



A CdSe/ZnS core/shell competitive quantum dot-based fluorescence-linked immunosorbent assay for the sensitive and accurate detection of aflatoxin B1 in corn sample

Yanbing Lv¹ · Yifan Yang¹ · Ruili Wu¹ · Yanxia Xu¹ · Jinjie Li¹ · Ning Li¹ · Huaibin Shen¹ · Yujuan Chai² · Lin Song Li¹

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Abstract

Aflatoxin B1 (AFB1), as the main metabolite, found in aflatoxin contaminated food is highly toxic and strictly controlled in many countries. Aiming to construct a simplified, highly sensitive, and accurate quantitative detection platform of AFB1 in ordinary conditions, a one-step competitive quantum dot-labeled immunosorbent assay (cQLISA) was developed using biocompatible CdSe/ZnS core/shell quantum dots (QDs). The quantitative detection of AFB1 can be achieved by adding food sample and QD-AFB1 antibody simultaneously, with an analytical range between 1 and 40 ng/mL. The half maximal inhibitory concentration (IC50) of this method is 542 pg/mL and the limit of detection (LOD) is 56 pg/mL for standard samples, demonstrating a twofold improvement in sensitivity compared with that of the commercial enzyme-linked immunosorbent assay (ELISA) test kit. The recovery rates of negative corn spike-in samples range from 87.01 to 114.7% (CV < 15%). A strong correlation was found between cQLISA and commercialized reference platform using real contaminated corns ($R^2 = 0.9916$). The current method demonstrating a shorter operation time due to reduced steps, and a higher accuracy and consistency. The cQLISA showed great potential for convenient, accurate, and sensitive quantitative detection of batch analytes in food safety monitoring in ordinary condition.

Keywords Aflatoxin B1 · Quantum dots · Fluorescent probe · Competitive quantum dot-labeled immunosorbent assay · Corn

Introduction

Aflatoxins are a class of highly toxic mycotoxins, which are metabolites of fungi such as aflatoxin and parasitic aspergillus [1]. Many kinds of aflatoxins have been discovered,

with 18 types having been isolated and identified, including aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1), aflatoxin G2 (AFG2), aflatoxin M1 (AFM1), and aflatoxin M2 (AFM2) [2]. Among them, AFB1 is generally recognized as one of the most carcinogenic natural substance, listed as a Group I carcinogen by the International Agency for Research in Cancer [3, 4]. Moreover, AFB1 is the most common hazard in contaminated agriculture crops, occasionally detected in the majority of food raw materials and manufactured products worldwide [5]. Due to the wide existence and high toxicity of AFB1, regulations on its level are increasingly strict in countries all over the world [6]. It is necessary to establish a feasible method to detect AFB1 with high sensitivity and accuracy that can be easily performed.

In recent years, the detection method of AFB1 has also made remarkable progress. The current methods are mainly divided into two categories: physicochemical analysis based on chromatographic analysis and immune analysis based on antibody-antigen response [7, 8]. High-performance liquid chromatography (HPLC) is the most commonly used

Yanbing Lv and Yifan Yang have contributed equally to this paper.

✉ Ruili Wu
wrl0378@henu.edu.cn

✉ Yujuan Chai
chaiyj@szu.edu.cn

¹ Key Lab for Special Functional Materials, Ministry of Education, National and Local Joint Engineering Research Center for High-Efficiency Display and Lighting Technology, School of Materials, and Collaborative Innovation Center of Nano Functional Materials and Applications, Henan University, Kaifeng 475004, Henan, China

² Department of Biomedical Engineering, Shenzhen University, Shenzhen 518000, China

standard method to detect AFB1 at home and abroad, and it can achieve qualitative or quantitative detection of AFB1 combined with ultraviolet absorption, fluorescence, or mass spectrometry [9–12]. The advantages of this method are high specificity, sensitivity, and overall accuracy. However, the application of this method is limited in food-processing industry due to tedious steps and expensive instruments. An alternative technique is immunoassay based on antibody-antigen immunoreaction, including enzyme-linked immunosorbent assay (ELISA) [13, 14], electrochemiluminescent immunoassay [15, 16], immunoturbidimetric assays [17, 18], fluoroimmunoassay [19, 20], and surface plasmon resonance [21, 22], etc. Among these methodologies, ELISA is the most important tool and commonly used for the quantitation of AFB1, numerous ELISA test kits have been developed for screening of AFB1 [23–26]. However, the traditional ELISA method is complex and time-consuming, and some researchers have used magnetic material or fluorescent quantum dots (QDs) as labels, to shorten the testing time of ELISA and improve the sensitivity and specificity [27, 28]. Given its excellent stability and efficient signal amplification performance, QDs as biological label nanomaterials can achieve quantitative detection.

