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Universal probe with oriented antibody to improve the immunochromatographic assay of lead ions in *Procambarus clarkii*

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Abstract

Objectives: Based on the information from the random inspection of foods by the China Food and Drug Administration in 2022, the contamination levels of lead ions are high in many edible products. Traditional methods of detecting lead ions cannot meet the requirements of on-site analysis of food due to the need for large equipment. The immunochromatographic assay (ICA) is an effective, rapid, on-site analytical technique for determining lead ions in foods. However, the performance of ICA based on the traditional probe (AuNP-mAb) is limited by ignoring the influence of the antibody orientation.

Materials and Methods: In this study, we developed an efficient technology for constructing a universal probe (AuNP-PrA-mAb) based on the oriented immobilization of antibody. The performance of ICA was largely improved due to specific binding of the Fc region of the antibody with recombinant protein A (PrA) on the surface of a gold nanoparticle (AuNP). The ICA based on a universal probe was applied for the qualitative and quantitative detection of lead ions in *Procambarus clarkii* within 30 min. Meanwhile, a simple and fast pretreatment method based on dilute acid extraction was developed for pretreating the *P. clarkii* containing lead ions.

Results: The visual limit of detection and the scanning limit of quantization of the developed ICA strip for lead ions were 0.5 ng/mL and 0.28 ng/mL, respectively. The sensitivity of ICA based on universal probe was 10-fold higher than that of the ICA using traditional probe. Furthermore, the detection results had no obvious difference between the ICA and ICP-MS with *t*-test statistical method.

Conclusions: The developed ICA based on a universal probe presented broad application prospects in detecting contaminants in foods.

Keywords: Oriented antibody; universal probe; immunochromatographic assay; dilute acid extraction; lead ions.

Introduction

Lead ions are highly toxic and widely distributed in nature. Various foods, including fruits, vegetables, meat, aquatic products, and beverages, may be contaminated by lead ions, which can ultimately accumulate in the human body through the food chain. Excessive intake of lead ions may cause serious damage to the nervous system, hematopoietic system, immune system, and digestive system in humans (Obiri-Nyarko *et al.*, 2021; Si *et al.*, 2021). There are many methods for detecting lead ions in foods, such as graphite furnace atomic absorption spectrometry (Perelonia *et al.*, 2021), flame atomic absorption spectrometry (Altunay *et al.*, 2020; Chaikhan *et al.*, 2022), and inductively coupled plasma mass spectrometer (ICP-MS; Mohamed *et al.*, 2020; Shchukin *et al.*, 2020), all of which have the advantages of high sensitivity and wide application. However, these detection procedures require large apparatus, and thus they are difficult to apply in rapid on-site analyses.

With the development of biochemical technology, rapid detection methods for lead ions have developed rapidly. At present, the common rapid detection methods include electrochemical biosensors (Sridharan and Mandal, 2022; Yin *et al.*, 2022; Zhu *et al.*, 2023), enzyme-linked immunosorbent assay (ELISA; Xu *et al.*, 2020), immunochromatographic assay (ICA; Sun *et al.*, 2018; Li *et al.*, 2020), and so forth. Among them, electrochemical biosensor and ELISA have the advantages of high sensitivity, strong specificity and a wide range of applications. However, the ICA displays better readability due to the visual detection by naked eyes. The ICA can achieve rapid on-site screening for various contaminants in foods, accompanied by the unique advantages of simple procedure, rapid detection, and portable read-out (Lin *et al.*,

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2020; Liang *et al.*, 2022). The performance of the ICA often depends on the construction approach of the probe (Lou *et al.*, 2018). According to the traditional strategy, the antibody is often attached to the nanoparticle surface via passive adsorption for the fabrication of an immune probe (Di Nardo *et al.*, 2019; Chang *et al.*, 2021). The passive adsorption method provides the most direct strategy, but it is relatively random and uncontrolled (Liu *et al.*, 2015; Li *et al.*, 2019). Covalent conjugation is another popular approach. Covalent bonds are mostly formed between the available functional groups that are located at the surface of the antibody and properly modified nanoparticle, thereby producing efficient and irreversible attachment (Tang *et al.*, 2021; Zhou *et al.*, 2021). However, covalent conjugation via randomly distributed active groups results in unspecified antibody immobilization, which further causes partial loss of activity as the active binding sites of the antibody are shielded (Gao *et al.*, 2021). In all the above methods, the antibody is immobilized in a random manner, so the antigen-binding fragment (Fab) region partially loses its access to the antigen.

