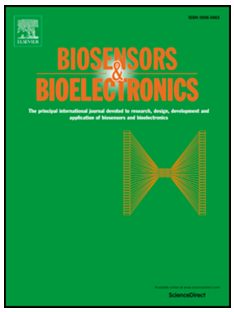

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Ultrasensitive and selective detection of *Staphylococcus aureus* using a novel IgY-based colorimetric platform

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Abstract

To develop a specific method for the detection of *S. aureus*, chicken anti-protein A IgY was adopted for specifically capturing *S. aureus*, depending on the specific recognition of staphylococcal protein A (SPA) by chicken anti-protein A IgY, which can eliminate the interference from protein G-producing *Streptococcus*. HRP labeled IgG, Fc region of which has a high affinity towards SPA, was paired with IgY for the colorimeter analysis of the system. By optimizing the system, a super-low detection limit of 11 CFU of *S. aureus* in 100 μL PBS without enrichment, with a linear range from 5.0 × 10^2 CFU mL^{-1} to 5.0 × 10^4 CFU mL^{-1} was obtained. The entire assay was accomplished in less than 90 min and no cross-reactivity with the other tested bacterial species was observed. Moreover, the developed assay has been applied for the detection of *S. aureus* in three different types of real samples (apple juice, urine and sodium chloride injection) with satisfactory results. To the best of our knowledge, it is the first time to report using chicken anti-protein A IgY and any IgG to detect *S. aureus* based on the dual-recognition mode of SPA. The novel method opened up a way for monitoring *S. aureus* in food samples with high sensitivity, specificity and simple operation.

Key words: *Staphylococcus aureus*; Dual-recognition mode; Staphylococcal protein A; IgY; Mammalian IgG; Specificity

1. Introduction

Staphylococcus aureus (*S. aureus*), which is one of the most commonly iatrogenic and foodborne pathogenic bacteria, and it is attributed to a wide range of infections in humans from skin infections to life-threatening diseases (Esteban-Fernandez de Avila et al. 2012; Lowy 1998; Tannert et al. 2018). In United States, around 241,000 cases of food-borne illnesses were caused by *S. aureus* each year, which represent a major public health problem (Yu et al. 2016). *S. aureus* imposes a great threat to food safety and public health (Juronen et al. 2018; Wollenhaupt et al. 1992; Zhao et al. 2018). Hence, it is critical to guarantee food safety and human health by the detection of *S. aureus* with high sensitivity and selectivity.

The gold standards for *S. aureus* detection rely on bacteria-culture, including pre-enrichment, selective enrichment, selective plating, biochemical screening and serological confirmation (Liu et al. 2019; Rubab et al. 2018; Song et al. 2016; Yang et al. 2019). The whole procedure consumes more than 2 days to acquire a confirmed result of bacterial cells, which could not meet the needs of fast screening food pathogens in food samples. Polymerase chain reaction (PCR) based methods exhibit high sensitivity and specificity for the targets by determining the specific sequences and they can avoid time-consuming bacterial culture and hence reduce the detection time (Brakstad et al. 1992; Hao et al. 2017; Schuelke 2000; Sun et al. 2015). However, the polymerases used in PCR are easily inhibited by the complex food matrix factors and cannot directly detect low levels of target bacteria in large volume of food samples and also PCR can only detect nucleic acid and cannot detect inactivated pathogens (Sung et al. 2013; Zhang et al. 2017). Antibody-based biosensor methods rely on specific-interactions between antigens and corresponding antibodies, presenting the merits of rapid speed, low cost, high sensitivity, high throughput screening and good specificity (Afkhami et al. 2017; Karami et al. 2019;

Khoshfetrat et al. 2018; Khoshfetrat et al. 2019). Recently, lots of antibody-based strategies, utilizing the binding behavior of Staphylococcal protein A (SPA) towards the Fc fragment of non-specific mammalian immunoglobulin G (IgG), have realized the diagnosis of *S. aureus* with high sensitivity. Molecule recognition agents such as antibiotics (e.g. vancomycin) or cell wall binding domains of bacteriophage endolysin have been reported to serve as paired recognition elements to form sandwich assays (Gao et al. 2015; Kong et al. 2015; Yang et al. 2016; Yu et al. 2016). However, a major hindrance to the specificity of these antibody-based immunoassays is the presence of protein G-producing *Streptococcus*, leading to the false-positive results for the detection of *S. aureus*. For example, protein G-producing *Streptococcus*, which can be recognized by vancomycin, a broad-spectrum antibiotic for Gram-positive microbes, interferes the detection of *S. aureus*. As well, protein G on the cell wall of protein G-producing *Streptococcus* could also bind with mammalian IgG, which will produce false positive signals for the detection of *S. aureus* by using IgG antibody as recognition element (Yan et al. 2017; Yang et al. 2017).

