

# Development of a sandwich ELISA and immunochromatographic strip for the detection of shrimp tropomyosin

Lu Zeng<sup>a</sup>, Shanshan Song<sup>a</sup>, Qiankun Zheng<sup>b</sup>, Pengjie Luo<sup>c\*</sup>, Xiaoling Wu<sup>a\*</sup> and Hua Kuang<sup>a</sup>

<sup>a</sup>State Key Lab of Food Science and Technology, School of Food Science and Technology, Jiangnan University, Wuxi, People's Republic of China; <sup>b</sup>Delishi Group, Weifang, People's Republic of China; <sup>c</sup>China National Center for Food Safety Risk Assessment, NHC Key Laboratory of Food Safety Risk Assessment, Beijing, People's Republic of China.

## ABSTRACT

Shrimp tropomyosin is one of the most important causes of shellfish allergy. The objective of this study was to develop a highly sensitive and specific immunoassay for the detection of shrimp tropomyosin in food. Ten monoclonal antibodies against shrimp tropomyosin were obtained by fusion and cell screening. We found two optimum monoclonal antibodies (mAbs); mAb 2 was used as the capture antibody, and HRP-labelled mAb 3 was used as the detection antibody. Using this pair of mAbs, a sandwich enzyme-linked immunosorbent assay (ELISA) was developed with the limit of detection (LOD) of 0.45 ng/mL. Cross-reactivity with other food allergens using this method was found to be negligible. An immunochromatographic assay strip was also developed for the rapid detection of shrimp tropomyosin with the LOD of 0.5 ng/mL. The results demonstrated that our developmental methods represent a useful tool for the detection of shrimp tropomyosin in food.

## ARTICLE HISTORY

Received 2 April 2019  
Accepted 14 April 2019

## KEYWORDS

Shrimp tropomyosin;  
monoclonal antibody;  
sandwich ELISA;  
immunochromatographic  
assay strip

## Introduction

There is a growing health concern over food allergies, which are abnormal reactions of the immune system to particular foods, and can affect up to 8% of children and approximately 2% of adults (Jiang et al., 2016). In 1999, the Joint FAO/WHO Codex Alimentary Commission Session recommended labelling eight kinds of foods containing allergenic ingredients (Seiki et al., 2007). Fourteen allergenic ingredients have been listed to food labels by the European Union Legislation (Koizumi, Shirota, Akita, Oda, & Akiyama, 2014). Shrimp tropomyosin, which belongs to one of the “big eight”, has become the most prevalent allergen in shellfish (FaberFaber et al., 2017). Shrimp tropomyosin is a water soluble, heat

**CONTACT** Pengjie Luo  pengjieluo@cfsa.net.cn  China National Center for Food Safety Risk Assessment, NHC Key Laboratory of Food Safety Risk Assessment, Beijing, People's Republic of China; Xiaoling Wu  wuxiaoling\_8@163.com  
\*Equally contributed authors.

© 2019 The Author(s). Published by Informa UK Limited, trading as Taylor & Francis Group

This is an Open Access article distributed under the terms of the Creative Commons Attribution-NonCommercial-NoDerivatives License (<http://creativecommons.org/licenses/by-nc-nd/4.0/>), which permits non-commercial re-use, distribution, and reproduction in any medium, provided the original work is properly cited, and is not altered, transformed, or built upon in any way.

stable protein with a molecular weight ranging from 36 to 38 kDa, and is associated with IgE-mediated hypersensitivity (Werner, Faeste, & Egaas, 2007). Shrimp tropomyosin can lead to a variety of clinical symptoms such as dermatitis, diarrhea, asthma, and intestinal syndrome (Du Toit et al., 2018; Scurlock, 2018; Sicherer & Leung, 2007; Sicherer & Sampson, 2018). Severe, responses to shrimp tropomyosin can cause anaphylactic shock and even endanger life. Therefore, a highly-efficient and specific analytical method for its detection and quantification is critical to ensure food safety and to improve public health.

Currently, there are a large number of analytical methods for the detection of trace amounts of shrimp tropomyosin in foodstuffs. Polymerase chain reaction (PCR) methods have been widely employed for the detection of food allergens (Eischeid, 2016; Eischeid & Stadig, 2018; Fernandes, Costa, Oliveira, & Mafra, 2018; Herrero, Vieites, & Espineira, 2014). These DNA-based methods are highly sensitive with a wide linear range for shrimp DNA. However, there are disadvantages, such as the complicated operation process, time-consuming analyses, and high false-positive rate, to these methods. High performance liquid chromatography coupled with tandem mass spectrometry has also been developed to accurately quantify food allergens (Chen, Pan, & Huang, 2017; Nagai, Minatani, & Goto, 2015; Rahman, Lopata, Randell, & Helleur, 2010). However, it is difficult to perform on-site detection, or use as a high-throughput screen because of the complicated sample pre-treatment procedures, expensive equipment, and trained personnel needed. Furthermore, various biosensors exhibiting high sensitivity and good specificity have been designed and developed to determine shrimp tropomyosin (Jiang et al., 2013; Jiang et al., 2015; Jiang et al., 2019; Tabrizi, Shamsipur, Saber, Sarkar, & Ebrahimi, 2017; Wang, Rao, Zhou, Zheng, & Fu, 2019). However, it also requires sophisticated sample preparation steps and expensive equipment. Therefore, increasing attention has focused upon monoclonal antibody (mAb)-based immunoassays (Peng, Song, Liu, Kuang, & Xu, 2015; Werner, Faeste, Levsen, & Egaas, 2011). Enzyme-linked immunosorbent assay (ELISA), an analytical method which is based on antibody-antigen interactions, has been widely used in the detection of shrimp tropomyosin due to its high sensitivity, specificity, and high sample throughput (Z. Li, Zhang, Lin, Haider, & Jiang, 2010). A sandwich ELISA based on a polyclonal rabbit anti-shrimp antibody has been successfully developed for the quantification of tropomyosin in eight shrimp species in Taiwan (Lin, Lee, Huang, Sheu, & Yu, 2018). Although this method showed high sensitivity and specificity, it was still limited by the fact that the polyclonal antibody had poor reproducibility and stability. Immunochromatographic assay strips have been widely employed for semi-quantitative, qualitative detection, and mass sample screening due to their sensitivity and convenience (Kong, Xie, Liu, Song, Kuang, & Xu, 2017; Kong, Xie, Liu, Song, Kuang, Cui et al., 2017; Lei, Xu, Song, Liu, & Kuang, 2018; Li, Wu, Song, Zheng, & Kuang, 2019).