The favorable orientation of the antibody could be achieved by utilizing its crystallizable fragment (Fc) for solid substrate immobilization in order to expose its Fab. This arrangement maintains antibody activity at its maximum (Iijima *et al.*, 2019; Coluccio *et al.*, 2021; Gao *et al.*, 2022). The recombinant protein A (PrA) is an illustrious immunoglobulin G (IgG)-binding protein that has a specific recognition of the Fc region of the antibody instead of the Fab region. This recognition reaction will not influence the bioactivity of the antibody (Wang *et al.*, 2020; Zhao *et al.*, 2022). At present, there have been many reports on ICA strips based on PrA-orientated antibody for the analysis of toxins and hormones in foods. However, few have been utilized for the detection of heavy metals or analyzed in depth how PrA improved ICA strip performance (Lu *et al.*, 2022; Zhao *et al.*, 2022). In this study, we constructed a universal probe based on PrA for the improvement of ICA for lead ions in *Procambarus clarkii*. It is noteworthy that we attempt to analyze the reason why the universal probe could improve the performance of the strip from the point of antibody orientation and bioactivity in comparison with the traditional probe. Using the Coomassie brilliant blue assay and ELISA, universal and traditional probes were compared for antibody orientation and bioactivity. In addition, the dilute acid extraction method was developed to pretreat the *P. clarkii* samples, which effectively shortened the detection time within 30 min. Qualitative analysis of the proposed ICA was performed by visual inspection, while the quantitative detection was accomplished using the scanner and ImageJ software. Comparisons between this developed ICA system and ICP-MS for lead ions were performed. This developed ICA based on universal probe is capable of being extensively applied to the analysis of various contaminants in foods.

Materials and Methods

Chemicals and equipment

P. clarkii was obtained from a Wuhan local aquatic wholesale market (Wuhan, China). Pb(II), Hg(II), Cd(II), Cr(VI), and As(III) standards were purchased from Guobiao (Beijing) Testing & Certification Co., Ltd. (Beijing, China). The recombinant protein A and polyclonal antibody (pAb, to bind monoclonal antibody) were provided by Shandong Lvdu Biotechnique Co., Ltd. (Binzhou, China). The anti-lead monoclonal antibody (mAb; http://www.wxdtm.com/) and lead antigens (lead ions binding keyhole limpet hemocyanin) were acquired from Wuxi Determine Biotechnology Co., Ltd. (Wuxi, China). Gold nanoparticles were obtained from Nanjing XFNANO Materials Tech Co., Ltd. (Nanjing, China). The horse radish peroxidase (HRP)-labeled Goat Anti-Mouse IgG, TMB (3,3′,5,5′-tetramethylbenzidine) chromogen solution and stop solution for TMB substrate were obtained from Beyotime Biotechnology Co., Ltd. (Shanghai, China). HNO_3, H_2SO_4, HCl, Na_2EDTA, KOH, K_2CO_3, sucrose, Tween-20, and bovine serum albumin (BSA) were purchased from Sigma-Aldrich Co., Ltd. (Urbana, IL, USA). Phosphate buffer solution (PBS; 10 mmol/L, pH 7.4) and carbonate buffer solution (CBS); 0.05 mmol/L, pH 9.6) were provided by Cytiva Co., Ltd. (Shanghai, China). The nitrocellulose membranes (NC; Sartorius CN140), polyvinyl chloride (PVC) backing pad (DB-7), glass fiber membrane (GL-B02), and absorbent pad (H5072) were obtained from Jieyi Biotechnology Co., Ltd. (Shanghai, China).

The XYZ 3050 Dispensing Platform and CM 4000 Guillotine Cutter were obtained from Bio Dot Instrument Co., Ltd. (Irvine, CA, USA). A high-speed refrigerated centrifuge (CF16RX) was purchased from Hitachi (Tokyo, Japan). The vortex oscillator was made by VELP Corporation (Bohemia, NY, USA). The DHG-9123A thermostatic drying chamber was obtained from Yiheng Scientific Instrument Co., Ltd. (Shanghai, China). The SB-5200D ultrasonic cleansing machine was made by SCIENTZ Biotechnology Co., Ltd. (Ningbo, China). The Enspire multimode plate reader was obtained from PerkinElmer Corporation (Waltham, MA, USA). The Python Quartz Crystal Microbalance (QCM) was provided by Boinst Technology Co., Ltd. (Beijing, China). Dynamic light scattering (DLS) was provided by Brookhaven Instrument Corporation (Holtsville, NY, USA).

Construction and characterization of the probes

The universal probe was produced as described by Zhao *et al.* (2022) with minor modification. First, AuNP-PrA conjugate was prepared by a passive adsorption method. Ten microliters of 0.1 mmol/L K_2CO_3 solution and 3 μg of PrA were added to 1 mL gold nanoparticle solution prior to incubation at 37 °C for 30 min, and then 50 μL of 10% BSA was dripped and incubated for another 30 min for blocking. The AuNP-PrA conjugate was purified by centrifugation and resuspended in 1 mL of PBS buffer. To save mAb, the antibody coupling density of the universal probe was optimized by adding a varying molar ratio of mAb to coupled PrA (1, 1.5, 2, 2.5, 3) and determined by the Coomassie bright blue method. Meanwhile, the antibody consumption of the two probes was analyzed by the Coomassie bright blue method. Then, the optimal additive amount of anti-lead mAb was added and incubated at 37 °C for 2 h, resulting in the formation of a universal probe via coupling the AuNP-PrA conjugate with anti-lead mAb. The probe was purified by centrifugation (10 000 r/min, 8 min) and resuspended in 100 μL of running buffer (PBS containing 0.1% Tween-20 and 1% sucrose).

