Food Analytical Methods (2022) 15:2470–2478
https://doi.org/10.1007/s12161-022-02302-6

A Fluorescence Immunochromatographic Strip Based on Quantum Dot Nanobeads for the Rapid Detection of Okadaic Acid

Han Wang[1] · Hong-Lin Ren[1] · Pan Hu[1] · Yan-Song Li[1] · Yu Zheng[1] · Qi Cao[1] · Zhan-Xu Liu[1] · Zeng-Shan Liu[1] · Yong Yang[1] · Shi-Ying Lu[1]

Received: 13 May 2021 / Accepted: 13 April 2022 / Published online: 25 April 2022

Abstract

A fluorescence immunochromatographic strip using quantum dot nanobeads (QDNBs)-monoclonal antibody (McAb) conjugates was developed to rapidly detecting okadaic acid (OA) in shellfish. Under optimal conditions, the linear working range was 0.62–20 ng·mL^{-1}, and the full competitive limit of detection (LOD) was 20 ng·mL^{-1}, corresponding to 160 μg·kg^{-1} per shellfish, meeting the regulatory limit of European Conformity (EC). The strips could show results in 20 min, had high reproducibility, and were effective within 6 months when stored sealed at 4 °C and 25 °C. Moreover, the strips successfully detected OA in spiked shellfish samples. The reliability of the test strips was further confirmed by enzyme-linked immunosorbent assay (ELISA) which was consistent well with Abraxis imported ELISA ($R^2 = 0.988$). The strips are sensitive, specific, replicable, and ideal for rapidly detecting OA, providing a potential tool that can be further developed to detect OA and other marine toxins in shellfish.

Keywords Okadaic acid · Quantum dot nanobeads · Fluorescence immunochromatographic strips · Diarrheic shellfish poisoning toxins

Abbreviations

LFIC	lateral flow immunochromatographic
ELISA	enzyme-linked immunosorbent assay
HPLC–MS	high-performance liquid chromatography-tandem mass spectrometry
SPR	surface plasmon resonance
QDNBs	quantum dot nanobeads
QDs	quantum dots
MNPs	magnetic nanoparticles
AuNPs	gold nanoparticles
McAb	monoclonal antibody
OA	okadaic acid
DSP	diarrheic shellfish poisoning
PPI	protein phosphatase inhibition
PP1	protein phosphatases of type 1
PP2A	protein phosphatases of type 2A
LOD	limit of detection
EC	European Conformity
BSA	bovine serum albumin
NHS	N-hydroxysuccinimide
DCC	N,N-dicyclohexylcarbodiimide
EDC	N-(3-dimethylaminopropyle)-N-ethyl-carbodiimide hydrochloride
N,N-DMF	N,N-dimethylformamide
HEPES	4-(2-hydroxyerhyl) piperazine-1-erhanesulfonic acid
PEG 20 000	polyethylene glycol 20 000
NC	nitrocellulose
PBS	phosphate-buffered saline
PB	phosphate buffered
TEM	transmission electron microscope

Han Wang, Hong-Lin Ren and Pan Hu contributed equally to this work.

✉ Shi-Ying Lu
lushiying1129@163.com

[1] State Key Laboratory for Zoonotic Diseases, Key Laboratory for Zoonosis Research of the Ministry of Education, Institute of Zoonosis, and College of Veterinary Medicine, Jilin University, Changchun 130062, People's Republic of China

Introduction

Okadaic acid (OA) is a form of lipophilic marine algal toxin with a low-molecular weight of approximately 805 Da, and it can accumulate in the internal organs of shellfish that accidently ingest toxin-producing dinoflagellates (Hu et al.

1992; Morton et al. 1998). Because of the high-temperature resistance, the toxicity of OA cannot be eliminated by cooking (T. An et al. 2010; Lee et al. 1989; Reguera et al. 2012). So, if contaminated seafood was ingested by humans, OA is able to inhibit serine/threonine protein phosphatases of type 1 (PP1) and 2A (PP2A) by binding to the receptor site; this results in the overphosphorylation of protein in cells (L. Q. Hu et al. 2013; Zhou et al. 2016), causing diarrheic shellfish poisoning (DSP) including diarrhea and vomiting (Prassopoulou et al. 2009; Sassolas et al. 2013). In order to guarantee the safety of seafood, the European Conformity (EC) put forward a specific regulation 853/2004/EC stating that the maximum permitted level was 160 μg of OA equivalents kg^{-1} (Anon 2004; European Food Safety 2008). Therefore, it is necessary to develop fast, sensitive, and reliable methods to detect OA.

A number of methods have been used to determine OA concentrations in shellfish, including mouse bioassays, HPLC techniques, protein phosphatase inhibition (PPI) assays, and immunological methods (S. Y. Lu et al. 2012a, b). While proven to be useful, these techniques still need a complex instrument, consume time, and also have ethical issues. Currently, immunoassays have become one of the most popular methods due to their high sensitivities, simplicity, and the small sample volumes required (L. Q. Hu et al. 2013). Among them, an immunochromatographic strip based on colloidal gold is commonly used because of the simplicity, rapid, low cost, and visualized result. However, the insufficient brightness makes the sensitivities low (Shen, et al. 2017a, b). In order to improve sensitivity, a series of materials and methods have been tested, including enzymes, fluorescent materials, thermal contrast readouts, and magnetic microspheres (Ren et al. 2016; Shen, et al. 2017a, b; Wang et al. 2016; Y. Yao et al. 2016). It is found that fluorescence materials producing highly sensitive fluorescence signals are more effective than the traditional colloidal gold (AuNPs) and magnetic nanoparticles (MNPs) that produce colorimetric signals (Guo et al. 2019). Among them, upconversion nanoparticles (UCNPs) can produce strong luminescence and have unique optical properties such as low toxicity, narrow emission spectra, chemical stability, and no self-fluorescence background compared with fluorescent dyes, but have low quantum yield (Liang et al. 2019); europium microspheres (EuNPs) have broad excitation spectrum, narrow emission peak, long fluorescence lifetime, high quantum yield, large stokes shift, high resolution, low environmental interference, and no photobleaching (H. Chen et al. 2020).