In this study, we successfully produced mAbs against shrimp tropomyosin, and a highly sensitive and specific sandwich ELISA method and immunochromatographic assay strip were developed for the determination of shrimp tropomyosin. The experimental results showed that this sandwich ELISA method and immunochromatographic assay strip not only exhibited high sensitivity and specificity, but also showed high-throughput accuracy, demonstrating that these methods have great potential for food safety evaluation.

## Materials and methods

### Reagents and materials

Freund's complete adjuvant, Freund's incomplete adjuvant, egg albumin (OVA), and bovine serum albumin (BSA) were purchased from Sigma-Aldrich (Shanghai, China). Gelatin, goat anti-mouse IgG antibody, and horseradish peroxidase (HRP) were supplied from Aladdin Chemistry Co., Ltd. (Shanghai, China). Other analytical grade reagents were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Nitrocellulose (NC) membranes (Pura-bind RP) were obtained from Whatman-Xinhua Filter Paper Co. (Hangzhou, China), and polyvinyl chloride (PVC) backing materials, sample pad, and absorbance pad were supplied by Goldbio Tech Co. (Shanghai, China). Shrimp was purchased from the local supermarket.

### Solutions

The buffers used in this study included (1) PBS buffer (0.01 M phosphate-buffered saline, pH 7.4), (2) PBS buffer (0.05 M phosphate-buffered saline, pH 7.4), (3) coating buffer (0.01 M sodium carbonate buffer, pH 9.6), (4) blocking buffer (coating buffer containing 0.2% w/v gelatin), (5) wash buffer (PBS buffer containing 0.05% v/v Tween-20), (6) antibody dilution buffer (PBS buffer containing 0.05% v/v Tween-20 and 0.1% w/v gelatin), (7) stop buffer (2 M sulfuric acid), (8) substrate solution (10 mL of 0.1 M citrate phosphate buffer which contained 1.8  $\mu$ L of 30% hydrogen peroxide mixed with 2 mL of 0.06% w/v TMB in glycol), and (9) 0.05 M carbonate buffer (pH 10.0).

### Preparation of shrimp tropomyosin

Shrimp tropomyosin was obtained using the method, as reported previously (Peng et al., 2013). The shrimp (20 g) were homogenized and degreased with petroleum ether (W/V 1:10). After stirring overnight at 4°C, the mixture was centrifuged at 6000 g for 15 min. The supernatant was discarded, and 0.05 M PBS buffer (W/V 1:20) was added to the pellet and left overnight. After centrifugation at 4°C for 20 min at 7000 g, the supernatant was transferred into a beaker and precipitated with 30% ammonium sulfate. After centrifugation at 4°C for 20 min at 8000 g, the supernatant was precipitated for a second time with ammonium sulfate, reaching a final concentration of 50%. After centrifugation at 4°C for 20 min at 8000 g, the supernatant was precipitated further with ammonium sulfate, reaching a final concentration of 70%. The precipitate was resuspended with 0.01 M PBS buffer and dialyzed against 0.01M PBS for 3 days. Following dialysis, the crude shrimp tropomyosin extract was purified further using gel filtration chromatography. The total protein content was determined by the Bradford assay, and the protein was confirmed by SDS-PAGE.

### Preparation of monoclonal antibodies against shrimp tropomyosin

The purified shrimp tropomyosin was used as the immunogen in mice to generate antibodies against shrimp tropomyosin. The mouse with the highest serum antibody titer was sacrificed, and its spleen was fused with Sp2/0 murine myeloma cells. The target

cells were selected by an indirect ELISA and obtained by the limiting dilution method. The mAbs against shrimp tropomyosin were purified by the caprylic acid-ammonium sulfate precipitation method.

### ***Labelling of monoclonal antibodies against shrimp tropomyosin***

The purified mAbs were conjugated to HRP using the modified sodium periodate method, as reported previously (Kuang et al., 2013). Firstly, HRP was oxidized with 0.06 M NaIO<sub>4</sub> for 30 min at 4°C, the reaction was terminated by adding 0.16 M glycol for 30 min at room temperature. Secondly, the purified antibody was added into the mixture, and the system was adjusted at pH 9.0 using 0.05 M carbonate buffer (pH 10.0) and incubated for 20 h at 4°C. After this, the stable HRP-labelled mAb was obtained by adding NaBH<sub>4</sub> for 2 h at 4°C. Finally, the conjugated antibody was precipitated by adding an equal volume of saturated ammonium sulfate solution. After incubating for 2 h at 4°C, the mixture was centrifuged, and the sediment was resuspended with 0.01 M PBS. The HRP-labelled mAb was dialyzed with 0.01M PBS for 3 days.