For further comparison, a traditional probe based on passive adsorption was developed as well. Five microliters of 0.1 mmol/L K_2CO_3 and 3.5 μL of 3.5 mg/mL anti-lead mAb were added and incubated at 37 °C for 30 min, followed

by BSA block as aforementioned procedure. This traditional probe was centrifuged at 9000 r/min for 12 min and redissolved in 100 μL of running buffer.

The universal probe (AuNP-PrA-mAb) and traditional probe (AuNP-mAb) were characterized via UV-Vis absorption spectra, QCM, 0.5% agarose gel electrophoresis analysis, and DLS.

Evaluation of antibody orientation and bioactivity of probes

In this section, we evaluated the antibody orientation of the probe by measuring the antigen-binding rate of the universal probe and traditional probe. The antigen-binding rate was the ratio of the immobilized antigens in the probes to the added antigens. In order to determine the antigen-binding rate of these two probes, 38 μL of 0.1 mg/mL antigen solution was added to 1 mL of 2.3 nmol/L AuNP-mAb and AuNP-PrA-mAb probes, respectively. After incubation at 37 °C for 1 h, the mixture were centrifuged at 9000 r/min for 12 min. The residual antigens in the supernatant was measured using the Coomassie brilliant blue assay and the antigen-binding rates of these two probes were determined by evaluating the difference of the antigen before and after the binding reaction.

In this paper, we detected the bioactivity of two probes based on ELISA with minor modification. The 96-well microtiter plate was coated with the lead antigen (1 μg/mL, 100 μL/well) in CBS buffer and incubated for 2 h at 37 °C. The plates were washed three times with PBST (PBS containing 0.05% Tween-20) and blocked with 1% BSA (200 μL/well) for 2 h at 37 °C. After washing, a series of concentrations (0.01, 0.02, 0.05, 0.1, 0.2, and 0.5 mg/mL, 100 μL/well) of probe were added and incubated for 1 h at 37 °C. Then, the washed plates were incubated with HRP-labeled Goat Anti-Mouse IgG (1.25 μg/mL, 100 μL/well), at 37 °C for 1 h, followed by the addition of TMB (100 μL/well) as substrate. After 15 min of incubation, a stop solution for the TMB substrate (100 μL/well) was added, and the absorbance was measured at 450 nm. With the absorbance as the abscissa and probe concentration as the ordinate, two Hill equation curves of the AuNP-PrA-mAb and AuNP-mAb probes were fitted. We evaluated the bioactivity of the probes by comparing the parameter (K_d) of the Hill equation (Shi *et al.*, 2015).

Sample pretreatment

The *P. clarkii* sample was shelled and homogenized. Half a gram of homogenous tissue was weighed into a centrifuge tube. Then, the sample was soaked in 5 mL of 15% nitric acid and ultrasonically extracted for 15 min. After the extraction procedure, the tube with the sample was centrifuged at 11 000 r/min for 10 min. The supernatant was passed through a 0.22-μm membrane to remove impurities. The filtrate collection was added to 18.6 mg of Na_2EDTA and then adjusted to a pH of 7.4 using 1 mol/L KOH.

Fabrication of the ICA strip

During the construction of the strip based on the universal probe, the dosage of AuNP probes and the spray density of lead antigens on T line were optimized together. The spray density of lead antigens on T line was set at 0.2, 0.3, and 0.4 mg/mL and the dosage of AuNP probes was set to 6, 7, and 8 μL. Under the corresponding conditions, ICA strips were used to detect lead ion standard solutions with concentrations of 0 ng/mL and 5 ng/mL. We took into account both the color development of the T line and the detection sensitivity as the optimum condition.

The pAb (0.5 mg/mL) and lead antigen of optimized spray density were sprayed onto the NC membrane as the control (C) line and test (T) line at a speed of 0.6 μL/cm using the dispensing platform instrument. The distance between the C and T lines was set to be 8 mm. The NC membrane was attached to the PVC baseplate and dried at 37 °C for 2 h. Next, the absorption and sample pads were sequentially pasted on both sides of the PVC baseplate with 2 mm overlaps with the NC membrane. Finally, the packed PVC baseplate was cut into 4.5 mm ICA strips and kept under a dry condition before use. The ICA strip based on the traditional probe was also constructed by the similar method as above, in which the spray density of lead antigens on T line was 0.4 mg/mL and the dosage of AuNP probes was 10 μL.