Immunoglobulin Y (IgY), a counterpart of mammalian IgG, exists in avian serum and egg yolk (Duan et al. 2009; Liu et al. 2017; Vikinge et al. 1998). Compared to mammalian IgG, chicken IgY does not non-specifically combine with SPA or protein G (Jin et al. 2013; Reddy et al. 2013). Chicken IgY might be a good candidate to detect *S. aureus* circumventing the interference from protein G generating *Streptococcus*. As well-known, plenty of SPA molecules are expressed only on the surface of *S. aureus* (Liu et al. 2007). Herein, SPA was chosen as an epitope of *S. aureus* in this study. IgY specific to protein A coupling with magnetic beads (MBs) was utilized to capture *S. aureus* in samples, and horseradish peroxidase (HRP) labeled non-specific mammalian IgG was employed to bind with "vacant" SPA molecules to establish a sandwich assay, where the oxidation of 3, 3', 5, 5' -

tetramethylbenzidine (TMB) was catalyzed to obtain colorimetric signals. The scheme of the method is illustrated in Fig. 1. The combination of chicken anti-protein A IgY with a non-specific mammalian IgG enhanced selectivity and sensitivity significantly. Chicken anti-protein A IgY as capturing antibody can greatly improve the specificity of immunoassay. Furthermore, non-specific mammalian IgG as detection antibody could amplify the detection signals through binding Fc region of IgG to SPA, where there are around 80, 000 SPA molecules on the surface of one bacterium of *S. aureus* (Xiong et al. 2016). What is more, one SPA molecule contains five homologous IgG-binding regions (Yang et al. 2008), improving the sensitivity of the proposed method. To our best knowledge, this was the first reported utilization of chicken anti-protein A IgY and non-specific mammalian IgG as the antibody pair to detect *S. aureus* with high sensitivity and selectivity based on the dual-recognition mode of SPA. The developed method has potential applications for the detection of *S. aureus* in multiple fields including medical and food areas.

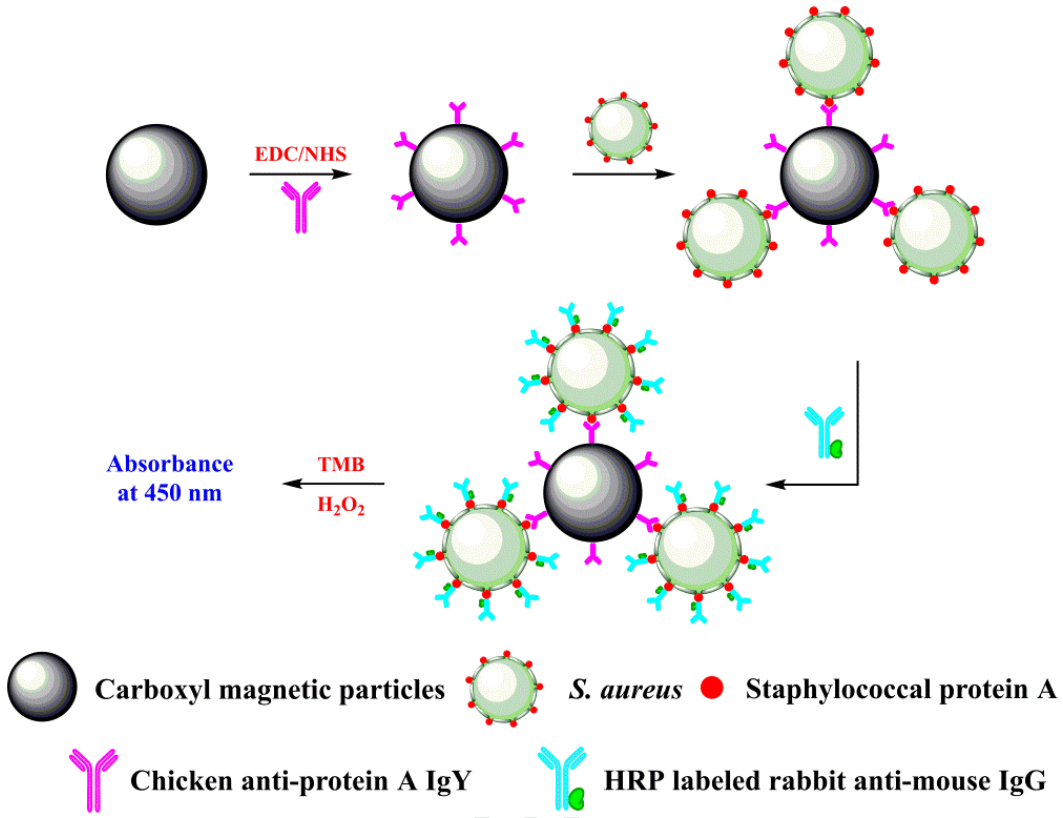

Fig. 1. Schematic illustration of the proposed sandwich colorimetric platform for *S. aureus* (not to scale).