### ***Indirect ELISA***

An indirect ELISA was developed to detect serum titers and select hybridoma cell lines. A 96-well microplate was coated with shrimp tropomyosin standards diluted in coating buffer (100 µL/well) and incubated at 37°C for 2 h. The plate was then washed three times with washing buffer. After washing, 200 µL of blocking buffer was added to each well and incubated at 37°C for 2 h. Next, 100 µL of mouse serum or cell supernatant diluted with antibody dilution buffer was added to each well and incubated for 30 min at 37°C. After washing, 100 µL of HRP-labelled goat anti-mouse IgG diluted with antibody dilution buffer was added to each well, and the plate was incubated for 30 min at 37°C. After washing the plate three times, 100 µL of substrate solution was added to each well and incubated for 15 min at 37°C in the dark. The reaction was stopped with the addition of stop buffer (50 µL/well) and the absorbance was measured at 450 nm.

### ***Development of the sandwich ELISA***

A 96-well microplate was coated with anti-shrimp tropomyosin mAb (4 µg/mL) diluted in coating buffer (100 µL/well) overnight at 4°C. The microplate was then washed three times with wash buffer. After the plate was washed, blocking buffer was added (200 µL/well) and incubated at 37°C for 2 h. After washing, the standard solution was added (100 µL/well) and the plate was incubated for 1 h at 37°C. After washing, HRP-labelled anti-shrimp tropomyosin mAb (2 µg/mL) was added (100 µL/well) for 1 h at 37°C. After washing, the substrate solution (50 µL/well) was added for 15 min at 37°C. Then, the reaction was stopped by the addition of stop buffer. The absorbance was measured at 450 nm.

### ***Pairwise interaction analysis***

To obtain the optimal mAb pairing for the sandwich ELISA for the detection of shrimp tropomyosin, anti-shrimp tropomyosin mAbs, which were generated separately from 10

cell lines and HRP- labelled mAbs, were analyzed in a sandwich ELISA. The capture antibody was paired with the other nine detection antibodies. The shrimp tropomyosin standard of 500 ng/mL (100  $\mu$ L/well), and 0.01M PBS (100  $\mu$ L/well) were added as positive and negative controls, respectively. The sandwich ELISA procedure was the same as mentioned above. The highest OD<sub>450</sub> ratio for the positive and negative controls (P/N value) was regarded as the optimal pair.

### **Optimization of the sandwich ELISA**

To obtain the optimal concentration of capture antibody and detection antibody in the sandwich ELISA, a checkerboard method was designed. Briefly, the plates were coated separately with capture antibody diluted to 8, 4, 2, and 1  $\mu$ g/mL. After blocking, 100  $\mu$ L of 500 ng/mL shrimp tropomyosin standard and 100  $\mu$ L of 0.01M PBS were added as positive and negative controls, respectively. For detection, a series of concentrations ranging from 8  $\mu$ g/mL to 1  $\mu$ g/mL was added. The highest OD<sub>450</sub> ratio for the positive and negative controls (P/N value) was regarded as the optimal pair.

### **Cross-reactivity**

The cross-reactivity was measured to determine the mAb specificity. Other food allergens including Casein, OVA, soybean globulin,  $\beta$ -conglycinin, wheat gliadin, Ara H1, Ara H2, parvalbumin, and cashew nut crude extract were analyzed by our sandwich ELISA.

### **Immunochromatographic assay strip for the detection of shrimp tropomyosin**

The immunochromatographic assay strip was developed and assembled in our laboratory as previously described (Song et al., 2019). The NC membrane was pasted onto the center of the PVC backing card. The sample and absorption pads were glued to the bottom and upper section of the PVC backing card with a 2-mm overlap of the NC membrane. Goat anti-mouse IgG and mAb 2 were sprayed onto the NC membrane (1  $\mu$ L/cm) as the control line and test line (C line and T line), respectively. After drying at 37°C, the card was cut into 3-mm wide individual test strips and stored in a desiccator until use.

Standard solution or sample solution (150  $\mu$ L) was mixed with 50  $\mu$ L of colloidal gold-labelled mAb 3 and allowed to react at room temperature for 5 min. The strips were then inserted into the reaction buffer, which was allowed to migrate by capillary action from the sample pad, where shrimp tropomyosin was combined with the colloidal gold-labelled mAb 3. Here, it reacted with mAb 2 on the T line and the goat anti-mouse IgG antibody on the C line, finally reaching the absorption pad. For the shrimp tropomyosin positive samples, both the red T line and the C line were visible. As the concentration of shrimp tropomyosin decreased, the colour intensity of the T line gradually decreased, and when the concentration of shrimp tropomyosin went below a threshold value, no colour appeared on the T line at all. In the shrimp tropomyosin negative samples, only a red C line was seen. The sensitivity of the immunochromatographic assay strip was determined using serial dilutions of the shrimp tropomyosin.

### ***Food sample preparation and analysis***

Minced fish that did not contain shrimp tropomyosin was used for spiking experiments. Shrimp tropomyosin was added to 1 g of minced fish at concentrations of 1, 5, and 20 ng/g. The mixture was added in 10 mL of 0.01M PBS and mixed. After centrifugation at 7000 g for 10 min, the supernatant was diluted ten times with 0.01M PBS to final concentrations of 0.1, 0.5, and 2 ng/g. The spiked sample was measured using our sandwich ELISA.

Food samples purchased from a local supermarket were prepared to test the usefulness of the system, and extracts were obtained as previously described. Briefly, each food sample was homogenized and degreased with petroleum ether. The supernatant was discarded, and 20 mL of 0.05M PBS buffer was added to the pellet and stirred overnight at 4°C. After centrifugation at 4°C for 20 min at 7000 g, the supernatant was serially diluted with 0.01 M PBS and measured using either the sandwich ELISA or strips.