The procedure for the ICA strip

The optimal dosage of the universal probe and 50 μL of analyte were added to running buffer to the mixture volume of 200 μL. The test strip was inserted into the above mixture and the color of the test strip was observed after 15 min of incubation at 37 °C for qualitative analysis, while the grayscale (GS) of the T and C lines was recorded by a scanner and ImageJ software for quantitative detection (Figure 1). Sample extraction (50 μL) was diluted 4-fold with running buffer to mitigate the matrix influence (Figure S3) and spiked with distinct concentrations (0, 0.1, 0.2, 0.5, 1, 2, 5, 10, and 20 ng/mL) of lead ions before being detected three times using the test strips. Visual limit of detection (vLOD) was defined as the minimum concentration at which the T line was significantly shallower than the negative control. The cut-off value was the minimum concentration where the T line completely disappeared. The ICA strip based on the universal probe was quantitatively analyzed using the scanner and ImageJ software. The GS_T/GS_C values of the negative control and spiked samples were defined as B_0 and B, respectively. The standard curve of the ICA strip based on the universal probe was constructed with B/B_0 as the ordinate and lead ions concentration logarithm as the abscissa. The scanning limit of quantization (sLOQ) was defined as the lead ion concentration corresponding to the B_0 mean plus 10-fold standard deviation (SD) of the negative samples (Pang *et al.*, 2022).

Specificity, accuracy, and practicability verification of the ICA strip

The specificity, accuracy, and practicability of the ICA strips based on the universal probe were determined. To evaluate the specificity of the ICA strip, 5 ng/mL standard solutions of Pb(II), Cd(II), Cr(VI), Hg(II), As(III), and the mixture of these five heavy metal ions were tested for cross-reactivity. The recovery was studied to evaluate the accuracy of the developed ICA strips. The spiked sample extract with distinct concentrations (0.1, 0.2, 1, and 2 ng/mL) was detected six times using the proposed test strips. The practicability of the ICA strip was evaluated by analyzing the spiked sample extracts with varying concentrations (0.3, 0.6, 1.2, and 1.8 ng/mL). Each sample was determined by the proposed ICA strips and ICP-MS with three replicates. The results of both methods were compared by *t*-test.

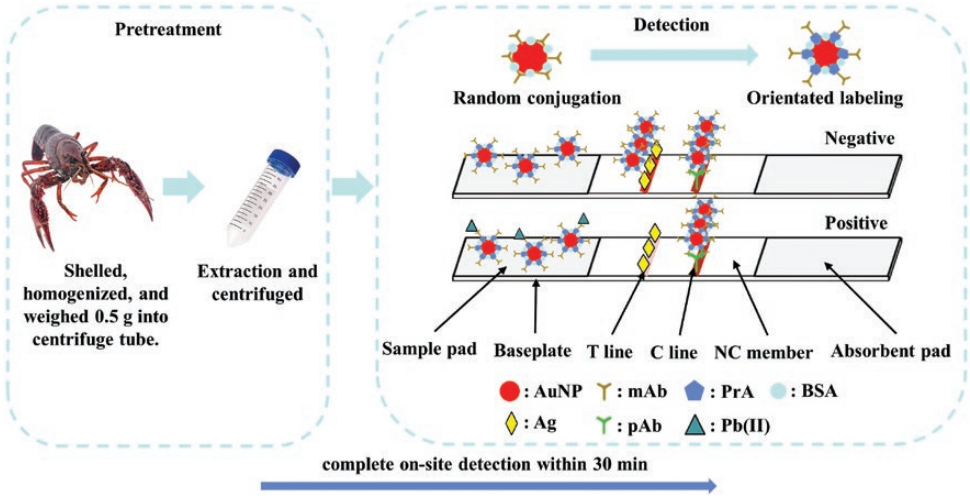

Figure 1. Procedure illustration for the immunochromatography assay strip.

Results and Discussion

Characterization of probes

Antibody with twice the molar concentration of coupled PrA was added into AuNP-PrA conjugate for the construction and characterization of the universal probe (Figure S1A). The antibody consumption of the universal and traditional probe is shown in Figure S1B. Due to the introduction of PrA as bridge protein, the antibody consumption of the universal probe was 12% less than that of the traditional probe, indicating that the PrA-oriented labeling method was more economical than traditional passive adsorption. We detected the QCM response of AuNP-PrA-mAb and AuNP-mAb probes (Figure 2A). The QCM frequency of these two probes decreased, because the mass of the probe increased after binding with the PrA and mAb. The UV-Vis absorption spectra exhibited maximum absorbance at wavelengths of 520, 527, 526, and 532 nm for AuNP, AuNP-mAb, AuNP-PrA, and AuNP-PrA-mAb conjugates, respectively (Figure 2B). The maximum absorbance of the AuNP-mAb and AuNP-PrA-mAb probes had a significant redshift in comparison to the free AuNP. This indicated that the prA and mAb were sequentially coated on the surface of the AuNP. As shown in Figure 2C, compared with the moving speed of free AuNP, the moving rates of AuNP-mAb, AuNP-PrA, and AuNP-PrA-mAb in agarose gel electrophoresis gradually decreased. This is due to the increased size of the probe and the reduced negative surface charge after binding the protein. In DLS characterization, the diameter size of the AuNP-mAb and AuNP-PrA-mAb probes increased compared to that of AuNP (Figure 2D). This finding indicated that PrA and mAb were successfully coated on the surface of AuNP. These results demonstrated that AuNP-PrA-mAb and AuNP-mAb probes were successfully prepared.

