Electronic supplementary information (ESI) for

Colorimetric and ultrasensitive immunosensor for one-step pathogen detection via the combination of nanoparticles-trigged signal amplification and magnetic separation

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Materials and methods

Apparatus

3K30-high speed freezing centrifuge (Sigma, USA) was used to prepare the HRP-Au- Ab conjugate. An orbital shaker (IKA Inc., Germany) was employed to mix the samples, AuNPs and MBs 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.

Chemicals

The carboxyl modified magnetic beads (1 μm, 10 mg/mL) were purchased from Invitrogen (Life Technologies) (USA). 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and horse reddish peroxidase (HRP) were purchased from Sigma. *S. enterica* monoclonal antibody (Ab1, 2 mg/mL) was purchased from Genway (San Diego, California, USA). Horseradish peroxidase (HRP)-labeled secondary antibody was purchased from Jackson Immuno Research Inc. (USA). Other reagents and solvents were of analytical grade and were purchased from Beijing Chemical Reagents Co. (Beijing, China). All solutions were prepared using18 MΩ water obtained by a Millipore-XQ system.

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 (pH=7.4, 0.01M) was used to adjust bacterial concentration.

Preparation of immune-magnetic beads (MBs-Ab1)

0.5 mg of MBs was suspended in 2 mL of activated buffer (80 nM MES, pH=5.2). Then, 50 μL of EDC (10 mg mL^{-1}) was added to the MBs solution. After activation for about 30 min, the excess EDC and byproducts were removed via magnetic separation using a magnetic scaffold. Then 2 mL of PBS buffer (pH=7.4, 0.01M) was added to re-suspend the activated MBs. Subsequently, 0.1 mg anti-S. enterica antibody (capture antibody: Ab_1) was added to the activated MBs solution. The mixture was gently stirred to react for 2 h at room temperature and then blocked with 3% (m/v) bovine serum albumin (BSA) for 0.5 h. The MBs-Ab1 conjugate was separated from the free Ab_1, re-suspended in 1000 μL of PBS and stored at 4 ^oC for further use.

Fabrication of HRP-Au- Ab2 conjugate

Citrate-stabilied Au NPs (20~30nm diameter) were prepared according to the previous work []. After that, the process of HRP-Au-Ab2 conjugate was followed an optimized procedure. The pH of 20 mL 0.01% Au NPs was adjusted into 8.2 by adding into 0.01M K_2CO_3 solution, and then different amount of detection antibody (Ab2) and HRP molecular ware added into the mixture solution. The resulting solution was incubated for 1 h at 650 rpm. Then 0.5 mL 10 mg/mL BSA aqueous solution was added and stirring was continued for another 0.5 h. The mixture solution was centrifuged at 14000 rpm and 4 ^oC for 20 min. The supernate was removed and the HRP-Au-Ab2 conjugate was re-suspended with 0.5 mL PBS solution (0.1% BSA),

and this solution was centrifuged at 14000 rpm and 4 ^{o}C for 20 min again. Finally, the supernate was removed and the HRP-Au-Ab2 conjugate was resuspended with 0.2 mL developing solvent.

The protocol of visual immunosensor assay

The procedure was illustrated in Scheme 1. First, 100 μL of MBs-Ab1 solution, 100 μL HRP -Au- Ab2 conjugate and 900 μL of different concentrations of Salmonella enterica solution, which was diluted using the PBS buffer solution (10^8, 10^7, 10^6, 10^5, 10^4, 10^3, 10^2 , 10 and 0 cfu/mL) were transferred to centrifuge tubes. Each mixture was gently shaken for 45 min at room temperature. Then all the tubes were put on a magnetic scaffold. After separation, the immune-complex was washed 3 times with PBST buffer (0.01 M, pH 7.4, Tween-20 0.01%). Following that, 200 μL tetramethylbenzidine (TMB) solution was added into the above immune-complex and re-suspended at gentle shake for 10 min at room temperature. Finally, the supernate was transferred into 96 well plate to observe the blue color of the sample.

Optimization of immunological reaction

Experimental factors, such as the concentration of MBs-Ab1 and the dilution ratios of HRP-Au-Ab2 conjugate, were optimized to improve the sensitivity and stability of the visual immunosensor. In this experiment, three concentrations of MBs-Ab1 (0.1, 0.05 and 0.01 mg/mL) and four dilution ratios of HRP-Au-Ab2 conjugate (1:20, 1:100, 1:200 and 1:500) were selected.

Real sample analysis.

The milk samples (positive samples and negative samples) were provided by Chinese Academy of Inspection and Quarantine (CAIQ) (Beijing, China).The negative samples and the positive samples were identified by RT-PCR method. These

milk samples (100 μL) were 10-folds diluted by PBS solution, 100 μL of MBs-Ab1 solution, 100 μL HRP-Au-Ab2 conjugate were added to the diluted milk samples. Each mixture was gently shaken for 45 min at room temperature. After magnetic separation, the immune-complex was washed 3 times with PBST buffer (0.01 M, pH 7.4, Tween-20 0.01%). Following that, 200 μL tetramethylbenzidine (TMB) solution was added into the above immune-complex and re-suspended at gentle shake for 10 min at room temperature. Finally, the supernate was transferred into 96 well plate to observe the blue color of the sample.