## **Results and discussion**

### ***Characterization of shrimp tropomyosin***

The shrimp tropomyosin was characterized by SDS-PAGE. [Figure 1](#) shows that there was an obvious protein band between 36 and 38 kDa in the shrimp tropomyosin crude extract precipitated with 50% ammonium sulfate. The 50% saturated ammonium sulfate component was further purified using gel filtration chromatography. The chromatogram of the purified shrimp tropomyosin is shown in [Figure 2](#). The expected bands for the final

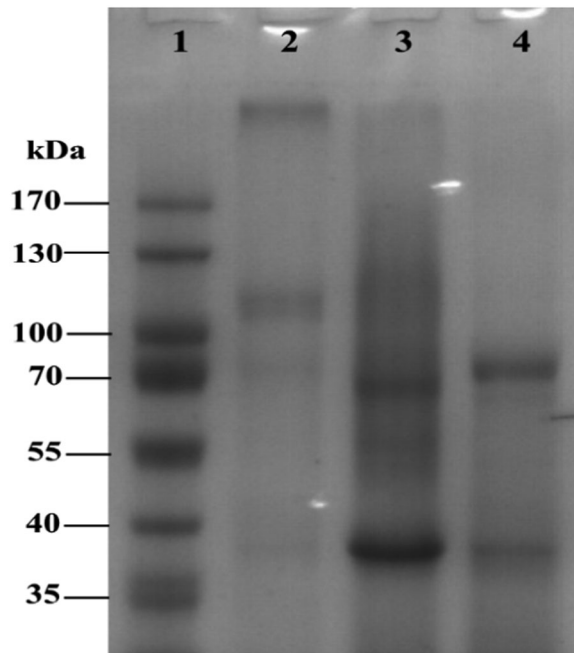


**Figure 1.** SDS-PAGE of 30,50, and 70% saturated ammonium sulfate component. Lane 1, Marker; Lane 2, 30% saturated ammonium sulfate component; Lane 3, 50% saturated ammonium sulfate component; and Lane 4, 70% saturated ammonium sulfate component.

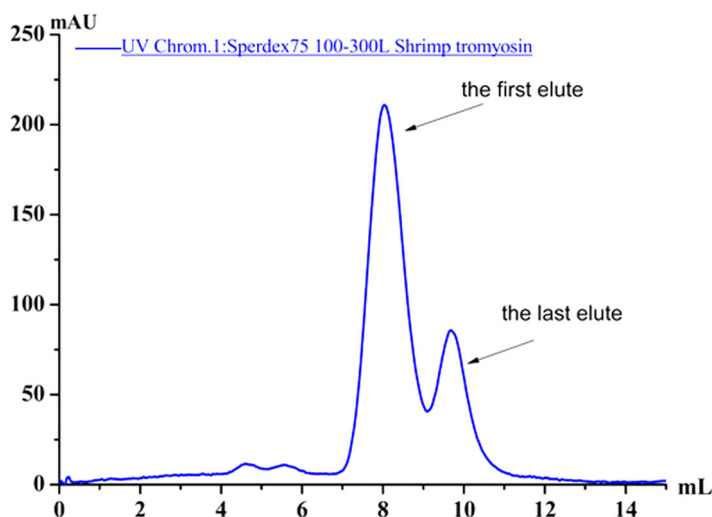


**Figure 2.** Gel filtration chromatography of 50% saturated ammonium sulfate component.

elution were between 36 and 38 kDa. As shown in Figure 3, purified shrimp tropomyosin displayed a clear single band when using SDS-PAGE, and its molecular weight was approximately 36 kDa. This shrimp tropomyosin was used for further experiments.

### *Pairwise interaction analysis*

After cell selection, 10 hybridoma cell lines, namely mAb 1 to mAb 10, were obtained. A sandwich ELISA was developed based on mAbs and HRP-labelled mAbs, which were used