Meanwhile, quantum dots (QDs) as nanoscale fluorescent labels attracted significant interest for biological and medical detection (Li et al. 2014) because of excellent optical properties, high quantum yields, tunable bandgaps, high efficiency, a broad absorption cross-section, and narrow emission bandwidth (Alivisatos 1996; F. P. An et al. 2015; Duan et al. 2017; Guo et al. 2019; Huang et al. 2016). But, they also have the problem of insufficient sensitivity caused by instability after phase-transfer procedures (T. Zhang et al. 2006). Therefore, QD beads were encapsulated into quantum dot nanobeads (QDNBs) as signal amplification label to gain stability (Dubertret et al. 2002). By now, the conjugation of QDNBs and monoclonal antibodies (McAbs) has become common practice for immunoassays (Ouyang et al. 2017). For instance, Zhang et al. developed a dot-blot immunoassay that the fluorescent signal of QDNBs could be amplified in just one step (P. F. Zhang et al. 2014). Yao et al. developed immunoassays based on a dual-signal system that combined QDNBs and fluorescent nanoparticles (S. Yao et al. 2019). In another study, Shen et al. developed a fluorescence-quenching immunochromatographic strip with QDNBs and AuNPs (Shen, et al. 2017a, b).

Considering that previous methods involving QDNBs have yet to be used to detect OA, in this study, we used carboxy-modified QDNBs as signal density reporters to label McAb and developed a fluorescence immunochromatographic strip for the rapid determination of OA in seafood samples.

Materials and Methods

Reagents and Materials

OA was purchased from Express Technology. Bovine serum albumin (BSA), N-hydroxysuccinimide (NHS), N, N-dicyclohexylcarbodiimide (DCC), N-(3-dimethylaminopropyle)-N-ethyl-carbodiimide hydrochloride (EDC), and N,N-dimethylformamide (N, N-DMF) were purchased from Sigma. Tween-20, polyethylene glycol 20 000 (PEG 20 000), D (+)-sucrose, and 4-(2-hydroxyerhyl) piperazine-1-erhan esulfonic acid (HEPES) were purchased from Beijing Dingguo Changsheng Biotech. Goat anti-mouse immunoglobulin G (IgG) antibody was purchased from Boster. The anti-OA monoclonal antibody (McAb) (IgG1 subclass) was produced and preserved by our laboratory (S.-Y. Lu et al. 2012a, b; S. Y. Lu et al. 2012a, b). Quantum dots nanobeads (QDNBs) were purchased from Kundao Biotech. MicroAmp 96-well reaction plate was purchased from ThermoFisher. Sample pad was purchased from Shanghai Kinbio Tech. Absorption pad, nitrocellulose (NC) membranes, plastic backing, desiccant, and aluminum foil bag were purchased from Shanghai Jiening Biotech. Other reagents were of analytical purity, and doubly distilled water was used throughout all of the experiments.

Apparatus

The apparatus was used as follows: the AKTA purifier 100 was purchased from General Electric Company (GE, USA); the T-Green transilluminator (OSE-470/470L) was purchased from Tiangen Biotech (Tiangen, Beijing); the fluorescence immunoassay analyzer (MD-100) was purchased from microdetection (MD, Nanjing); the XYZ 3060 platform and CM 4000 cutting system were purchased from Biodot (Biodot, China); the H-7650 transmission electron microscope (TEM) was purchased from Hitachi (Hitachi, Japan); the 200 PRO multifunction microplate reader was purchased from Tecan Infinite (Tecan, Switzerland); the microplate spectrophotometer epoch was purchased from Bio-Tek Instruments (Bio-Tek, USA); the nanodrop 2000 ultra-micro spectrophotometer was purchased from Thermo Fisher (Thermo, USA).

Preparation of Coating Antigen

OA-BSA was prepared according to the established methodology with some modifications (Lin et al. 2014). OA (0.5 mg), NHS (0.08 mg), and DCC (0.15 mg) were added into 60 μL of N,N-DMF and reacted for 2 h at 20–25 °C. Then, the mixture was transferred in 50 μL of $NaHCO_3$ (0.1 M) containing 2.0 mg of BSA and reacted for 2 h at 20–25 °C. The mixture was purified by centrifugal ultrafiltration. Finally, OA-BSA was diluted to 1 mg·mL^{-1} with phosphate-buffered saline (PBS, 0.01 M, pH 7.4) and then was preserved at − 20 °C.

Preparation of QDNBs-McAb

The QDNBs-McAb were generated in two steps: activation of carboxyl groups on the surface of QDNBs and conjugation of activated QDNBs and McAb. First, QDNBs (1 mg) and EDC (0.0742 mg) were added to 500 μL of HEPES buffer (0.03 M, pH 6.75). The solution was blended for 30 min at 20–25 °C and was washed three times (11 000 rpm, 15 min) with 500 μL of HEPES buffer. Subsequently, McAb (100 μg) was added and blended for 30 min at 20–25 °C. Then, the washed solution was resuspended in 500 μL of blocking buffer (0.03 M HEPES, 0.07 M BSA, pH 6.75) for 30 min at 20–25 °C. Finally, the washed solution was resuspended in 1 mL of preservation solution (0.03 M HEPES, 50 mM Tris, 0.154 M NaCl, 13.8 mM NaN3, 36.8 mM BSA, pH 7.25) and was stored at 4 °C.

Performance of QDNBs-McAb

Direct ELISA and direct competitive ELISA were used to confirm whether the QDNBs and McAb were successfully conjugated. The OA-BSA diluted to 1 μg·mL^{-1} with bicarbonate buffer (pH 9.6) was immobilized on micro-well plates and incubated at 4 °C overnight. The QDNBs-McAb was diluted with different concentrations (10, 5, 2.5, 1.25, 0.62, 0.31, 0.15 μg·mL^{-1} of QDNBs) for direct ELISA. The QDNBs-McAb diluted to 5 μg·mL^{-1} of QDNBs was mixed with an equal volume of different concentrations of OA (100, 50, 25, 12.5, 6.25, 3.12, 1.56 ng·mL^{-1}) for direct competitive ELISA. The dilutions were added into coated microplates and incubated for 1 h at 37 °C. Then, the fluorescence intensity of each well was detected repeatedly by a microplate reader, and the average readings were used for calculation and graphing.

Preparation of QDNB Strips

The test strips were prepared as follows: The goat anti-mouse IgG and OA-BSA were immobilized on NC membrane as control (C) line and test (T) line with 7-mm interval. Then, the sample pad, NC membrane, and absorbent pad were laminated and pasted on a PVC plate and cut into 3-mm-wide and 60-mm-long strips by strip cutter.

Immunochromatographic (Strip) Assays for Okadaic Acid (OA)

Forty microliters of sample buffer was mixed with 2.5 μL of QDNBs-McAb and 37.5 μL of loading buffer (0.2 mM PEG 20 000, 0.196 M BSA, and 0.01 PB); then, the mixture was loaded on the sample pad. Results could be observed with a T-Green transilluminator and calculated by a fluorescence reader.