2. Experimental

2.1. Materials and reagents

S. aureus (ATCC 12598), *E. coli* O157: H7 (EDL 933), *Salmonella* (ATCC 14028) and *Listeria monocytogenes* (ATCC 19115), *Streptococcus dysgalactiae* (ATCC 35666) and *Streptococcus agalactiae* (isolated from raw milk) were used for this study. Carboxyl MBs (0.82 μm in diameter) of 25 mg mL^{-1} were purchased from Spherotech (USA). (3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) were purchased from Aladdin (Shanghai, China). Chicken anti-protein A IgY (1 mg mL^{-1}) and biotin-chicken anti-protein A IgY (1 mg mL^{-1}) were supplied by Abcam (UK). HRP labeled rabbit anti-mouse IgG (HRP-IgG) (1 mg mL^{-1}), Alexa

fluor 568 labeled rabbit anti-mouse IgG (1 mg mL^{-1}) and TMB solution were obtained from Biodragon immunotechnologies (Beijing, China). *S. aureus* ELISA kit was purchased from Meimian biotechnology (Jiangsu, China). Streptavidin-FITC (SA-FITC) was purchased from Biolegend (USA). Tween-20 and skim milk powder were acquired from Solarbio (Beijing, China) and BD Difco (USA), respectively. 96-well serological microplates were bought from Jet bio-filtration (Guangzhou, China). Sodium chloride injection and apple juice were obtained from local market. Human urine was provided by healthy volunteers. All other reagents were of analytical grade and supplied by local commercial suppliers unless specified otherwise. All buffers were prepared using the deionized water (18.2 MΩ).

2.2. Apparatus

Magnetic separation racks, bought from Goldmag biotech (Xi'an, China), were utilized to isolate the MBs from the solutions to the side wall of Eppendorf tube. An Oscillator Vortex Genie 2 (USA) was employed to disperse MBs in Eppendorf tube. An incubator shaker ZWY-100H (Labwit scientific, China) was used for bacterial incubation and antigen-antibody reaction. The deionized water (18.2 MΩ) was produced by Ulupure UPR-II-10T (Sichuan, China). Fluorescence micrographs were captured by an Eclipse Ti-S fluorescence microscope (Nikon, Japan). Optical measurements were performed utilizing a Multiskan MK3 microplate reader (Thermo Scientific, USA).

2.3. Bacteria culture and Counting

S. aureus, *E. coli* O157: H7 and *Salmonella* were inoculated in Luria-Bertani broth medium, and *Streptococcus agalactiae* and *Listeria monocytogenes* were grown in brain heart infusion broth medium. After incubation at 37 °C overnight with continuous shaking, pure cultures were collected by centrifuge

131 at 3000 r min^{-1} for 10 min to remove culture medium and washed twice by sterile phosphate-buffered
132 saline (PBS, 10 mmol L^{-1}, pH 7.4, containing 4.3 mmol L^{-1} Na_2HPO_4, 1.4 mmol L^{-1} KH_2PO_4, 137
133 mmol L^{-1} NaCl and 2.7 mmol L^{-1} KCl). The collected bacteria were re-suspended in sterile PBS (10
134 mmol L^{-1}, pH 7.4) with 20 % glycerol. The bacterial concentration was determined by standard colony
135 counting method. Colony-forming units (CFU) of bacteria strains were counted as ~ 10^8 CFU mL^{-1},
136 and aliquots of the bacteria in sterile Eppendorf tubes were kept at -20 °C until use.
137
138 **2.4. Characterization of specific combination of anti-protein A IgY or mammalian IgG towards *S.***
139 ***aureus***
140 To demonstrate the specific combination of anti-protein A IgY or mammalian IgG with *S. aureus*,
141 *S. aureus* was incubated for 30 min with Alexa fluor 568 labeled rabbit anti-mouse IgG and
142 biotin-chicken anti-protein A IgY, respectively. Then, the antibodies labeled *S. aureus* cells were
143 centrifuged and washed three times with PBST to remove the unbound antibodies. The biotin-chicken
144 anti-protein A IgY labeled *S. aureus* required a further incubation with SA-FITC to load FITC on *S.*
145 *aureus* through biotin-streptavidin affinity. The scheme of the preparation of fluorochrome-stained *S.*
146 *aureus* is illustrated in supplementary Fig. S1. Ultimately, the fluorescence micrographs of the
147 fluorochrome-stained *S. aureus* were observed and recorded utilizing a fluorescence microscope.
148
149 **2.5. Preparation of immuno-magnetic beads**
150 Immuno-magnetic beads (IMBs) were prepared according to our previously reported method
151 (Zhang et al. 2019). Briefly, 40 μL of Carboxyl magnetic beads were first pipetted into Eppendorf tube
152 and placed on a magnetic separation racks to remove the supernatant. After washed twice by PBS (10