Analysis of antibody orientation and bioactivity of probes

The higher antigen-binding rate indicated better antibody orientation of the probe. As shown in Figure 3A, the antigen-binding rate of AuNP-PrA-mAb probe was higher than that of AnNP-mAb, because the AuNP-PrA-mAb probe immobilized more oriented antibodies that could bind antigens effectively. This result indicated that the antibody orientation of the AuNP-PrA-mAb probe was better. Additionally, we utilized the Hill equation to evaluate the bioactivity of the probes. In this equation, a lower K_d value indicates the stronger bioactivity of the probe. As shown in Figure 3B, the K_d value of the AuNP-PrA-mAb probe was much lower than that of the AuNP-mAb probe, showing that the bioactivity of the AuNP-PrA-mAb probe was stronger. In the AuNP-mAb probe, the antibody was randomly labeled and might be partly deactivated, resulting in decreased antibody orientation and bioactivity. In contrast, the universal probes were capable of exposing more antigen-binding sites owing to the oriented immobilization of antibody, giving rise to enhanced antibody orientation and bioactivity.

Optimization of sample pretreatment

Sample pretreatment was a pivotal process in the analysis method. ICA is a rapid assay method that requires convenient pretreatment. As the traditional extraction process of lead ions from aquatic products is complex and time-consuming, a dilute acid extraction method was established to simply and rapidly extract lead ions from *P. clarkii*. The extraction solution (HCl, H_2SO_4, HNO_3), concentration (2%, 5%, 10%, 15%, 20%), and extraction time (5, 10, 15, 20, 25, and 30 min) were optimized to determine the ideal reaction conditions. In the optimization of extraction reagent types, the concentration of extraction reagents was set at 20%. We found that HNO_3 was the optimal extraction solution according to the recovery rate (Figure 4A). Then, we further optimized the extraction concentration of HNO_3. As shown in Figure 4B, the recovery rate reached a maximum when the concentration of HNO_3 was 15%. Thus, we used 15% HNO_3 to optimize the extraction time. When the extraction time was increased to 15 min, the recovery rate reached a maximum (Figure 4B). In summary, the extraction conditions were determined as follows: the extraction reagent was HNO_3, the reagent concentration was 15%, and the extraction time was 15 min.

Optimization of the ICA strip

The optimization of the ICA strip based on a universal probe was similar to the checkerboard method in ELISA. We found that when the dosage of AuNP probes was 7 μL and the spray density of lead antigens on the T line was 0.3 mg/mL, the test