Reproducibility and Stability of QDNB Strips

To test the reproducibility, three concentrations of standard OA solutions were prepared (0, 10, and 20 ng·mL^{-1}), and each concentration was tested with three test strips. To test the stability, the strips made from the same bunch were sealed and preserved separately at 4 °C and 25 °C, and then were used to detect OA every month for the next 6 months.

Detection of Okadaic Acid (OA) in Spiked Samples

First, the composition of the sample extracting solution was optimized. Two types of OA-free shellfish sample (*Rapana venosa* and *Argopecten irradians*) were screened by ELISA, and each type was used to prepare spiked (20 ng·mL^{-1}) and non-spiked (0 ng·mL^{-1}) groups. Also, two types of sample extracting solution were prepared: solution 1 consisted of methanol and water (8:2) and solution 2 consisted of methanol, water, and acetic acid (80:19:1). Then, each group was extracted by two types of extracting solution as follows: 1 g of homogenized tissue was mixed with 2 mL of sample

extract, sonicated for 10 min, and centrifuged at 10 000 rpm for 10 min. Then, the supernatant was diluted four times as a sample solution and tested by the test strip.

In spiking study, three types of OA-free shellfish (*Glossaulax didyma*, *Rapana venosa*, and *Haliotis discus hannai*) were spiked at 20, 80, and 160 ng·mL^{-1} and detected with QDNB strips. Results were confirmed by ELISA developed earlier by our group (S.-Y. Lu et al. 2012a, b).

Results and Discussion

Confirmation of QDNBs-McAb

QDNBs and QDNBs-McAb were scanned by TEM; results showed that the diameter of QDNB was about 80 nm (Fig. 1a); this represented an appropriate size to conjugate with antibodies. The absorbance spectrum of QDNBs, McAb, and QDNBs-McAb were shown in Fig. 1b; QDNBs had no absorption peak at 280-nm; McAb showed an absorption peak appeared at 283-nm and shifted to 277-nm after conjugating with QDNBs. In direct ELISA, the fluorescence density changed with the dilution factor and was clearly different from the blank, thus indicating that after conjugating with QDNBs, McAb still was capable of binding to coated OA-BSA (Fig. 1c); in direct competitive ELISA, the more free OA existed, the less QDNBs-McAb bound to the coated OA-BSA, this indicated that competitive immunoreactions were successfully conducted between free and coated OA to bind to QDNBs-McAb (Fig. 1d). Collectively, the QDNBs-McAb had been prepared successfully.

Principle of QDNB Strips

First, the conjugation of McAb and QDNBs was shown in Fig. 2a. Then, OA-BSA and goat anti-mouse IgG were immobilized separately in the T and C regions. When the sample solution was free of OA, QDNBs-McAb would bind to the immobilized OA-BSA and goat anti-mouse IgG (Fig. 2b). Thus, fluorescence signals could be observed in both T and C regions due to the accumulation of QDNBs-McAb. Conversely, when free OA existed, it

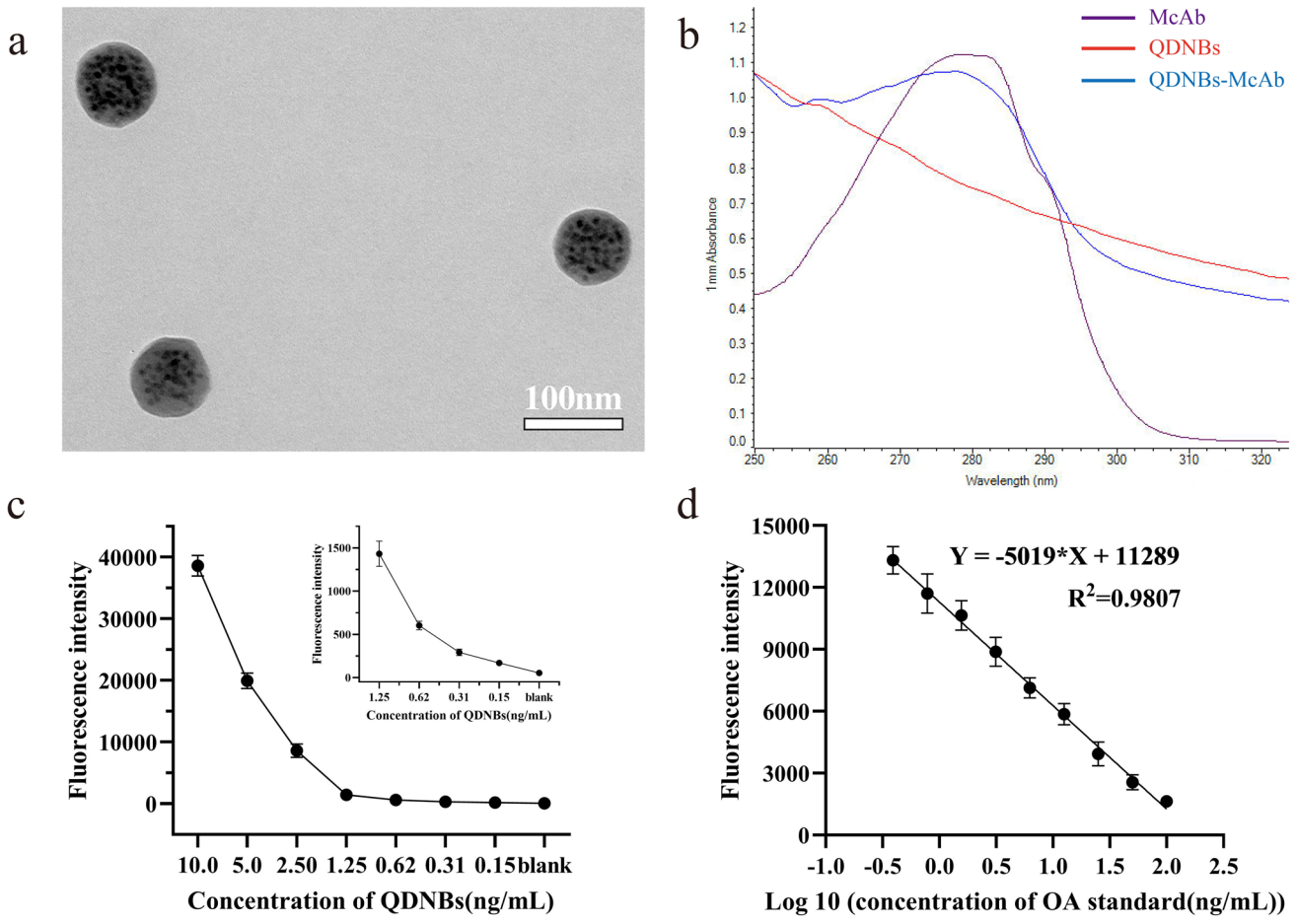

Fig. 1 Confirmation of QDNBs-McAb. **a** TEM image of QDNBs. **b** Absorbance spectrum of QDNBs and QDNBs-McAb. **c** Direct ELISA results of QDNBs-McAb (the measured value represented relative fluorescence intensity). **d** Standard curve of direct competitive ELISA