153 mmol L^{-1}, pH 6.8), MBs were dispersed in 120 μL of PBS (10 mmol L^{-1}, pH 6.8). Afterwards, 80 μL of
154 solution containing 25 mg mL^{-1} EDC and 12.5 mg mL^{-1} NHS was added to react with carboxyl of MBs
155 through the EDC/NHS amidization reaction. After 30 min, 50 μg of chicken anti-protein A IgY was
156 added and incubated under constant shaking (180 r min^{-1}) at 37 °C for 4 h. The resulted
157 immuno-magnetic beads (IMBs) were washed twice by 200 μL of phosphate-buffered saline with
158 Tween 20 (PBST, PBS at pH 7.4 containing 0.05 % Tween-20) and then blocked with blocking buffer
159 (PBS at pH 7.4 containing 5 % BSA and 15 % skim milk powder) for 2 h. Finally,
160 antibody-immobilized MBs were prepared and stored in 1 mL PBS (10 mmol L^{-1}, pH 7.4) containing
161 0.1 % BSA and 0.1 % NaN_3 at 4 °C for further use.
162
163 **2.6. Detection procedure**
164 The principle and procedure of the developed IgY-based colorimetric detection of *S. aureus* were
165 depicted in Fig. 1. Certain amount of IMBs were mixed with 100 μL of *S. aureus* under vigorous
166 shaking (180 r min^{-1}) at 37 °C. Following 30 min of incubation, where IMBs captured the target *S.*
167 *aureus*. Following that, the complexes of IMBs/*S. aureus* were magnetically separated. After discarding
168 the supernatant, the complexes were washed four times with 200 μL of PBST (Yan et al. 2017; Zhang
169 et al. 2016). The beads were re-suspended with 100 μL of HRP-IgG and the mixture was incubated
170 under the rotation (180 r min^{-1}) at 37 °C for 30 min. Finally, the formed immuno-complexes (IMBs/ *S.*
171 *aureus*/ HRP-IgG) were rinsed four times with 200 μL of to remove the unbound enzyme marker.
172 Afterwards, the immuno-complexes were mixed with 100 μL of chromogenic substrate containing
173 TMB and H_2O_2 at 37 °C in darkness for 10 min. Then, 100 μL of 2 mol L^{-1} H_2SO_4 was added to cease
174 the reaction. After the magnetic separation, the supernatant was transferred into the 96-well serological

175 microplate for the absorbance (450 nm) measurement.

176

177 **3. Results and Discussion**

178 **3.1. Recognition of chicken anti-protein A IgY and mammalian IgG towards *S. aureus***

179 *S. aureus* was stained with green fluorescence using SA-FITC combined with biotin-chicken
180 anti-protein A IgY through biotin-streptavidin reaction. Alexa fluor 568 labeled mammalian IgG was
181 utilized to serve as red fluorescence when SPA bound with Fc region of mammalian IgG. The
182 bright-field images of the stained *S. aureus* (as shown in supplementary Fig. S2A and S2B) indicated
183 the location of bacteria. Red fluorescence of *S. aureus* tagged with Alexa fluor 568 and green
184 fluorescence of *S. aureus* labelled with FITC can be observed in supplementary Fig. S2C and S2D,
185 respectively. The results of the fluorescence microscopy confirmed that chicken anti-protein A IgY and
186 mammalian IgG can successfully combine with *S. aureus*, which provided a crucial evidence for
187 utilizing chicken anti-protein A IgY and mammalian IgG as good recognition elements and good
188 labeling molecules for the detection of *S. aureus*.

189 **3.2. Optimization of the experimental conditions**

190 Generally speaking, the sensitivity of immunoassay could be severely influenced by the amount of
191 relative immuno-reagents (Mani et al. 2009; Yang and Li 2006). Hence, the amount of IMBs addition
192 and the concentration of HRP-IgG were tested to get ideal detection performance of immunoassay in
193 this study.

194 The amount of IMBs addition ranging from 3 μg to 20 μg was tested. In Fig. 2, the signal A
195 represented the absorbance intensity at 450 nm with 5.0×10^4 CFU mL^{-1} *S. aureus* and A_0 was obtained
196 for a blank of PBS using the same process as the samples containing *S. aureus*. As illustrated in Fig. 2A,

197 the signal *A* increased with the enhancing amount of the IMBs and almost reached a plateau when the
198 addition of IMBs was 10 μg. While A_0 ascended gradually with the increasing amount of IMBs,
199 resulting from the addition of the more IMBs into immunoassay and correspondingly the more
200 HRP-IgG non-specifically absorbed onto IMBs (Zhang et al. 2019; Zhang et al. 2017), which led to the
201 highest value of A/A_0 appearing in the middle of the amount of the IMBs of 10 μg as revealed in Fig.
202 2B (The mean values of *A*, A_0 and A/A_0 along with standard deviations (n=3) displayed in Fig. 2A and
203 2B were listed in supplementary Table S1). Therefore, excess IMBs were not necessary, even abating
204 the sensitivity of the assay. The amount of 10 μg IMBs was selected as a compromise for the
205 subsequent optimization.