Result and discussion

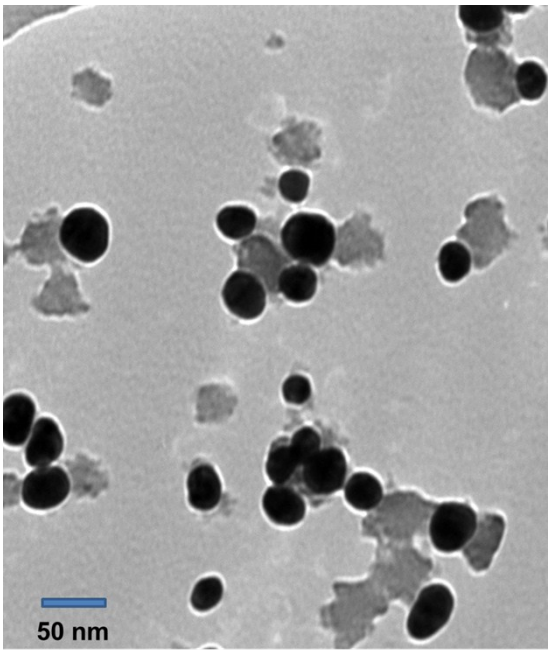

Figure S1 The characterizations of HRP-Au-Ab2 conjugate using biological electron microscope.

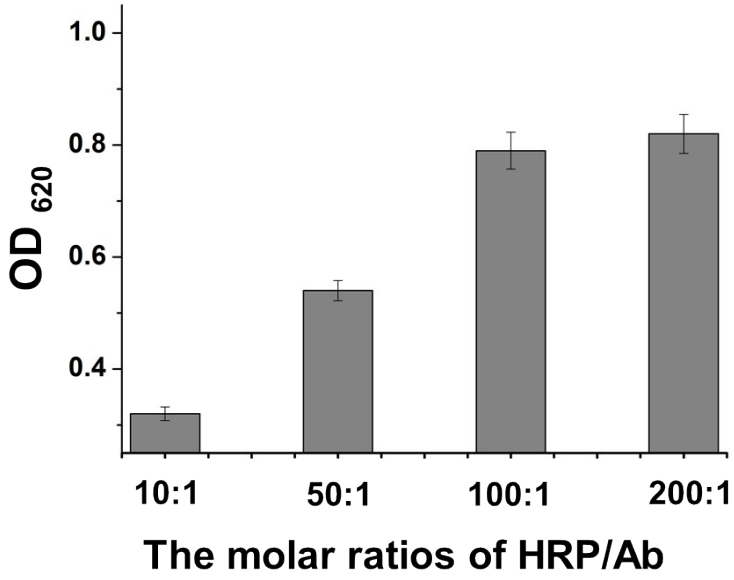

Figure S2 The relationship between the OD_{620} value and the molar ratios of HRP/Ab on the surface of Au NP_S.

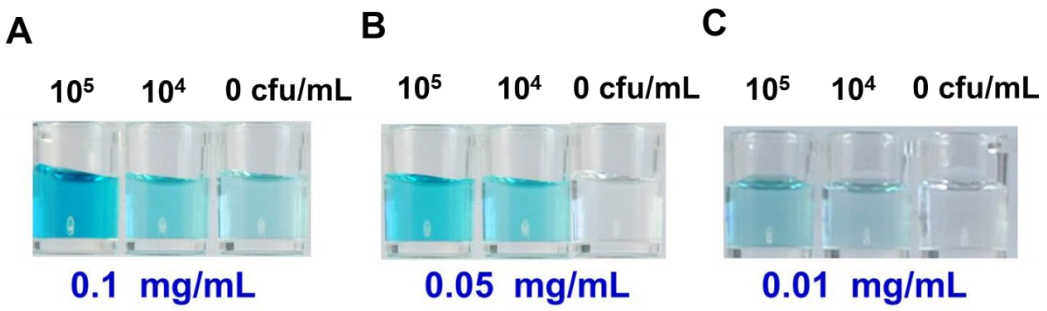

Figure S3. Optimization of concentration of MBs-Ab1. The concentration of S. enterica is 10^5, 10^4, and 0 cfu/mL and the diluted ratio of HRP-Au-Ab2 conjugate is 1:20. The time for immuno-reaction is 60 min.