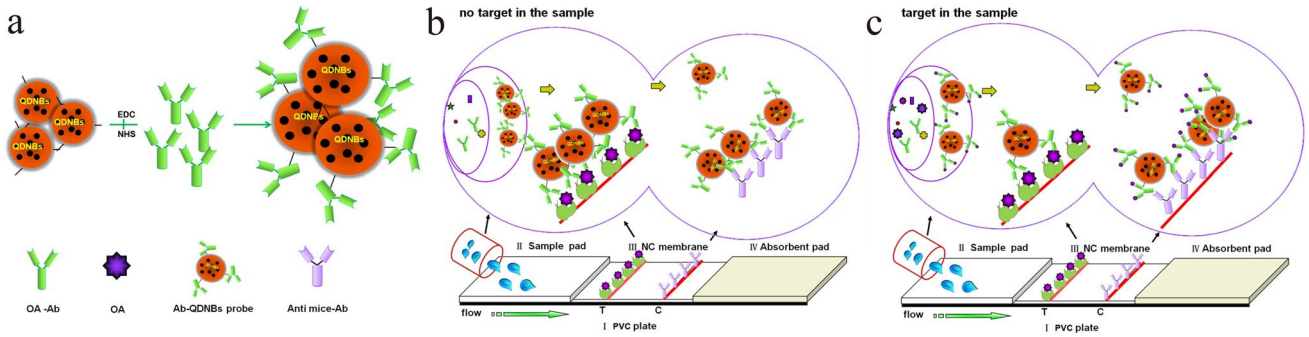

Fig. 2 Principle of QDNB strips. **a** QDNBs-McAb probe. **b** Negative reaction. When a negative sample (without OA) was tested, T-line would have the same fluorescence density as C-line. **c** Positive reaction. When a positive sample (OA present) was tested, the fluorescence density of the T-line would weaken or disappear

would combine with a limited number of QDNBs-McAb (Fig. 2c). The more free OA existed, the less QDNBs-McAb combined with immobilized OA-BSA, thus diminishing the fluorescence intensity of the test line (T-line). Therefore, a negative result could be judged when T-line had the same fluorescence density as the control line (C-line); a weakly positive result could be judged when the fluorescence density of T-line was weaker; a positive result could be judged when only C-line left. The result was worthless when no C line existed.

Optimization of Parameters

In optimization, parameters were selected when T-line and C-line were clear enough and showed similar and sufficient fluorescence intensity for observation. Results were shown in Fig. 3. The transilluminator capable of emitting light at 470-nm wavelength were shown in Fig. 3a. The optimal concentrations of Tween-20 and sucrose in the loading buffer were 0.10 and 5 % (Fig. 3b, c). The optimal volume of QDNBs-McAb (1 mg·mL^{-1} of QDNBs) was 0.25 μL (Fig. 3d). The optimal concentrations of immobilized OA-BSA and goat anti-mouse IgG on T and C regions were 0.2

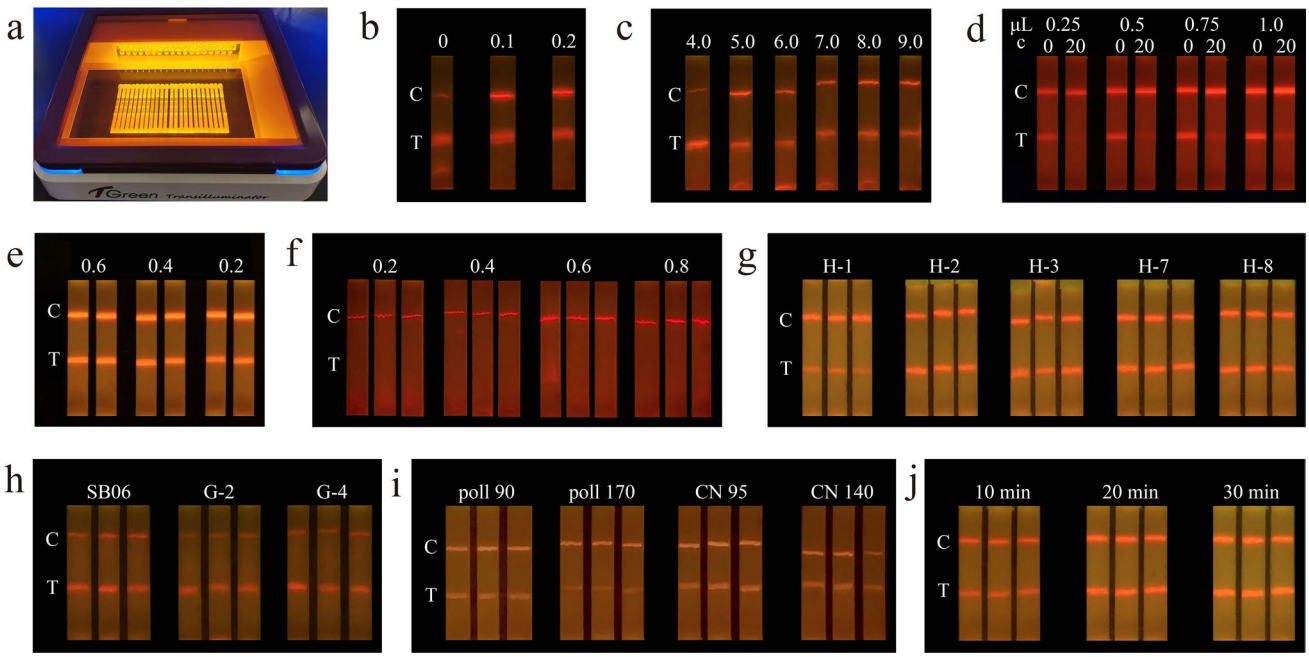

Fig. 3 Optimization of parameters. **a** T-Green Transilluminator (OSE-470/470L). **b** Optimal concentration of Tween-20. **c** Optimal concentration of D (+)-sucrose. **d** Optimal volume of QDNBs-McAb (1 mg·mL^{-1} of QDNBs). **e** Optimal concentration of immobilized OA-BSA. **f** Optimal concentration of immobilized goat anti-mouse IgG (H+L). **g** Optimal type of absorption pad. **h** Optimal type of sample pad. **i** Optimal type of NC membrane. **j** Chromatography time of QDNB strips

and 0.4 mg·mL^{-1} (Fig. 3e, f); this enable C-line to show the same fluorescence density as T-line. The appropriate material type for absorbent pad, sample pad, and NC membrane were H-7, SB06, and CN 95, respectively (Fig. 3g, h, i). Moreover, the chromatography time was within 20 min (Fig. 3j).