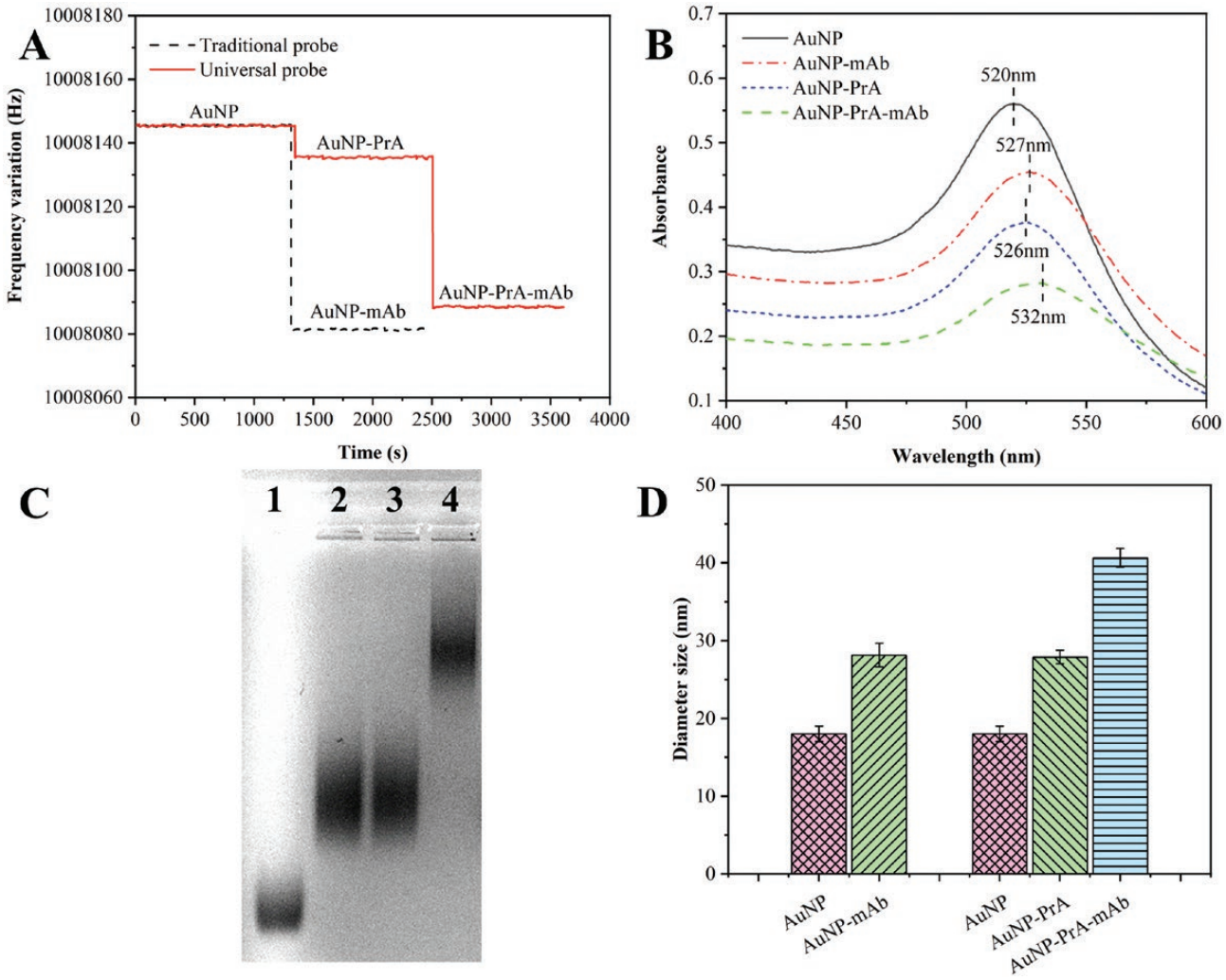

Figure 2. (A) Quartz Crystal Microbalance characterization of probes (AuNP-mAb and AuNP-PrA-mAb), AuNP-PrA, and free AuNP. (B) UV-Vis absorption spectra of probes, AuNP-PrA, and free AuNP. (C) Agarose 0.5% gel electrophoresis analysis of the probes, AuNP-PrA, and free AuNP, lane 1 represents free AuNP, lane 2 represents AuNP-mAb, and lanes 3 and 4 represent AuNP-PrA and AuNP-PrA-mAb. (D) Diameter size distribution of probes, AuNP-PrA, and free AuNP.

strip based on the universal probe could ensure both obvious color development of the T line and good sensitivity (Figure S2).

Analytical performance of the ICA strip

In this study, vLOD was used to evaluate the sensitivity of the developed methods. As illustrated in Figure 5A, an increase in lead ions was accompanied by a decrease in the color of the T line. The vLODs of the ICA strip based on the universal probe and the traditional probe were 0.5 ng/mL and 5 ng/mL, respectively. Correspondingly, the cut-off values were 5 ng/mL and 10 ng/mL, respectively. Because the antibody orientation and bioactivity of the universal probe were stronger than those of the traditional probe, the sensitivity of the ICA strip based on the universal probe was better. Some published ICA methods for lead ion detection are compared in Table 1. It can be seen that the developed ICA strip accompanied by oriented antibody has great advantages. The linear dynamic range of the quantification detection of the ICA strip based on the universal probe was 0.1 to 2 ng/mL with an acceptable correlation coefficient (R^2=0.991; Figure 5B). The sLOQ of the ICA strip based on the universal probe for lead ions was 0.28 ng/mL. The sLOQ of the ICA strip using a universal probe met the requirement for the quantitative detection of lead ions in *P. clarkii* (Mo *et al.*, 2021).

The specificity of the designed ICA strip was evaluated with four other common heavy mental ions, including Cd(II), Cr(VI), Hg(II), and As(III). The results illustrated that Pb(II) could be specifically identified by the ICA strip, and there was no cross-reaction with other common metal ions, suggesting that the proposed ICA strip exhibited excellent selectivity for lead ions (Figure 6). Table 2 displays the recoveries of lead ions in the spiked samples, which were quantitatively analyzed by the proposed ICA strip based on the universal probe. The mean recovery ranged from 97.5% to 106.0%, suggesting that the developed ICA strip possessed good accuracy.

Method verification of the ICA strip

Procambarus clarkii samples spiked with different concentrations of lead ions were used to evaluate the practicability of the ICA strip based on the universal probe. As shown in Table 3, the results had no significant difference between the ICA strip and ICP-MS with the *t*-test statistical method ($|t|$: 0.032, $t_{\alpha/2}$: 1.943, $|t|<t_{\alpha/2}$), which confirmed the reliability and practicability of the ICA method. Thus, the ICA strip based