Semiconductor nanocrystal QDs have been widely used as fluorescence probes in *in vitro* diagnostics (IVD) recently [29–32]. Based on the design of traditional ELISA, QDs can be adopted to replace the enzymes as labeling materials and achieve the sensitive detection of low molecular weight analytes such as toxins, with a competitive quantum dot-based fluorescence-linked immunosorbent assay (cQLISA). Different from enzymes which are vulnerable to environmental conditions, QDs possess good photostability and thermostability. This nanomaterial can directly amplify the detection signals, shorten the chromogenic reaction time of enzymatic assay, and improve the performance of test [33, 34]. Ding's group realized ng level detection of enrofloxacin residue by using CdTe quantum dot-based fluoroimmunoassay method [19]. Song et al. used a multi-color quantum dots-based fluorescence immunoassay array for the detection of multiple antibiotic residues in milk, and the detection sensitivity reached pg level [35]. It can be seen that QDs as fluorescent labels have reached ng or pg level detection sensitivity. However, the real sample composition for toxins detection is very complex, which often influences the reliability and accuracy of detection results. Therefore, the development of a probe with high stability is very important for the detection of AFB1.

In this paper, highly luminescent CdSe/ZnS QDs were prepared by the “green” synthesis method developed by our group [36]. By encapsulating amphiphilic oligomers (polymaleic acid *n*-hexadecanol ester, PMAH), the free carboxylic acid groups were modified on the surface of QDs, which allowed the prepared QDs to maintain high stability

in the aqueous environment [37]. The prepared QD fluorescent probe has good stability and high quantum yield (QY), which being suitable for the harsh physiological environments and laying a foundation for *in vitro* detection in different environment [38]. A one-step quantitative bioassay without the need of chromogenic reaction and termination reaction was developed for a more efficient AFB1 detection. Compared with commercial ELISA kits, the use of high-quality QDs as fluorophores for AFB1 detection can achieve a twofold improvement in sensitivity (56 pg/mL). In addition, the proposed cQLISA method offers good stability and accuracy for corn samples, which has immense potential for the development of convenient, rapid, accurate, and sensitive quantitative detection of batch analytes in food safety monitoring.

Materials and methods

Materials and reagents

Cadmium oxide (CdO, 99.99%, powder), zinc oxide (ZnO, 99.99%, powder), sulfur (S, 99.98%, powder), selenium (Se, 99.99%, powder), oleic acid (OA, 90%), 1-octadecene (ODE, 90%), proclin-300, bovine serum albumin (BSA), ethanolamine, the aflatoxin B1 monoclonal antibody (mAb), AFB1-BSA, and AFB1 antigen were purchased from Sigma-Aldrich (St. Louis, USA). NaOH, HCl, NaCl, KCl, Na₂CO₃, NaHCO₃, KH₂PO₄, and Na₂HPO₄ were purchased from Hushi Ltd (Shanghai, China). H₃BO₃, Na₂B₄O₇·10H₂O, Tris, Hepes, methanol, and Tween-20 were purchased from Sangon Biotech Ltd (Shanghai, China). 1-ethyl-3-(3-(dimethylamino) propyl) carbodiimide (EDC), sulfo-N-Hydroxysulfosuccinimide (sulfo-NHS), and the 96-well microplates were purchased from Thermo Fisher Scientific (Waltham, USA). The ELISA kit was purchased from Helica (California, USA). The fluorescence spectra were measured using SpectraMaxi3 (Molecular Devices, Sunnyvale, USA). The sizes and zeta potential of QDs and the QD-AFB1 antibody probe were recorded using dynamic light scattering (Nano-ZS, Malvern Instruments, Worcestershire, UK). Purified water (18.2 mΩ) was used by Mill-Q system in all experiments. The negative corn samples were obtained from Zhenghai Biotech Corp (Shenzhen, China).