LOD of the QDNB Strips

Different concentrations of standard OA were tested. In semi-quantitative assays (Fig. 4a), when testing OA at 5, 10, and 20 ng·mL^{-1}, QDNBs strips showed negative, weakly positive and positive results. Therefore, the full competitive LOD was 20 ng·mL^{-1} in the sample solution, corresponding to 160 μg·kg^{-1} in the shellfish sample, which was exactly European Conformity (EC) edible safety limit.

In quantitative assays, with 0.8 μL of QDNBs probe (0.1 mg·mL^{-1} of QDNBs), the results of strips were shown in Fig. 4b, and the standard curve was established based on machine measurement results as shown in Fig. 4c, indicating the linear working range of 0.62–20 ng·mL^{-1}. The more OA existed in sample solution, the lower and higher fluorescence density T- and C-lines had, the more obvious the variation was. Moreover, the sum of fluorescence intensities of T- and C-lines always remained in an approximate range (data not shown). It might be because the amount of QDNBs had not

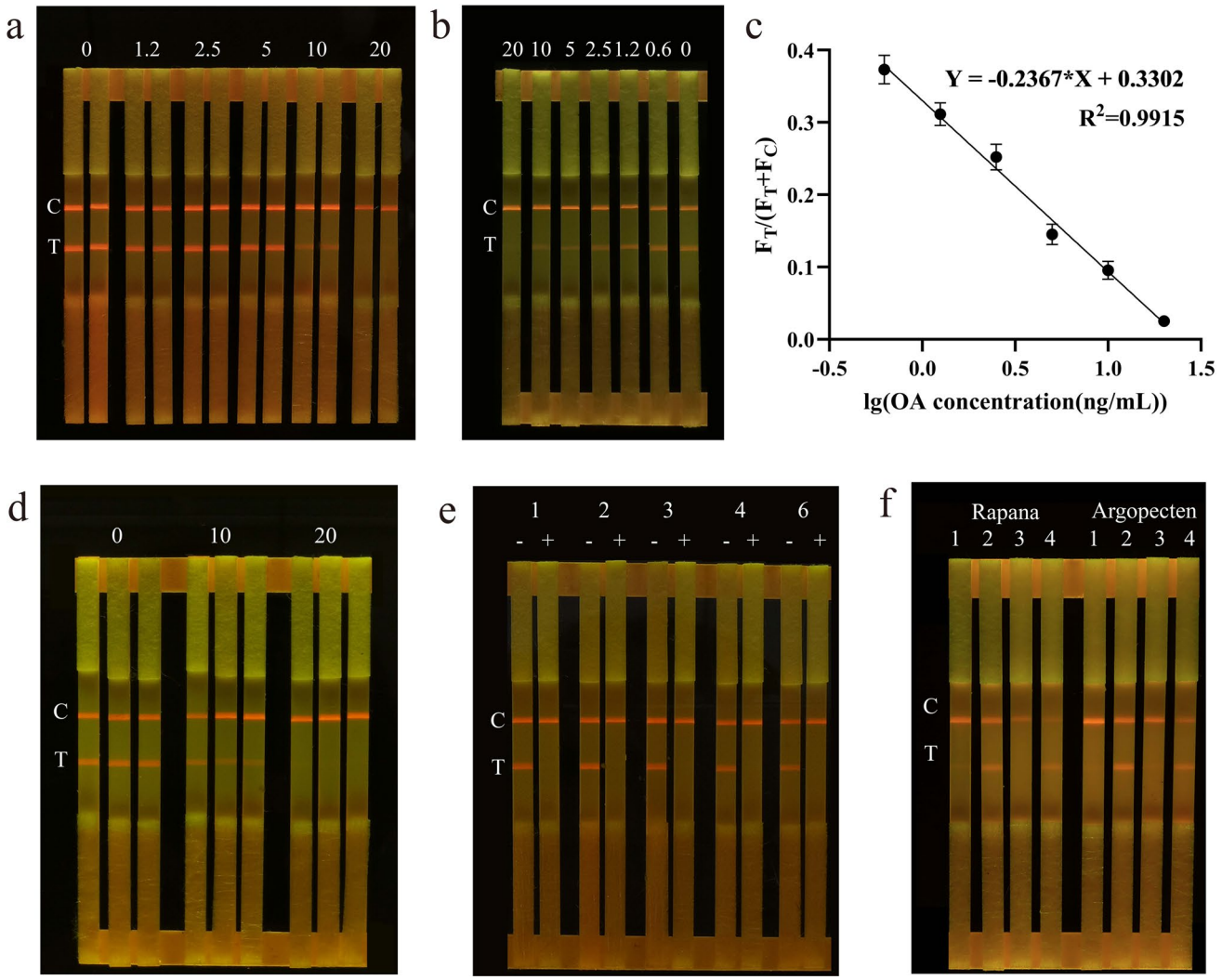

Fig. 4 Characteristics of QDNBs strip. **a** Semi-quantitative assays. The full competitive LOD was 20 ng·mL^{-1}. **b** Quantitative assays. **c** Standard curve. The minimum LOD was 0.62 ng·mL^{-1}. **d** Reproducibility. QDNB strips had high reproducibility and consistent characteristics. **e** Stability. QDNB strips could remain valid for at least 6 months, and the test results could also remain stable for at least 5 months. **f** Optimization of sample extracting composition. The extraction results using sample extract 1 (80% methanol and 20% water) and 2 (80% methanol, 19% water, and 1% acetic acid) were respectively shown in strips 1, 2 and 3, 4. Results of spiked and non-spiked groups were shown in strips 1, 3 and 2, 4. T and C-lines of strips 1 and 2 had high fluorescence density and clear boundary. In contrast, T and C-lines of strips 3 and 4 had significantly weaker fluorescence density and blurred boundary

reached the saturation value, making all the added QDNB probes binding to T- or C-lines and the sum of fluorescence intensities of T- and C-lines always in an approximate range so the fluorescence intensity of C-line no longer remained stable, but varied according to the number of bound QDNBs probes; therefore, when OA in sample solution decreased, the QDNBs on T-line increased and the QDNBs on C-line decreased, making the fluorescence intensity of T- and C-lines increased and decreased.