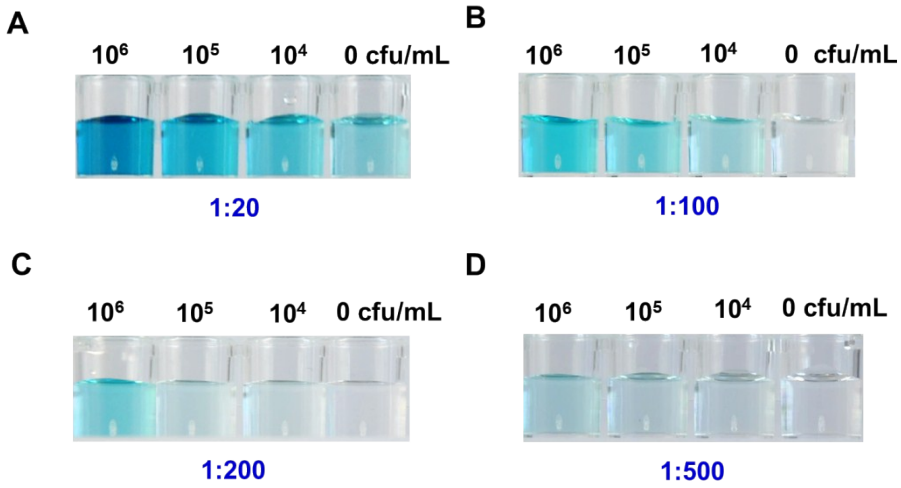

Figure S4. Optimization of concentration of HRP-Au-Ab2 conjugate. The molar ratio of HRP/Ab2 is 100:1 and the time for immuno-reaction is 60 min.

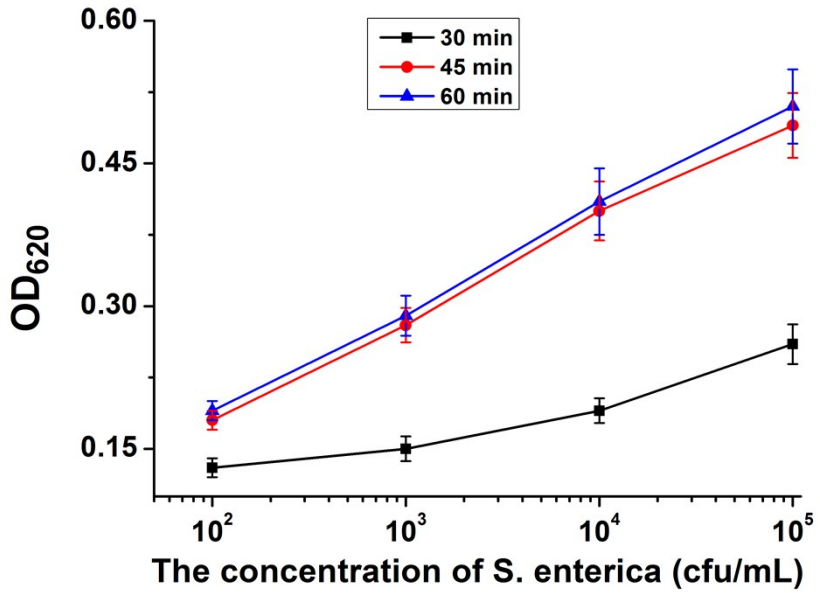

Figure S5. Optimization of immuno-reaction time of this immunosensor. The concentration of MB-Ab1 is 0.05 mg/mL and the diluted ratio of HRP-Au-Ab2 conjugate is 1:100.

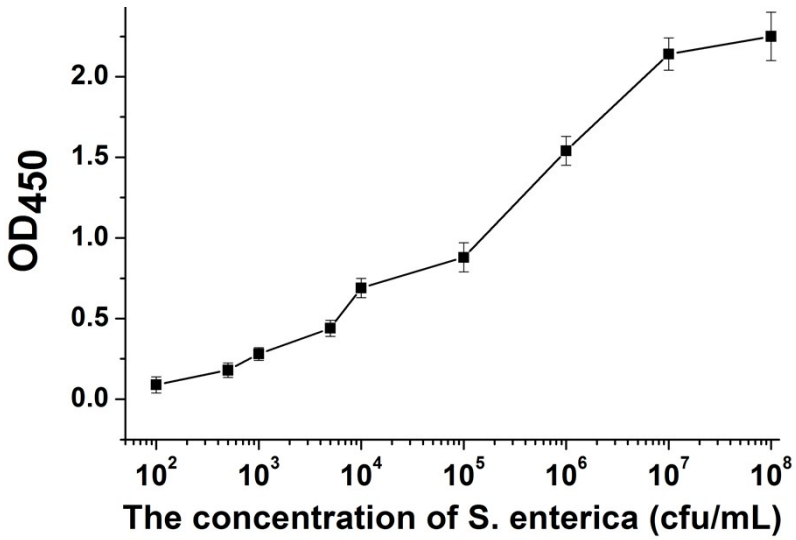

Figure S6. The sensitivity of ELISA method for the detection of S. enterica in PBS solution (pH=7.4, 3% BSA). The concentration of S. enterica is 0, 10^2, 5 $\times 10^2$, 10^3, 5×10^3, 10^4, 10^4，10^5，10^6，10^7 and 10^8 cfu/mL.

Table.S1 The comparison of ELISA, GLFT and this visual sensor for detection of S. enterica.

	Sensitivity	Detection time	Operation
ELISA	500 cfu/mL	2-4 h	complex
GLFS	10^4 cfu/mL	10 min	easy
Visual immunosensor	10^2 cfu/mL	1 h	easy