Preparation of QD-AFB1 antibody probe

The synthesis of CdSe/ZnS core/shell QDs (Photoluminescence [PL] = 623 nm) and aqueous PMAH-coated QDs were prepared according to the previous literature from our group [36, 37]. The AFB1 antibody was conjugated to aqueous QDs according to the previously described protocol [34]. Briefly, 0.75 mg of aqueous QDs nanocrystals, 0.868 mg of

EDC, and 2.45 mg of sulfo-NHS were dissolved by sodium borate solution (5 mM, pH 7.2, BS buffer), respectively, and added into a 1.5 mL-centrifuge tube. Then, the mixture was activated to react at 4 °C for 10 min under ultrasound. Therewith, the unreacted reagents were discarded by centrifugation at 20,000 rpm for 30 min. The mixture was dissolved in 400 μ L BS buffer (5 mM, pH 8.0), and a 100 μ g AFB1 antibody was added into the solution. Then, the mixture was incubated at 4 °C overnight. Afterward, the mixture was blocked by 1 wt% BSA solution and stopped by ethanolamine for 30 min, respectively. Next, the mixture was washed with 5 mM BS buffer (pH 9.0) by centrifugation at 20,000 rpm for 30 min. The QD-antibody probe was re-dispersed with 50 μ L of BS solution (5 mM, pH 8.0) containing 0.1 wt% BSA and 0.05% Proclin-300.

Fabrication of cQLISA

As shown in Fig. 1, the capture AFB1-BSA antigen (6.2 mg/mL) was diluted with carbonate-bicarbonate buffer (0.05 M, pH 9.6, CB buffer), and 100 μ L of the diluted AFB1-BSA was added to a 96-well microplate and incubated at 4 °C overnight. To avoid nonspecific binding, the

microplate was washed with PBST buffer (0.01 M, pH 7.4 phosphate buffered saline (PBS) containing 0.05% Tween-20) three times, and the excess binding sites were blocked by 0.01 M PBS buffer containing 1 wt% BSA at 4 °C overnight. The microplate was dried in a constant temperature humidity chamber for 24 h and then was stored at 4 °C until use. The schematic diagram of the cQLISA procedure was followed as Fig. 1. Firstly, the AFB1 antigen (100 ng/mL) was diluted to ensure the concentration (1, 5, 10, 20, and 40 ng/mL) by the methanol–water solution (70:30, v/v). Then, a series of concentrations of AFB1 samples were mixed with 0.01 M PBS (pH 7.4) at a ratio of 1:6 (the AFB1 standard sample was diluted 7 times by 0.01 M PBS). Subsequently, 50 μ L of different concentrations of AFB1 antigens or AFB1-spiked samples extract and 50 μ L of the QD-AFB1 antibody probe diluted with the probe buffer were added into the corresponding microplate and incubated for 60 min at 37 °C. To avoid nonspecific binding, the microplate was then washed with PBST buffer five times. At last, all the fluorescence intensity in every well was measured by SpectraMaxi3 when 450 nm was used as excitation wavelength.