The LOD of QDNB strips had been improved based on the previous period (S. Y. Lu et al. 2012a, b) and was similar to that of surface plasmon resonance (SPR) and colloidal gold strip method (Le Berre et al. 2015; Ling et al. 2019). Although other methods had more sensitive LOD (Hui Chen et al. 2019; Pang et al. 2019; Peng et al. 2020), based on meeting EC edible safety limit, the QDNB strip had the advantages of simplicity, rapid, visualized result, and easiness of field operation, making it a potential mean for rapid detection of OA in seafood samples.

Reproducibility and Stability of QDNB Strips

The reproducibility was tested by detecting OA at 0, 10, and 20 ng·mL^{-1} with the same bunch of strips. Figure 4d shows that QDNB strips had high reproducibility and consistent characteristics.

To test the stability, the same batch of strips were sealed and stored at 4 and 25 °C, then were used to detect OA every month. In the sixth month, all used strips stored at 4 °C were shown in Fig. 4e: in 6 months, when detecting OA at 0 ng·mL^{-1}, the fluorescence density of T- and C-line was always maintained at a high level, with clear boundaries that could be readily observed; when detecting OA at 20 ng·mL^{-1}, only C-line left. Strips stored at 25 °C were also stable (results not shown). Therefore, QDNB strips could remain valid for at least 6 months, and the test results could also remain stable for at least 5 months.

Testing of Spiked Sample

Results of optimal sample extracting composition were shown in Fig. 4f. The extraction results using sample extracts 1 and 2 were respectively shown in strips 1, 2 and 3, 4. Results of spiked and non-spiked groups were shown in strips 1, 3 and 2, 4. T and C-lines of strips 1 and 2 had high fluorescence density and clear boundary. In contrast, T and C-lines of strips 3 and 4 had significantly weaker fluorescence density and blurred boundary. Although the T-lines of strip 3 disappeared, the corresponding extracting composition cannot be considered valid. There exists a possibility that the spiked OA was not fully extracted and the free OA in the sample solution did not reach 20 ng·mL^{-1}, the T-line should have appeared. However, the decrease in fluorescence intensity prevents the T-line from being observed by the naked eye, giving false-positive results. This indicated that acetic acid would significantly reduce the fluorescence density of QDNBs, rendering the results unfavorable for visual observation.

Results of testing spiked samples were shown in Table 1. In semi-quantitative assays, when the QDNB strip showed a negative result, it meant that the OA in the sample was 0–80 μg·kg^{-1} (0–10 ng·mL^{-1} in sample solution), and the shellfish sample was safe for consumption; when showed a weakly positive result, the OA in the sample was 80–160 μg·kg^{-1} (10–20 ng·mL^{-1} in sample solution), and the shellfish sample was edible but still had potential risk;

Table 1 Detection results for OA in shellfish by LFIC and ELISA

Shellfish species	OA added (ng mL^{-1})	LFIC ($n=3$)					ELISA ($n=3$)		
		Showing test line	Visual results	Found (μg kg^{-1})	CV (%)	Recovery (%)	Found (μg kg^{-1})	CV (%)	Recovery (%)
Glossaulax didyma	20	Y	−	16.168 ± 0.853	5.27%	80.84%	17.303 ± 0.981	5.66%	86.52%
	80	Y	±	68.614 ± 3.125	4.55%	85.76%	86.284 ± 4.588	5.32%	107.85%
	160	N	+	138.946 ± 9.849	7.09%	86.84%	145.964 ± 9.508	6.52%	91.23%
Rapana venosa	20	Y	−	14.719 ± 0.727	4.93%	73.59%	18.043 ± 0.899	4.99%	90.22%
	80	Y	±	70.930 ± 3.616	5.09%	88.66%	77.934 ± 4.476	5.75%	97.42%
	160	N	+	134.639 ± 9.368	6.95%	84.41%	143.714 ± 9.251	6.44%	89.82%
Haliotis discus hannai	20	Y	−	15.629 ± 0.814	5.20%	78.14%	18.406 ± 0.912	4.94%	92.03%
	80	Y	±	65.579 ± 2.992	4.56%	81.97%	79.511 ± 4.781	6.01%	99.39%
	160	N	+	130.768 ± 9.047	6.91%	81.73%	138.41 ± 9.246	6.68%	86.51%

Note: Y represents "yes" when the T-line developed a fluorescence signal at the T region; N represents "no" when no T-line was developed at the T region; a minus sign (−) represents a negative result when the fluorescence density of the T-line was the same as that of the C-line; a plus/minus sign (±) represents a weakly positive result when the fluorescence density of the T-line was weaker than that of the C-line; a plus sign (+) represents a positive result when no T-line developed at the T region; results are shown as means ± standard deviations ($n=3$)

when showed a positive result, the OA in the sample was above 160 μg·kg^{-1} (above 20 ng·mL^{-1} in sample solution), and the shellfish sample was unsafe and unsuitable for consuming. In quantitative assays, the recovery was around 80%, the CV was 5–7 %. Moreover, the ELISA method established by our team with proven reliability (Lin et al. 2015) was used for repeat validation, and the results were consistent with QDNB strips. It meant that the QDNB strip could be further developed as an effective tool for screening OA in seafood samples.

In addition, because heavy metals like cadmium used in QDs have biological toxicity and pollution on the environment, QDs synthesized from natural materials such as saccharides and lipids with high photostability, biocompatibility, low toxicity, and easy tunability for physicochemical properties (Ahuja et al. 2022) will be considered in the future to establish detection methods.

Conclusion

A new fluorescence immunochromatographic strip was developed for the rapid detection of OA. It was developed based on previous research (S. Y. Lu et al. 2012a, b), and using QDNBs instead of AuNPs. The full competitive LOD had been improved to 20 ng·mL^{-1}, which could meet the requirement of the EU edible safety limit, and the linear working range was 0.62–20 ng·mL^{-1}. Also, the strips were user-friendly, required no complex equipment, and could screen numerous samples within 20 min, gave semi-quantitative and quantitative results with low background and clear bands, may be useful as a rapid on-site screening method for environmental monitoring when using shaking and filtering instead of sonication and centrifugation.

