Fullerton, K. E., Mahon, B. E., Hoekstra, R. M., Griffin, P. M., Tauxe, R. V., & Bhattarai, A. (2010). Preliminary FoodNet data on the incidence of infection with pathogens transmitted commonly through food-10 States, 2009. *Morbidity and Mortality Weekly Report*, 59, 418-422.

Min, C., Shao, H. L., Liong, M., Yoon, T. J., Weissleder, R., & Lee, H. (2012). Mechanism of magnetic relaxation switching sensing. *ACS Nano*, 6, 6821-6828.

Nagpal, R., Behare, P. V., Kumar, M., Mohania, D., Yadav, M., Jain, S., Menon, S., Parkash, O., Marotta, F., Minelli, E., Henry, C. J. K., & Yadav, H. (2012). Milk, milk products, and disease free health: An updated overview. *Critical Reviews in Food Science and Nutrition*, 52, 321-333.

Perelle, S., Dilasser, F., Malorny, B., Grout, J., Hoorfar, J., & Fach, P. (2004). Comparison of PCR-ELISA and LightCycler real-time PCR assays for detecting *Salmonella* spp. in milk and meat samples. *Molecular and Cellular Probe*, 18, 409-420.

Perez, J. M., Josephson, L., O'Loughlin, T., Hogemann, D., & Weissleder, R. (2002). Magnetic relaxation switches capable of sensing molecular interactions. *Nature Biotechnology*, 20, 816-820.

Perez, J. M., Simeone, F. J., Saeki, Y., Josephson, L., & Weissleder, R. (2003). Viral-induced self-assembly of magnetic nanoparticles allows the detection of viral particles in biological media. *Journal of the American Chemical Society*, 125, 10192-10193.

Peterson, V. M., Castro, C. M., Lee, H., & Weissleder, R. (2012). Orthogonal Amplification of Nanoparticles for improved diagnostic sensing. *ACS Nano*, 6, 3506-3513.

Reed, B. A., & Grivetti, L. E. (2000). Controlling on-farm inventories of bulk-tank raw milk - An opportunity to protect public health. *Journal of Dairy Science*, 83, 2988-2991.

Riyaz-Ul-Hassan, S., Verma, V., & Qazi, G. N. (2013). Real-time PCR-based rapid and culture-independent detection of *Salmonella* in dairy milk--addressing some core issues. *letters in Applied Microbiology*, 56, 275-282.

Scallan, E., Hoekstra, R. M., Angulo, F. J., Tauxe, R. V., Widdowson, M. A., Roy, S. L., Jones, J. L., & Griffin, P. M. (2011). Foodborne illness acquired in the United States-major pathogens. *Emerging Infectious Diseases*, 17, 7-15.

Son, J. R., Kim, G., Kothapalli, A., Morgan, M. T., & Ess, D. (2007). Detection of *Salmonella enteritidis*

385 using a miniature optical surface plasmon resonance biosensor. *Journal of Physics: Conference*
386 *Series*, 61, 1086-1090.

387 Tassa, C., Shaw, S. Y., & Weissleder, R. (2011). Dextran-Coated Iron Oxide Nanoparticles: A versatile
388 platform for targeted molecular imaging, molecular diagnostics, and therapy. *Accounts of*
389 *Chemical Research*, 44, 842-852.

390 Ullal, A. V., Reiner, T., Yang, K. S., Gorbатов, R., Min, C., Issadore, D., Lee, H., & Weissleder, R. (2011).
391 Nanoparticle-mediated measurement of target-drug binding in cancer cells. *ACS Nano*, 5,
392 9216-9224.

393 Urata, M., Iwata, R., Noda, K., Murakami, Y., & Kuroda, A. (2009). Detection of living *Salmonella* cells
394 using bioluminescence. *Biotechnology Letters*, 31, 737-741.

395 Xu, Z., Kuang, H., Yan, W. J., Hao, C. L., Xing, C. R., Wu, X. L., Wang, L. B., & Xu, C. L. (2012). Facile and
396 rapid magnetic relaxation switch immunosensor for endocrine-disrupting chemicals. *Biosensors*
397 *and Bioelectronics*, 32, 183-187.

Figure captions

Fig. 1. Schematic of MRS immunosensor for detecting *S. enterica*. Before immunoreaction, the state of SMNs was dispersed, and T_2 was in a high value. Dispersed SMNs then became aggregated SMNs and T_2 decreased after immunoreaction by Ag–Ab recognition. The change in T_2 positively correlated with *S. enterica* concentration, which was the quantitative basis of this MRS immunosensor.

Fig. 2. Effect of SMN concentration on the sensitivity of the MRS immunosensor to detect *S. enterica*. The dilution ratios of SMN–Ab1 were 1:20, 1:50 and 1:100.

Fig. 3. Relationship between the size of SMNs and the sensitivity of the MRS immunosensor. The sizes of SMNs were 20, 30, and 50 nm.

Fig. 4. Sensitivity of the MRS immunosensor and ELISA in detecting *S. enterica* (A) MRS immunosensor (*S. enterica* concentrations were 10^2 , 5×10^2 , 10^3 , 5×10^3 , 10^4 , 5×10^4 , 10^5 , 10^6 and 10^7 CFU/mL) (B) ELISA (*S. enterica* concentrations were 10^2 , 10^3 , 10^4 , 2×10^4 , 5×10^4 , 10^5 , 10^6 , 10^7 and 10^8 CFU/mL).

Fig. 5. Specificity of the MRS immunosensor for *S. enterica* detection. Concentration of each bacteria sample was 5×10^4 CFU/mL.

Fig. 6. Efficiency of (A) MRS immunosensor and (B) ELISA for *S. enterica* detection in milk samples.

(I) Samples 1–3 were blank milk samples. Samples 4–10 were spiked with different concentrations of *S. enterica* (10^2 , 10^3 , 10^4 , 10^5 , 10^6 , 10^7 and 10^8 CFU/mL). (II) Samples 11–30 were real samples [obtained from the Chinese Academy of Inspection and Quarantine](#). The dot line in this figure was used as a cut off value to distinguish positive or negative samples.

Figure 1
[Click here to download high resolution image](#)

Figure 2
[Click here to download high resolution image](#)

Figure 3
[Click here to download high resolution image](#)

Figure 4
[Click here to download high resolution image](#)

Figure 5
[Click here to download high resolution image](#)

Figure 6
[Click here to download high resolution image](#)