206 Effect of the concentration of HRP-IgG on the sensitivity of the proposed method was also
207 investigated. As shown in Fig. 2C, both of *A* and A_0 revealed an increase when the concentration of
208 HRP-IgG growing from 0.0625 to 1 μg mL^{-1}. At the low concentration range of 0.0625-0.25 μg mL^{-1}, *A*
209 increased faster than A_0, because in the low range of HRP-IgG concentrations, the reaction sites were
210 unsaturated. While at the high concentration range of 0.25-1 μg mL^{-1}, *A* increased more slowly than A_0,
211 owing to too high concentration of HRP-IgG promoting non-specific adsorption of HRP-labeled IgG
212 onto IMBs and leading to high background signals (The mean values of *A*, A_0 and A/A_0 along with
213 standard deviations (n=3) presented in Fig. 2C and 2D were listed in supplementary Table S2). Thus, as
214 displayed in Fig. 2D, optimal A/A_0 was achieved for HRP-IgG at the concentration of 0.25 μg mL^{-1}.
215 Thus this condition was adopted for further experiments.
216

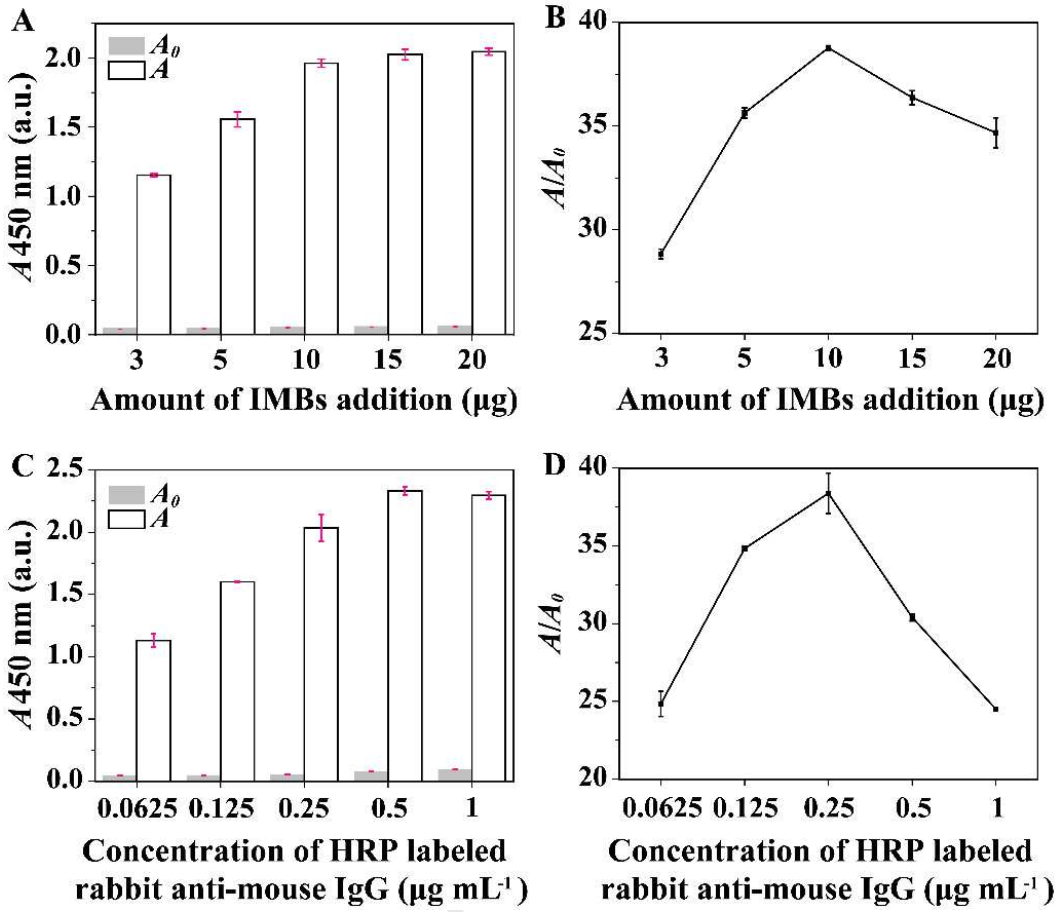

Fig. 2. Optimization of experimental conditions. (A) Effects of the amount of IMBs addition on A_0 and A. (B) Effects of the amount of IMBs addition on A/A_0. The corresponding blank was kept same as the corresponding amount of the IMBs. (C) Effects of the concentration of HRP-IgG on A_0 and A. (D) Effects of the concentration of HRP-IgG on A/A_0. The corresponding blank was kept same as the corresponding concentration of HRP-IgG. Three independent measurements were taken from three individual preparations for each condition. Error bars indicated the standard deviations.

3.3. Analytical performance for *S. aureus* detection

Under optimal conditions, the concentrations of *S. aureus* ranging from 5.0×10^2 to 5.0×10^4 CFU mL^{-1} were tested. As shown in Fig. 3, with increasing of the concentration of *S. aureus*, the absorbance intensity at 450 nm rose linearly. The regression equation was $Y = 4.0819 \times 10^{-5} X + 0.0602$ with the correlation coefficient (R^2) of 0.9916, where Y and X represented the absorbance intensity (450