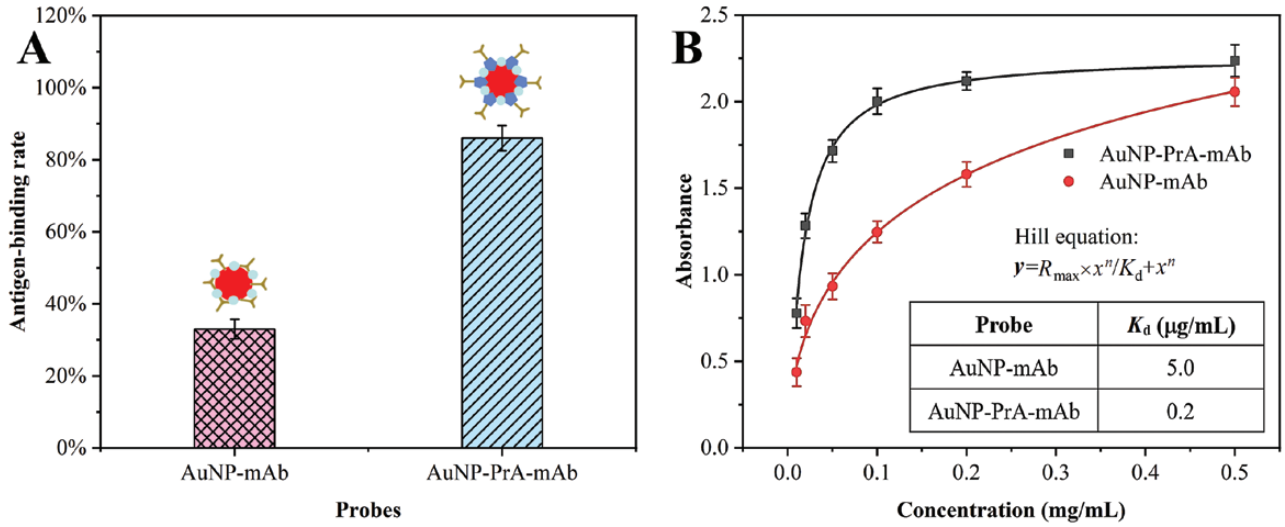

Figure 3. (A) Antigen-binding rate of two probes; (B) Hill equation curve and K_d of probes (K_d: dissociation equilibrium constant).

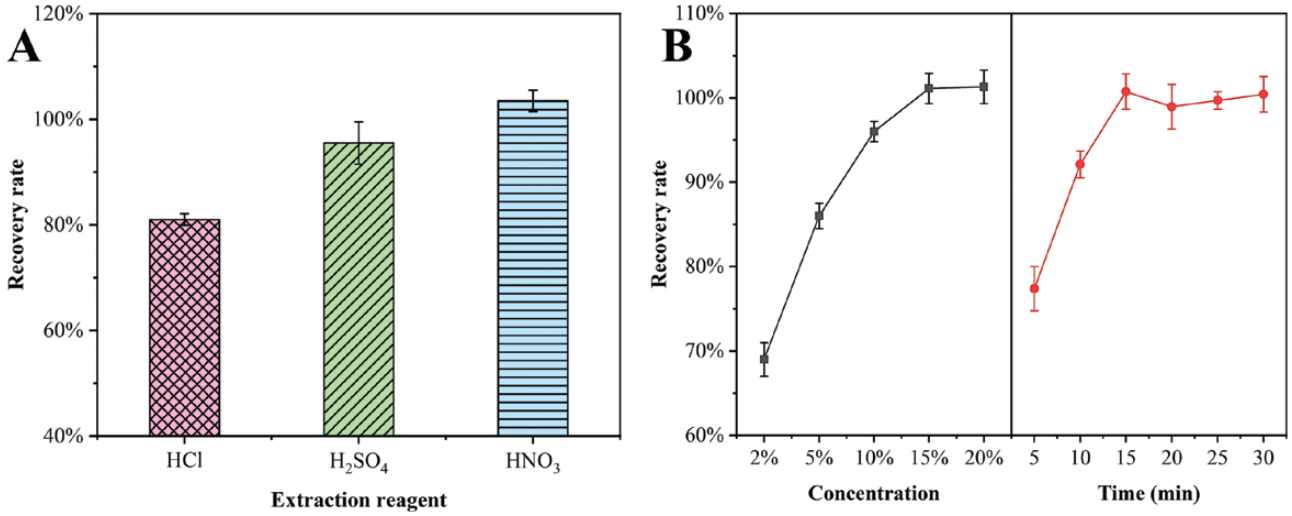

Figure 4. (A) Optimization of extraction reagent; (B) optimization of HNO_3 concentration and extraction time of 15% HNO_3.