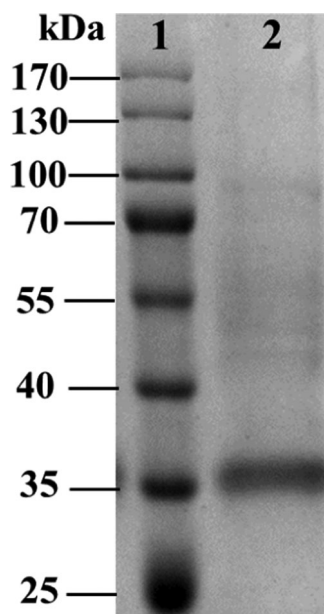


**Figure 3.** SDS-PAGE of 50% component of elute. Lane 1, Marker; Lane 2, the last eluate.

**Table 1.** P/N value in sandwich ELISA for pairwise analysis.

| Detection mAb | Capture mAb |       |      |       |       |       |       |       |      |       |
|---------------|-------------|-------|------|-------|-------|-------|-------|-------|------|-------|
|               | 1           | 2     | 3    | 4     | 5     | 6     | 7     | 8     | 9    | 10    |
| 1-HRP         | –           | 13.00 | 8.52 | 7.45  | 4.24  | 9.97  | 3.98  | 2.66  | 4.55 | 13.96 |
| 2-HRP         | 2.61        | –     | 4.01 | 4.40  | 1.92  | 9.37  | 3.50  | 1.52  | 1.99 | 11.06 |
| 3-HRP         | 6.27        | 18.82 | –    | 0.00  | 7.46  | 16.77 | 5.86  | 3.48  | 2.79 | 10.02 |
| 4-HRP         | 2.94        | 15.79 | 9.41 | –     | 5.34  | 17.06 | 4.16  | 2.29  | 2.61 | 10.32 |
| 5-HRP         | 2.12        | 2.04  | 5.70 | 5.00  | –     | 13.87 | 12.71 | 3.41  | 3.02 | 13.24 |
| 6-HRP         | 5.06        | 12.28 | 6.40 | 4.42  | 10.78 | –     | 9.81  | 5.82  | 5.10 | 3.94  |
| 7-HRP         | 7.28        | 16.16 | 8.54 | 10.49 | 17.28 | 7.35  | –     | 12.65 | 8.66 | 11.87 |
| 8-HRP         | 4.15        | 3.60  | 7.79 | 7.42  | 8.15  | 2.92  | 8.86  | –     | 7.12 | 7.23  |
| 9-HRP         | 3.36        | 5.48  | 6.87 | 6.62  | 7.26  | 1.61  | 7.01  | 9.95  | –    | 5.44  |
| 10-HRP        | 3.18        | 4.82  | 7.46 | 7.17  | 8.27  | 1.30  | 8.21  | 6.03  | 5.95 | –     |

Note: P/N value was the ratio of OD<sub>450</sub> value of the positive to the negative control.

separately as capture and detection antibodies, respectively. As shown in Table 1, 100 pairwise mAb combinations were obtained. When mAb 2 was used as a capture antibody and HRP-labelled mAb 3 was used as the detection antibody, the highest OD<sub>450</sub> ratio for the positive and negative controls (P/N value) was obtained. Therefore, this combination was used for further experiments.

### Optimization of the sandwich ELISA

Table 2 shows that the highest P/N value was found when using mAb 2 and mAb 3. Therefore, the concentration of mAb 2 is 4 µg/mL and the concentration of HRP-labelled mAb 3 is 2 µg/mL.

### Establishment and cross-reactivity of the mAb-based sandwich ELISA

After optimization, the concentration of the capture antibody, detection antibody, and Tween-20 in the antibody dilution buffer was 4, 2 µg/mL and 0.1% (v/v), respectively, and a standard curve was established for the detection of shrimp tropomyosin. As shown in Figure 4, the detection limit of the developed sandwich ELISA was 0.45 ng/mL. The cross-reactivity was also measured for the sandwich ELISA. Figure 5 shows that the absorbance value at 450 nm of other food allergens was below 0.25, indicating that the mAbs against shrimp tropomyosin exhibited high specificity.

### Recovery test

As shown in Table 3, the intra- and inter-assay recoveries ranged from 80% to 96% and 89% to 94%, respectively. The intra- and inter-assay coefficients of variance ranged

**Table 2.** P/N value of different concentrations of capture antibody and detection antibody in sandwich ELISA optimization.

|                            | P/N value | mAb2 (µg/mL) |       |      |       |
|----------------------------|-----------|--------------|-------|------|-------|
|                            |           | 8            | 4     | 2    | 1     |
| HRP-labelled mAb 3 (µg/mL) | 8         | 5.21         | 4.91  | 4.89 | 4.45  |
|                            | 4         | 8.61         | 7.91  | 7.73 | 7.75  |
|                            | 2         | 8.11         | 12.11 | 9.76 | 11.03 |
|                            | 1         | 8.74         | 9.52  | 9.01 | 8.80  |

Note: P/N value was the ratio of OD<sub>450</sub> value of the positive to the negative control.