229 nm) of the reaction solution and the concentration of *S. aureus* (CFU mL^{-1}), respectively (The mean
230 values along with standard deviations (n=3) exhibited in Fig. 3 were listed in supplementary Table S3).
231 The detection limit (3σ, where σ is the standard deviation of the detection of the blank (PBS), n=10,
232 data were shown in supplementary Table S4) was calculated to be 1.1×10^2 CFU mL^{-1}, which is 11
233 CFU in 100 μL sample, where there was no enrichment. If the enrichment of magnetic beads is
234 considered, the detection limit can be much lower (1.1 CFU in 100 μL can be determined if the
235 detection volume is 1 mL.). PCR based method can ideally detect 1 CFU, but the detection volume is
236 tiny (the highest is 5 μL). Hence the detection limit is 1 CFU in 5 μL, which is 2.0×10^2 CFU mL^{-1},
237 whereas in reality, it is hard to detect 1 CFU for PCR. Therefore, we can say that the sensitivity of the
238 method is much higher than PCR. The sensitivity of the proposed method was better than that of the
239 reported colorimetric immunoassays listed in Table 1. The super-low detection limit may be due to the
240 choice of SPA as an epitope of *S. aureus*. Since there are plentiful SPA molecules on one cell of *S.*
241 *aureus* and five homologous IgG-binding regions for one SPA molecule, HRP-IgG acting as a detection
242 antibody could amplify the colorimetric signals and improve the sensitivity of the proposed method.
243 Compared with these reported colorimetric methods, the proposed method exhibited ultra-high
244 sensitivity, little interference from the sample matrix and no disturbance from the protein G producing
245 *Streptococcus*, which did not require either rare metals, complicated nanomaterial preparations, trained
246 staff or sophisticated instruments. In addition, relative standard deviation (RSD) test employed *S.*
247 *aureus* cells at 5.0×10^4 CFU mL^{-1} as a standard sample to investigate the reproducibility of the
248 developed immunoassay. The resulted RSD value was calculated to be 2.94 % (detailed data were
249 shown in supplementary Table S5, n=11), manifesting a good reproducibility.
250

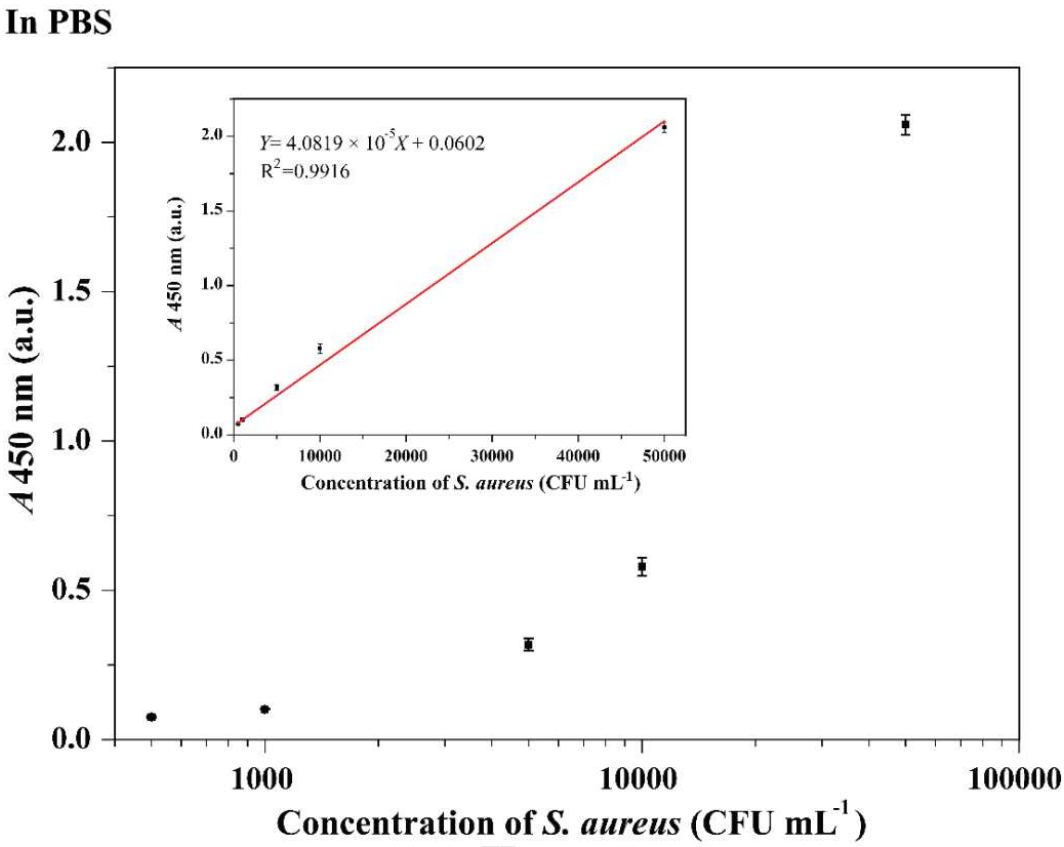

Fig. 3. The plot of absorbance intensity at 450 nm versus concentration of *S. aureus* in PBS. The calibration curve for *S. aureus* detection was shown in inset. Three independent measurements were taken from three individual preparations for each condition. Error bars indicated the standard deviations.

Table 1 Colorimetric methods for the detection of *S. aureus*

Methods	Detection limit (CFU mL^{-1})	Ref.