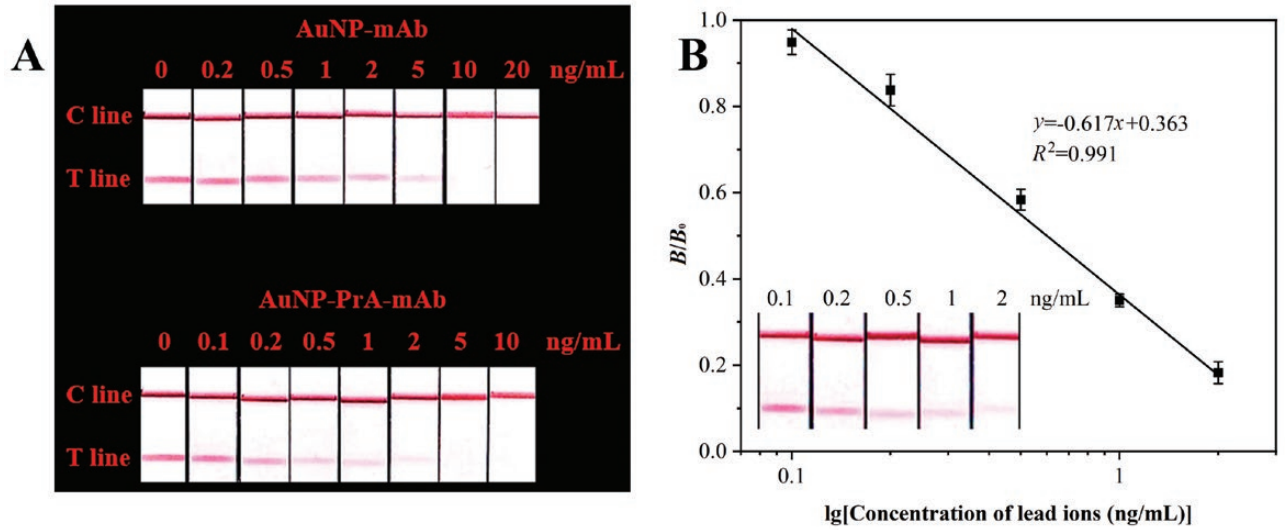

Figure 5. (A) Sensitivity of the immunochromatography assay (ICA) strip. (B) The standard curve of lead ions of the ICA strip based on the universal probe.

on the universal probe that was established in this experiment could be effectively applied for the on-site screening of lead ions in *P. clarkii*.

Conclusions

We have developed a universal probe with oriented antibodies to improve the ICA performance for the detection of lead ions. In comparison to the traditional probe synthesized by passive adsorption, this proposed universal probe possesses several appealing features. First, the introduction of the PrA enhances the number of the oriented antibodies on AuNP, which is probably beneficial for improving the sensitivity. Second, the AuNP-PrA-mAb probe could be readily adapted for the detection of various analytes by conjugating to the corresponding mAb, which avoids the trouble in preparing multiple immune probes. The developed ICA strip could detect the lead ions in *P. clarkii* within 30 min under optimal conditions. Our results suggest that the proposed ICA strip based on universal probes could be used as a reliable, simple, and sensitive measuring tool for the on-site detection of lead ions in food. However, the limitation that still requires improvement is that the PrA on the universal probe was randomly labeled, which results in the loss of some antibody binding sites of PrA.

Table 1. Comparison of the immunochromatography assay strip for the detection of lead ion

Label	Probe	Visual limit of detection (ng/mL)	Reference
AuNP	AuNP-mAb	30	Tang *et al.*, 2010
AuNP	AuNP-mAb	10	Sun *et al.*, 2018
Dual AuNP	Dual AuNP-mAb	2	Kuang *et al.*, 2013
AuNP	AuNP-PrA-mAb	0.5	Our method

Dual AuNP: two heterogeneously sized gold nanoparticles.

Supplementary material

Supplementary material is available at *Food Quality and Safety* online.

Author Contributions

Conceptualization and methodology: Zhengwei Zhu, and Lin Xu; Validation: Yangyang Li, Shuo Duan, and Qiao Wang; Resources: Lin Xu; Data curation: Yangyang Li and Wenli Qu; Writing original draft preparation: Yangyang Li; Writing, review and editing: Yan Liu, Qing Yang, Jine Wu, and Zhiyong Gong. All authors have read and agreed to the published version of the manuscript.

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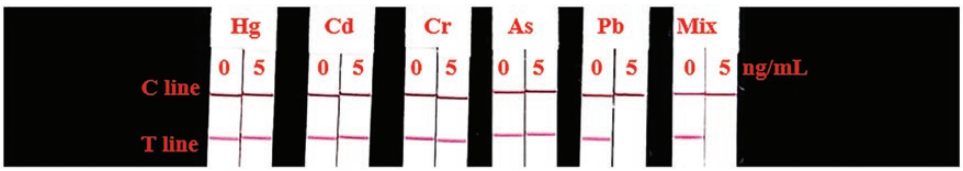

Figure 6. Specificity analysis of the immunochromatography assay strip using the universal probe.

Table 2. Accuracy test results of the immunochromatography assay strip based on the universal probe (*n*=6)

Spiked (ng/mL)	Found (ng/mL)	Recovery (%)	RSD (%)
0.1	0.106	106.0	6.5
0.2	0.205	102.5	6.5
1	0.975	97.5	5.7
2	1.993	99.7	4.5

Table 3. Results of the immunochromatography assay (ICA) strip of the universal probe compared with inductively coupled plasma mass spectrometer (ICP-MS) in *Procambarus clarkii* (*n*=3)

Sample	Spiked (ng/mL)	Mean (ng/mL)±SD (ng/mL)	
		ICA	ICP-MS
Procambarus clarkii	0.3	0.296±0.013	0.311±0.016
	0.6	0.602±0.041	0.617±0.026
	1.2	1.195±0.066	1.209±0.054
	1.8	1.787±0.086	1.803±0.068

Conflict of Interest

The authors declare no conflict of interest.

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