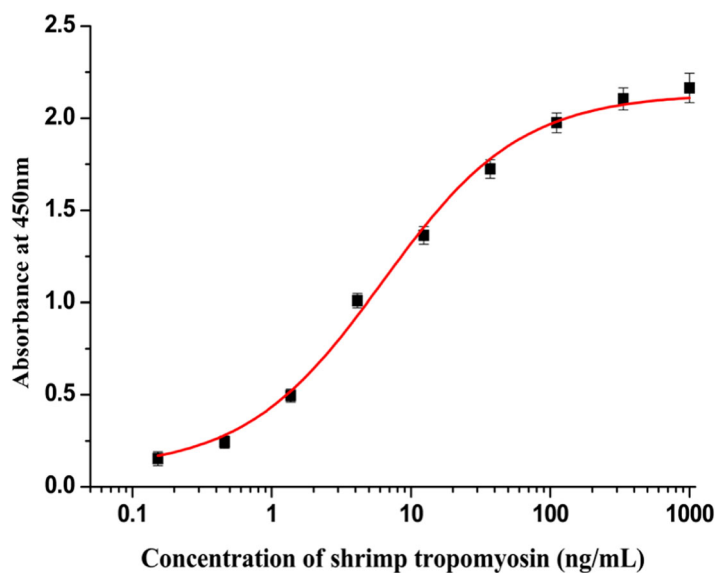


**Figure 4.** Standard curve of shrimp tropomyosin in PBST.

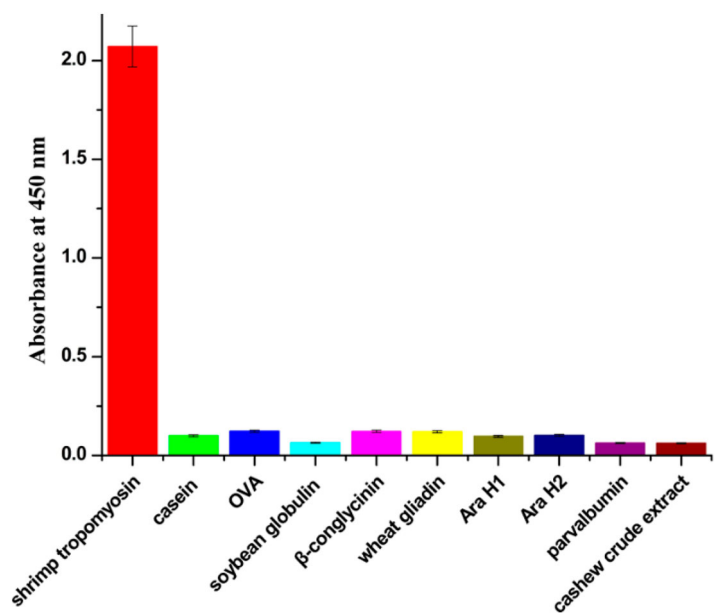


**Figure 5.** Specificity of the shrimp tropomyosin by the developed sandwich ELISA.

**Table 3.** Recovery of shrimp tropomyosin from spiked minced fish.

| Spiked level (ng/g) | Intra-assay (n = 6) |                | Inter-assay (n = 6) |                |
|---------------------|---------------------|----------------|---------------------|----------------|
|                     | Mean SD (ng/g)      | Recoveries (%) | Mean SD (ng/g)      | Recoveries (%) |
| 0.1                 | 0.08 ± 0.006        | 80.00          | 0.09 ± 0.008        | 90.00          |
| 0.5                 | 0.48 ± 0.019        | 96.00          | 0.47 ± 0.026        | 94.00          |
| 2                   | 1.76 ± 0.023        | 88.00          | 1.78 ± 0.033        | 89.00          |

from 3.5% to 9.0% and from 4.8% to 10.8%, respectively. These results indicate that the established ELISA method exhibited good stability and high accuracy.

### **Optimization and characterization of the immunochromatographic assay strip**

The concentration of mAb 2 and the composition of the suspension buffer were optimized to improve the analytical performance and sensitivity of the immune-chromatographic assay strip. Three concentrations of mAb 2 were (0.25, 0.5, and 1 ng/mL) were sprayed onto the NC membrane. The immunochromatographic assay strip was used to analyze a shrimp tropomyosin negative sample (0 ng/mL) and a shrimp tropomyosin positive sample (1 ng/mL). As shown in Figure 6(A). No colour on the T line was obtained with the shrimp tropomyosin negative sample at any concentration. A concentration of 0.5 ng/mL gave the deepest colour on the T line with the shrimp tropomyosin positive sample. Therefore, mAb 2 at a concentration of 0.5 ng/mL was used for subsequent experiments.

The flow rate, sharpness, background colour, and intensity of the T line was affected by the composition of the suspension buffer. The basic suspension buffer contained 20 mM Tris (pH 8.2), 0.1% PEG, 0.1% Tween, 5% sucrose, 5% trehalose, and 0.2% BSA, with 1% PVP, 1% PEG, and 1% BSA added separately. The shrimp tropomyosin negative sample (0 ng/mL) and shrimp tropomyosin positive sample (1 ng/mL) were analyzed (Figure 6(B)). The basic suspension buffer containing 1% PEG contributed to the deepest red on the T line with the shrimp tropomyosin positive sample. Therefore, the suspension buffer containing 1% PEG was used for further experiments.

PBST samples spiked with different concentrations of shrimp tropomyosin (0, 0.25, 0.5, 1, 2.5, 5, 10, 25, 50, and 100 ng/mL) were analyzed by the immune-chromatographic assay strip (Figure 7), and the sensitivity of the immunochromatographic assay strip was found to be 0.5 ng/mL.

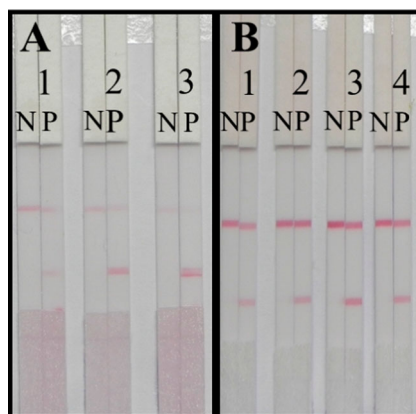


**Figure 6.** The optimization of the immunochromatographic assay strip. (A) Optimization the concentration of capture antibody: (1) 0.25 ng/mL; (2) 0.5 ng/mL; (3) 1 ng/mL; (B) Optimization the suspension solution for sample pad: (1) basic suspension buffer; (2) 1% PVP; (3) 1% PEG; (4) 1% BSA; N, shrimp tropomyosin negative sample (0 ng/mL); P, shrimp tropomyosin positive sample (1 ng/mL).

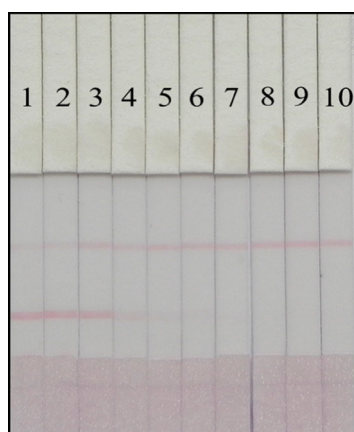


**Figure 7.** Shrimp tropomyosin detection by the immunochromatographic assay strip. The PBS samples: (1) 100 ng/mL; (2) 50 ng/mL; (3) 25 ng/mL; (4) 10 ng/mL; (5) 5 ng/mL; (6) 2.5 ng/mL; (7) 1 ng/mL; (8) 0.5 ng/mL; (9) 0.25 ng/mL; (10) 0 ng/mL.