Data Availability Statement

The datasets generated during the current study are available from the corresponding author on reasonable request.

Acknowledgements The authors would like to express their gratitude to EditSprings (https://www.editsprings.com/) for the expert linguistic services provided.

Funding This work was financially supported by grants from the National Key Research and Development Program of China (grant number 2017YFC1601205) and the National Nature Science Foundation of China (NSFC, No 32072943).

Declarations

Ethical Approval This article does not contain any studies with human participants or animals performed by any of the authors.

Informed Consent Not applicable.

Conflict of Interest Han Wang declares that she has no conflict of interest. Hong-Lin Ren declares that he has no conflict of interest. Pan Hu declares that she has no conflict of interest. Yan-Song Li declares that he has no conflict of interest. Yu Zheng declares that she has no conflict of interest. Qi Cao declares that he has no conflict of interest. Zhan-Xu Liu declares that he has no conflict of interest. Zeng-Shan Liu declares that he has no conflict of interest. Yong Yang declares that he has no conflict of interest. Shi-Ying Lu declares that he has no conflict of interest.

References

Ahuja V, Bhatt AK, Varjani S, Choi KY, Kim SH, Yang YH, Bhatia SK (2022) Quantum dot synthesis from waste biomass and its applications in energy and bioremediation. Chemosphere 293:133564. https://doi.org/10.1016/j.chemosphere.2022.133564

Alivisatos AP (1996). Semiconductor clusters, nanocrystals, and quantum dots. *Science, 271*(5251), 933–937. Retrieved from www.jstor.org/stable/2889983

An T, Winshell J, Scorzetti G, Fell JW, Rein KS (2010) Identification of okadaic acid production in the marine dinoflagellate Prorocentrum rhathymum from Florida Bay. Toxicon 55(2):653–657. https://doi.org/10.1016/j.toxicon.2009.08.018

An FP, Balantekin AB, Band HR, Beriguete W, Bishai M, Blyth S, Zou JH (2015) The muon system of the Daya Bay Reactor antineutrino experiment. Nucl Instrum Methods Phys Res, Sect A 773:8–20. https://doi.org/10.1016/j.nima.2014.09.070

Anon. (2004) Regulation (EC) No. 853/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific hygiene rules for food of animal origin. Off J Eur Union 139:60–61

Chen H, Huang C, Zhang W, Ding Q, Gao J, Zhang L (2019) Ultrastable nitrogen-doped carbon nanotube encapsulated cobalt nanoparticles for magnetic solid-phase extraction of okadaic acid from aquatic samples. J Chromatogr A 1608:460404. https://doi.org/10.1016/j.chroma.2019.460404

Chen H, Zhang XL, Jin ZY, Huang LP, Dan HB, Xiao W, Tang Y (2020) Differential diagnosis of PRV-infected versus vaccinated pigs using a novel EuNPs-virus antigen probe-based blocking fluorescent lateral flow immunoassay. Biosens Bioelectron 155:112101. https://doi.org/10.1016/j.bios.2020.112101

Duan H, Huang X, Shao Y, Zheng L, Guo L, Xiong Y (2017) Size-dependent immunochromatographic assay with quantum dot nanobeads for sensitive and quantitative detection of ochratoxin A in corn. Anal Chem 89(13):7062–7068. https://doi.org/10.1021/acs.analchem.7b00869

Dubertret, B., Paris, S., Norris, D. J., Noireaux, V., & et al. (2002). In vivo imaging of quantum dots encapsulated in phospholipid micelles. *Science, 298*(5599), 1759. Retrieved from https://search.proquest.com/docview/213593262?accountid=11718 http://www.yidu.edu.cn/educhina/educhina.do?artifact=&svalue=Science&stype=2&s=on http://pqdt.calis.edu.cn/Detail.aspx?pid= http://159.226.100.141/Reader/union_result.jsp?title=1&word=Science

European Food Safety A (2008) Marine biotoxins in shellfish - okadaic acid and analogues - Scientific Opinion of the Panel on Contaminants in the Food chain. EFSA J 6(1):589. https://doi.org/10.2903/j.efsa.2008.589

Guo L, Shao Y, Duan H, Ma W, Leng Y, Huang X, Xiong Y (2019) Magnetic quantum dot nanobead-based fluorescent immunochromatographic assay for the highly sensitive detection of aflatoxin B1

in dark soy sauce. Anal Chem 91(7):4727–4734. https://doi.org/10.1021/acs.analchem.9b00223

Hu LQ, Liu JX, Wang Q, Zhang Y, Jia R, Cai CE, He PM (2013) Development of an immunochromatographic strip test for the rapid detection of okadaic acid in shellfish sample. J Appl Phycol 25(4):1091–1099. https://doi.org/10.1007/s10811-012-9949-3

Hu T, Doyle J, Jackson D, Marr J (1992) Isolation of a new diarrhetic shellfish poison from Irish mussels. J Chem Soc Chem Commun (1). https://doi.org/10.1039/c39920000039

Huang X, Aguilar ZP, Xu H, Lai W, Xiong Y (2016) Membrane-based lateral flow immunochromatographic strip with nanoparticles as reporters for detection: A review. Biosens Bioelectron 75:166–180. https://doi.org/10.1016/j.bios.2015.08.032

Le Berre M, Kilcoyne M, Kane M (2015) Generation of a panel of high affinity antibodies and development of a biosensor-based immunoassay for the detection of okadaic acid in shellfish. Toxicon 103:169–175. https://doi.org/10.1016/j.toxicon.2015.06.030

Lee J-S, Igarashi T, Fraga S, Dahl E, Hovgaard P, Yasumoto T (1989) Determination of diarrhetic toxins in various dinoflagellate species. J Appl Phycol 1:147–152. https://doi.org/10.1007/BF00003877

Li X, Li W, Yang Q, Gong X, Guo W, Dong C, Chang J (2014) Rapid and quantitative detection of prostate specific antigen with a quantum dot nanobeads-based immunochromatography test strip. ACS Appl Mater Interfaces 6(9):6406–6414. https://doi.org/10.1021/am5012782

Liang MY, Zhao B, Xiong Y, Chen WX, Huo JZ, Zhang F, Li Y (2019) A "turn-on" sensor based on MnO2 coated UCNPs for detection of alkaline phosphatase and ascorbic acid. Dalton Trans 48(43):16199–16210. https://doi.org/10.1039/c9dt02971k

Lin C, Liu Z-S, Wang D-X, Ren H-L, Li Y-S, Hu P, Lu S-Y (2014) Sensitive and reliable micro-plate chemiluminescence enzyme immunoassay for okadaic acid in shellfish. Anal Methods 6(18):7142–7148. https://doi.org/10.1039/C4AY01063A