Mammalian Antibody/ gold nanoparticle/ magnetic nanoparticle nanocomposites based immunoassay	1.5×10^3	(Sung et al. 2013)
Double-site recognition of *S. aureus* utilizing antibiotic-affinity strategy	6.7×10^3	(Gao et al. 2015)
Enzyme linked cell wall binding domain of phage lysin	4×10^3	(Yu et al. 2016)
Phagomagnetic immunoassay	2.5×10^3	(Yan et al. 2017)
Lateral flow immunoassay	5.0×10^2	(Li et al. 2011)
Gold based immunosensor	1.0×10^5	(Souhir Boujday et al. 2008)
The proposed method	1.1×10^2	

3.4. Specificity for *S. aureus* detection

Specificity of the proposed method was investigated to testify its application reliability. *E. coli* O157: H7, *Salmonella*, *Listeria monocytogenes*, *Streptococcus agalactiae* and *Streptococcus dysgalactiae* all at 1.0×10^6 CFU mL^{-1} for each were employed as interfering bacteria. As exhibited in Fig. 4, the signals obtained from five interfering bacteria were close to that from PBS, and the signals obtained from their mixtures with *S. aureus* (5.0×10^4 CFU mL^{-1}) were similar to that from *S. aureus* (5.0×10^4 CFU mL^{-1}) (The mean values along with standard deviations (n=3) shown in Fig. 4 were listed in supplementary Table S6). Note that the interference from *Streptococcus* was negligible due to the adoption of chicken anti-protein A IgY as a capture agent. Since chicken IgY possesses a different structure of Fc region from mammalian IgG, chicken anti-protein A IgY does not bind to protein G producing *Streptococcus*. In addition, chicken anti-protein A IgY could specifically bind with SPA on

the surface of *S. aureus* through antibody-antigen affinity (Jin et al. 2013; Reddy et al. 2013). Hence, the proposed method can exhibit excellent selectivity towards *S. aureus* and avoid interference from common pathogenic microorganisms.