**Table 4.** Detection of shrimp tropomyosin in processed foods by the optimized sandwich ELISA.

| Processed foods              | ELISA test         | Strip test |
|------------------------------|--------------------|------------|
| Shrimp cracker               | 0.688 <sup>a</sup> | Positive   |
| Shrimp flakes                | 0.217 <sup>a</sup> | Positive   |
| Cup noodles (shrimp flavour) | 5.639 <sup>a</sup> | Positive   |
| Laver soup (seafood flavour) | 0.066 <sup>a</sup> | Positive   |
| Fish ball                    | Negative           | Negative   |

<sup>a</sup>Means shrimp tropomyosin/food (ug/g).

### Sample analysis

Four different kinds of processed foods, three labelled with shrimp ingredients and one without, were tested by the sandwich ELISA and the strip method. As shown in Table 4, when using our developed sandwich ELISA, we found the highest concentration of shrimp tropomyosin in cup noodles (5.639 µg/g), whereas the lowest was detected in laver soup (0.066 µg/g). Four samples with a shrimp label were found to be positive for shrimp tropomyosin when using the strip assay. The test strip was negative, however, when determining shrimp tropomyosin in fish balls.

The results indicate that the highly sensitive and specific sandwich ELISA was capable of identifying and quantifying shrimp tropomyosin in food. The strip assay results could estimate whether a processed food contained shrimp tropomyosin or not. The sandwich ELISA test process required 2 h, while the results could be obtained by the naked eye within 10 min using the immunochromatographic assay strip, which was faster than sandwich ELISA. The strip assay was suitable for semi-quantitative detection, on-site detection and mass sample screening. These results demonstrate that our developed sandwich ELISA and immunochromatographic assay strip met the requirements for the detection of shrimp tropomyosin.

### Conclusion

In this study, we selected the optimal pair of mAbs, mAb 2, and HRP-labelled mAb 3. These were used separately as the capture and detection antibodies. A highly sensitive

and specific sandwich ELISA was established for the detection of shrimp tropomyosin in food, with an LOD for the assay of 0.45 ng/mL. The cross-reactivity with other food allergens using this method was negligible. The immunochromatographic assay strip was developed with an LOD of 0.5 ng/mL. These methods for the quantitation of shrimp tropomyosin could determine the presence or absence of shrimp tropomyosin in different food products. Therefore, our developed mAb-based sandwich ELISA and immunochromatographic assay strip are effective for the on-site detection of shrimp tropomyosin.

## Disclosure statement

No potential conflict of interest was reported by the authors.

## Funding

This work is financially supported by National Key R&D Program (2017YFC1600902), the National Natural Science Foundation of China (21673104) and grants from Natural Science Foundation of Jiangsu Province, MOF and MOE (BE2014672, BE2013613, CMB21S1614, WX18LVJN003). This work is also financially supported by the Innovative Project of Postgraduate Education in Jiangsu Province (SJLX15\_0542).

## References

- Chen, Q., Pan, X.-D., & Huang, B.-F. (2017). Authentication of shrimp muscle in complex foodstuff by in-solution digestion and high-resolution mass spectrometry. *Rsc Advances*, 7(52), 32903–32908.
- Du Toit, G., Sampson, H. A., Plaut, M., Burks, A. W., Akdis, C. A., & Lack, G. (2018). Food allergy: Update on prevention and tolerance. *Journal of Allergy and Clinical Immunology*, 141(1), 30–40.
- Eischeid, A. C. (2016). Development and evaluation of a real-time PCR assay for detection of lobster, a crustacean shellfish allergen. *Food Control*, 59, 393–399.
- Eischeid, A. C., & Stadig, S. R. (2018). A group-specific, quantitative real-time PCR assay for detection of crab, a crustacean shellfish allergen, in complex food matrices. *Food Chemistry*, 244, 224–231.
- Faber, M. A., Pascal, M., El Kharbouchi, O., Sabato, V., Hagendorens, M. M., Decuyper, I. I., ... Ebo, D. G. (2017). Shellfish allergens: Tropomyosin and beyond. *Allergy*, 72(6), 842–848.
- Fernandes, T. J. R., Costa, J., Oliveira, M. B. P. P., & Mafra, I. (2018). A new real-time PCR quantitative approach for the detection of shrimp crustaceans as potential allergens. *Journal of Food Composition and Analysis*, 72, 7–14.
- Herrero, B., Vieites, J. M., & Espineira, M. (2014). Development of an in-house fast real-time PCR method for detection of fish allergen in foods and comparison with a commercial kit. *Food Chemistry*, 151, 415–420.
- Jiang, D., Ge, P., Wang, L., Jiang, H., Yang, M., Yuan, L., ... Ju, X. (2019). A novel electrochemical mast cell-based paper biosensor for the rapid detection of milk allergen casein. *Biosensors & Bioelectronics*, 130, 299–306.
- Jiang, D., Ji, J., An, L., Sun, X., Zhang, Y., Zhang, G., & Tang, L. (2013). Mast cell-based electrochemical biosensor for quantification of the major shrimp allergen Pen a 1 (tropomyosin). *Biosensors & Bioelectronics*, 50, 150–156.
- Jiang, H., Jiang, D., Zhu, P., Pi, F., Ji, J., Sun, C., ... Sun, X. (2016). A novel mast cell co-culture microfluidic chip for the electrochemical evaluation of food allergen. *Biosensors & Bioelectronics*, 83, 126–133.