Lin C, Liu Z-S, Tan C-Y, Guo Y-P, Li L, Ren H-L, Lu S-Y (2015) Contamination of commercially available seafood by key diarrhetic shellfish poisons along the coast of China. Environ Sci Pollut Res 22(2):1545–1553. https://doi.org/10.1007/s11356-014-3494-3

Ling SM, Li XL, Zhang DP, Wang K, Zhao WW, Zhao Q, Wang SH (2019) Detection of okadaic acid (OA) and tetrodotoxin (TTX) simultaneously in seafood samples using colloidal gold immunoassay. Toxicon 165:103–109. https://doi.org/10.1016/j.toxicon.2019.04.011

Lu S-Y, Zhou Y, Li Y-S, Lin C, Meng X-M, Yan D-M, Ren H-L (2012a) Production of monoclonal antibody and application in indirect competitive ELISA for detecting okadaic acid and dinophytoxin-1 in seafood. Environ Sci Pollut Res 19(7):2619–2626. https://doi.org/10.1007/s11356-012-0819-y

Lu SY, Lin C, Li YS, Zhou Y, Meng XM, Yu SY, Liu ZS (2012b) A screening lateral flow immunochromatographic assay for on-site detection of okadaic acid in shellfish products. Anal Biochem 422(2):59–65. https://doi.org/10.1016/j.ab.2011.12.039

Morton SL, Moeller PDR, Young KA, Lanoue B (1998) Okadaic acid production from the marine dinoflagellate Prorocentrum belizeanum Faust isolated from the Belizean coral reef ecosystem. Toxicon 36(1):201–206. https://doi.org/10.1016/S0041-0101(97)00054-8

Ouyang S, Zhang Z, He T, Li P, Zhang Q, Chen X, Zhang W (2017) An on-site, ultra-sensitive, quantitative sensing method for the determination of total aflatoxin in peanut and rice based on quantum dot nanobeads strip. Toxins 9(4):137. https://doi.org/10.3390/toxins9040137

Pang L, Quan H, Sun Y, Wang P, Ma D, Mu P, Hammock BD (2019) A rapid competitive ELISA assay of Okadaic acid level based on epoxy-functionalized magnetic beads. Food Hydrocolloids 30(1):1286–1302. https://doi.org/10.1080/09540105.2019.1689231

Peng J, Zhao Z, Zheng M, Su B, Chen X, Chen X (2020) Electrochemical synthesis of phosphorus and sulfur co-doped graphene quantum dots as efficient electrochemiluminescent immunomarkers for monitoring okadaic acid. Sens Actuators, B Chem 304:127383. https://doi.org/10.1016/j.snb.2019.127383

Prassopoulou E, Katikou P, Georgantelis D, Kyritsakis A (2009) Detection of okadaic acid and related esters in mussels during diarrhetic shellfish poisoning (DSP) episodes in Greece using the mouse bioassay, the PP2A inhibition assay and HPLC with fluorimetric detection. Toxicon 53(2):214–227. https://doi.org/10.1016/j.toxicon.2008.11.003

Reguera B, Velo-Suárez L, Raine R, Park MG (2012) Harmful dinophysis species: a review. Harmful Algae 14:87–106. https://doi.org/10.1016/j.hal.2011.10.016

Ren W, Cho I-H, Zhou Z, Irudayaraj J (2016) Ultrasensitive detection of microbial cells using magnetic focus enhanced lateral flow sensors. Chem Commun 52(27):4930–4933. https://doi.org/10.1039/C5CC10240E

Sassolas A, Catanante G, Hayat A, Stewart LD, Elliott CT, Marty JL (2013) Improvement of the efficiency and simplification of ELISA tests for rapid and ultrasensitive detection of okadaic acid in shellfish. Food Control 30(1):144–149. https://doi.org/10.1016/j.foodcont.2012.05.028

Shen H, Xu F, Xiao M, Fu Q, Cheng Z, Zhang S, Tang Y (2017a) A new lateral-flow immunochromatographic strip combined with quantum dot nanobeads and gold nanoflowers for rapid detection of tetrodotoxin. Analyst 142(23):4393–4398. https://doi.org/10.1039/C7AN01227F

Shen H, Zhang S, Fu Q, Xiao W, Wang S, Yu S, Tang Y (2017b) A membrane-based fluorescence-quenching immunochromatographic sensor for the rapid detection of tetrodotoxin. Food Control 81:101–106. https://doi.org/10.1016/j.foodcont.2017.06.001

Wang Y, Qin Z, Boulware DR, Pritt BS, Sloan LM, González IJ, Bischof JC (2016) Thermal contrast amplification reader yielding 8-fold analytical improvement for disease detection with lateral flow assays. Anal Chem 88(23):11774–11782. https://doi.org/10.1021/acs.analchem.6b03406

Yao Y, Guo W, Zhang J, Wu Y, Fu W, Liu T, Chang J (2016) Reverse fluorescence enhancement and colorimetric bimodal signal readout immunochromatography test strip for ultrasensitive large-scale screening and postoperative monitoring. ACS Appl Mater Interfaces 8(35):22963–22970. https://doi.org/10.1021/acsami.6b08445

Yao S, Xiao W, Chen H, Tang Y, Song Q, Zheng Q, Deng N (2019) The combined detection of ovarian cancer biomarkers HE4 and CA125 by a fluorescence and quantum dot dual-signal immunoassay. Anal Methods 11(37):4814–4821. https://doi.org/10.1039/C9AY01454C

Zhang T, Stilwell JL, Gerion D, Ding L, Elboudwarej O, Cooke PA, Chen FF (2006) Cellular effect of high doses of silica-coated quantum dot profiled with high throughput gene expression analysis and high content cellomics measurements. Nano Lett 6(4):800–808. https://doi.org/10.1021/nl0603350

Zhang PF, Lu HQ, Chen J, Han HX, Ma W (2014) Simple and sensitive detection of HBsAg by using a quantum dots nanobeads based dot-blot immunoassay. Theranostics 4(3):307–315. https://doi.org/10.7150/thno.8007

Zhou J, Qiu XX, Su KQ, Xu GX, Wang P (2016) Disposable poly (o-aminophenol)-carbon nanotubes modified screen print electrode-based enzyme sensor for electrochemical detection of marine toxin okadaic acid. Sensors Actuators B-Chem 235:170–178. https://doi.org/10.1016/j.snb.2016.05.067