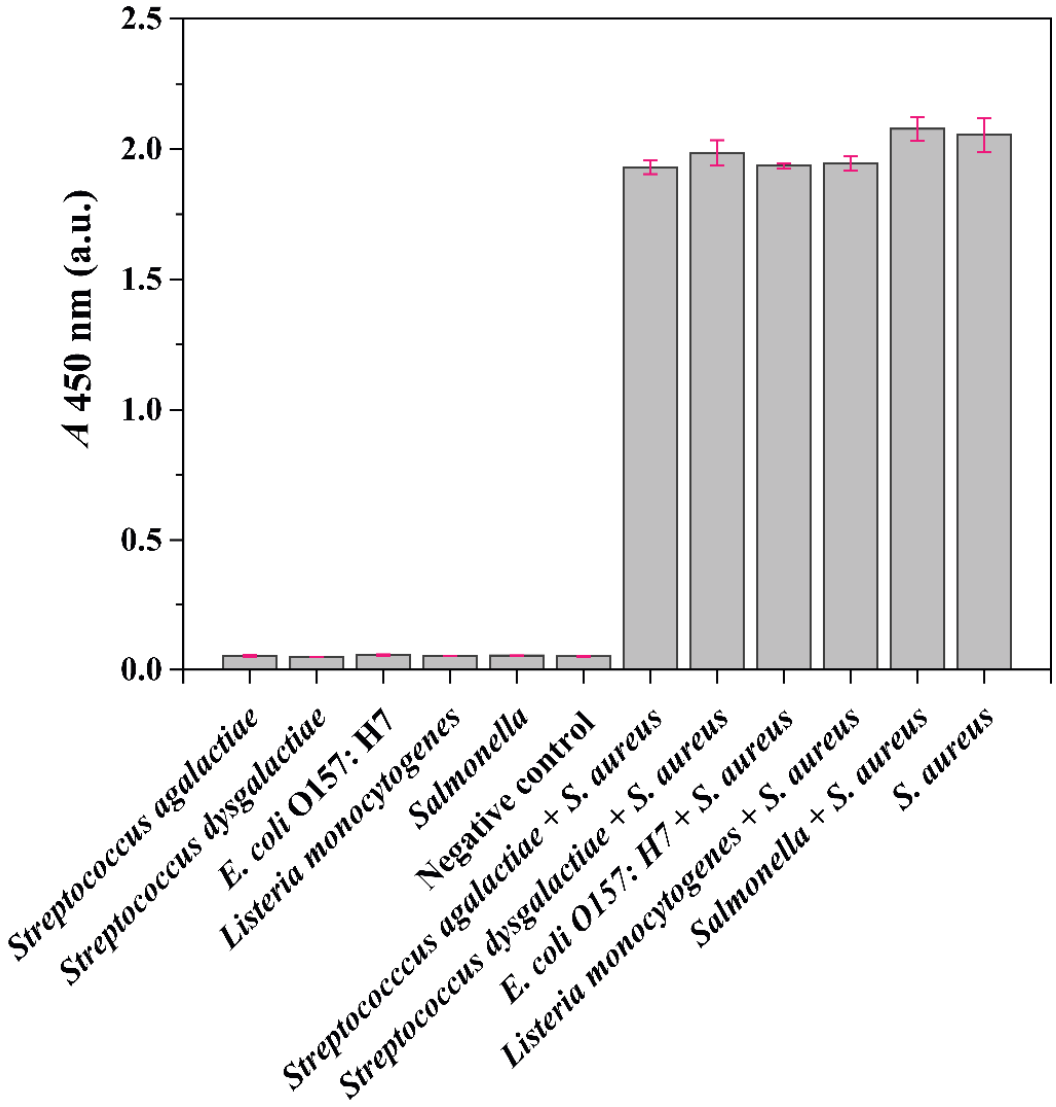

Fig. 4. Absorbance intensity at 450 nm by the developed method for the detection of *S. aureus* and the interfering bacteria. Three independent measurements were taken from three individual preparations for each condition. Error bars indicated the standard deviations.

3.5. Real sample detection

Spiked sodium chloride injection, apple juice and human urine were used as drug, food and clinical samples to access the application potential. All these samples were prepared using the standard addition method. Calibration curves in spiked apple juice was shown in supplementary Fig. S3, S4 and S5. The recoveries were listed in Table 2 and found to range from 89.1 % to 121.1 %, which revealed satisfactory potential of the proposed method in real sample detection.

Table 2 Recovery tests of *S. aureus* spiked in real samples (n = 3)

Samples	Spiked (CFU mL^{-1})	Found (CFU mL^{-1})	RSD (%)	Recovery (%)
Sodium chloride injection	2.0×10^3	1.8×10^3	2.50	89.1
	8.0×10^3	8.3×10^3	2.32	103.2
	2.0×10^4	2.1×10^4	3.04	104.9
Apple juice	2.0×10^3	2.0×10^3	5.58	101.4
	8.0×10^3	8.1×10^3	4.45	101.8
	2.0×10^4	2.1×10^4	4.06	102.8
human urine [a]	2.0×10^3	2.3×10^3	4.78	113.9
	8.0×10^3	9.4×10^3	2.18	118.1
	2.0×10^4	2.4×10^4	3.71	121.1

[a] The sample was 10-time diluted.

In order to compare the proposed method with a common-used method, several real samples (sodium chloride injection, apple juice and human urine) spiked by *S. aureus* were collected to test by the developed method and a standard method enzyme linked immunosorbent assay (ELISA). The results were shown in Table S7 in the supplementary material, which indicated that the results obtained

293 from the method were consistent with those obtained from ELISA. The data proved that the novel
294 colorimetric method offers good precision and has high potential for the analysis of *S. aureus* in various
295 kinds of samples. However, there is a major limitation of the proposed method, which is the presence
296 of IgG in real samples (e.g. serum). In the proposed method, the binding of anti-protein A IgY and
297 HRP-IgG to protein A on the surface of *S. aureus* was adopted, so IgG in samples may interact with
298 protein A on the surface of *S. aureus*, which would cause the capture efficiency of IMBs decreasing
299 rapidly and much less SPA molecules of *S. aureus* left for HRP-IgG recognition.
300
301 **4. Conclusion**
302 Through the dual-recognition mode of SPA, chicken anti-protein A IgY and non-specific
303 mammalian IgG were served as an antibody pair to detect *S. aureus* with high sensitivity and selectivity
304 in this study. Chicken anti-protein A IgY as a capture antibody has highly selective and specific binding
305 to the target bacteria, and non-specific mammalian IgG as a detection antibody to form a sandwich
306 assay. Because one SPA molecule can bind with five mammalian IgG molecules, the sensitivity of the
307 proposed method can be improved. Under optimal conditions, *S. aureus* can be readily detected at an
308 ultralow detection limit **(**1.1×10^2 CFU mL^{-1}**)** in less than 90 min. Moreover, this assay was validated
309 to be compatible with spiked samples, benefited from ideal sensitivity and selectivity. Therefore, this
310 immunoassay provided an effective tool for *S. aureus* detection in various samples (except for IgG
311 containing samples) and exhibited great potentials in developing a commercial kit for *S. aureus*
312 screening.
313
314

Acknowledgments

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Highlights

- Chicken anti-protein A IgY was utilized to capture *S. aureus* with high selectivity.
- HRP-IgG, with high affinity to SPA of *S. aureus*, was adopted for colorimetry.
- 11 CFU of *S. aureus* in 100 μL samples were detected with high specificity.
- No interference from protein G producing *Streptococcus* was observed.
- The method has been applied to detect *S. aureus* in various types of samples.

Credit author statement

Yun Zhang: Conceptualization, methodology, funding acquisition. **Wenqing Tan:** Software, investigation, formal analysis, data curation. **Yang Zhang:** Writing-original draft. **Huili Mao:** Visualization. **Shuyou Shi:** Resources. **Liangwei Duan:** Writing-original draft, funding acquisition. **Hui Wang:** Validation, investigation. **Junping Yu:** Writing-review & editing, supervision.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.