- Jiang, D., Zhu, P., Jiang, H., Ji, J., Sun, X., Gu, W., & Zhang, G. (2015). Fluorescent magnetic bead-based mast cell biosensor for electrochemical detection of allergens in foodstuffs. *Biosensors & Bioelectronics*, 70, 482–490.
- Koizumi, D., Shirota, K., Akita, R., Oda, H., & Akiyama, H. (2014). Development and validation of a lateral flow assay for the detection of crustacean protein in processed foods. *Food Chemistry*, 150, 348–352.
- Kong, D., Xie, Z., Liu, L., Song, S., Kuang, H., Cui, G., & Xu, C. (2017). Development of indirect competitive ELISA and lateral-flow immunochromatographic assay strip for the detection of sterigmatocystin in cereal products. *Food and Agricultural Immunology*, 28(2), 260–273.
- Kong, D., Xie, Z., Liu, L., Song, S., Kuang, H., & Xu, C. (2017). Development of ic-ELISA and lateral-flow immunochromatographic assay strip for the detection of vancomycin in raw milk and animal feed. *Food and Agricultural Immunology*, 28(3), 414–426.
- Kuang, H., Wang, W., Xu, L., Ma, W., Liu, L., Wang, L., & Xu, C. (2013). Monoclonal antibody-based sandwich ELISA for the detection of Staphylococcal Enterotoxin A. *International Journal of Environmental Research and Public Health*, 10(4), 1598–1608.
- Lei, X., Xu, L., Song, S., Liu, L., & Kuang, H. (2018). Development of an ultrasensitive ic-ELISA and immunochromatographic strip assay for the simultaneous detection of florfenicol and thiamphenicol in eggs. *Food and Agricultural Immunology*, 29(1), 254–266.
- Li, S., Wu, X., Song, S., Zheng, Q., & Kuang, H. (2019). Development of ic-ELISA and an immunochromatographic strip assay for the detection of aristolochic acid ROMAN NUMERAL ONE. *Food and Agricultural Immunology*, 30(1), 140–149.
- Li, Z., Zhang, Y., Lin, H., Haider, S., & Jiang, J. (2010). Quantitative analysis of shrimp allergen in food matrices using a protein chip based on sandwich immunoassay. *European Food Research and Technology*, 231(1), 47–54.
- Lin, S.-Y., Lee, C.-H., Huang, E. S., Sheu, S.-C., & Yu, H.-S. (2018). Quantification of crustacean tropomyosin, a major food allergen, in eight species of Taiwanese shrimp based on immunoassay. *Food Analytical Methods*, 11(9), 2607–2613.
- Nagai, H., Minatani, T., & Goto, K. (2015). Development of a method for crustacean allergens using liquid chromatography/tandem mass spectrometry. *Journal of Aoac International*, 98(5), 1355–1365.
- Peng, J., Song, S., Liu, L., Kuang, H., & Xu, C. (2015). Development of sandwich ELISA and immunochromatographic strip for the detection of peanut allergen Ara h 2. *Food Analytical Methods*, 8(10), 2605–2611.
- Peng, J., Song, S., Xu, L., Ma, W., Liu, L., Kuang, H., & Xu, C. (2013). Development of a monoclonal antibody-based sandwich ELISA for peanut allergen Ara h 1 in food. *International Journal of Environmental Research and Public Health*, 10(7), 2897–2905.
- Rahman, A. M. A., Lopata, A. L., Randell, E. W., & Helleur, R. J. (2010). Absolute quantification method and validation of airborne snow crab allergen tropomyosin using tandem mass spectrometry. *Analytica Chimica Acta*, 681(1–2), 49–55.
- Scurlock, A. M. (2018). Oral and sublingual immunotherapy for treatment of IgE-mediated food allergy. *Clinical Reviews in Allergy & Immunology*, 55(2), 139–152.
- Seiki, K., Oda, H., Yoshioka, H., Sakai, S., Urisu, A., Akiyama, H., & Ohno, Y. (2007). A reliable and sensitive immunoassay for the determination of crustacean protein in processed foods. *Journal of Agricultural and Food Chemistry*, 55(23), 9345–9350.
- Sicherer, S. H., & Leung, D. Y. M. (2007). Advances in allergic skin disease, anaphylaxis, and hypersensitivity reactions to foods, drugs, and insects. *Journal of Allergy and Clinical Immunology*, 119(6), 1462–1469.
- Sicherer, S. H., & Sampson, H. A. (2018). Food allergy: A review and update on epidemiology, pathogenesis, diagnosis, prevention, and management. *Journal of Allergy and Clinical Immunology*, 141(1), 41–58.
- Song, S., Suryoprabowo, S., Liu, L., Kuang, H., Xu, L., Ma, W., & Wu, X. (2019). Development of monoclonal antibody-based colloidal gold immunochromatographic assay for analysis of halo-fuginone in milk. *Food and Agricultural Immunology*, 30(1), 112–122.

- Tabrizi, M. A., Shamsipur, M., Saber, R., Sarkar, S., & Ebrahimi, V. (2017). A high sensitive visible light-driven photoelectrochemical aptasensor for shrimp allergen tropomyosin detection using graphitic carbon nitride-TiO<sub>2</sub> nanocomposite. *Biosensors & Bioelectronics*, 98, 113–118.
- Wang, Y., Rao, Z., Zhou, J., Zheng, L., & Fu, L. (2019). A chiral assembly of gold nanoparticle trimer-based biosensors for ultrasensitive detection of the major allergen tropomyosin in shellfish. *Biosensors & Bioelectronics*, 132, 84–89.
- Werner, M. T., Faeste, C. K., & Egaas, E. (2007). Quantitative sandwich ELISA for the determination of tropomyosin from crustaceans in foods. *Journal of Agricultural and Food Chemistry*, 55(20), 8025–8032.
- Werner, M. T., Faeste, C. K., Levsen, A., & Egaas, E. (2011). A quantitative sandwich ELISA for the detection of Anisakis simplex protein in seafood. *European Food Research and Technology*, 232(1), 157–166.