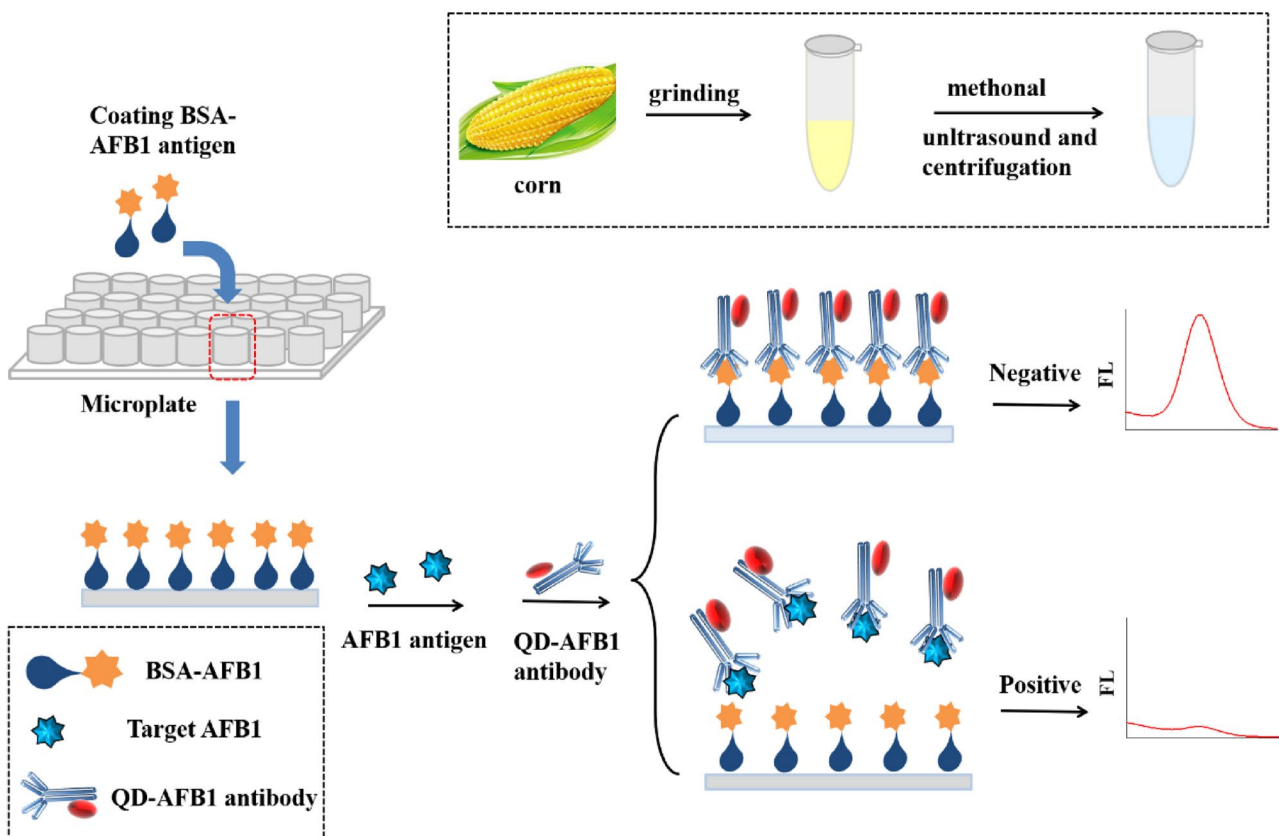


Fig. 1 Diagram of one-step cQLISA operation procedure

ELISA procedure

Follow manufacturer's instructions: The AFB1 antigens were diluted to 1, 2, 5, 10, and 20 ng/mL by the sample dilution buffer, and then diluted five times with 0.01 M PBS (pH 7.4) containing 20% methanol. HRP-AFB1 (1:1,000) and AFB1 samples were mixed in a 2:1 ratio (v/v), and 100 μ L of the mixture was added to the microplate and incubated for 15 min at room temperature. After washing with distilled water five times, 100 μ L of the tetramethylbenzidine (TMB) substrate system was added and incubated for 5 min at room temperature in the dark. The reaction was terminated by adding 100 μ L sulfuric acid (2 M) and the absorbance was measured with SpectraMaxi3 at 450 nm.

Spike in corn samples

The negative corn samples (AFB1 content < 1.0 ppb) determined by the HPLC method were obtained from Zhenghai Biotech Corp, Shenzhen, China. The negative corn samples served as the base sample were grinded with a grinder and filtered with 20 mesh screens, and 1 g of corn flour was added into 1 mL methanol–water solution (70:30, v/v). 1 mL of the AFB1 standard samples (2, 10, 20, 40, and 80 ng/mL) diluted by methanol–water solution (70:30, v/v) were added to the negative corn sample (mix 1:1 by volume). Subsequently, the mixture was oscillated on a vortex for 5 min and centrifuged at 6,000 g for 1 min. Five spiked corn flour with AFB1 concentrations of 1, 5, 10, 20, and 40 ng/mL were prepared for accuracy and precision analysis according to the above process.

Comparative evaluation with commercial ELISA Kit

A total of 20 random corn samples were obtained from farms, and the concentration of AFB1 in these samples were reported to be positive within the range of 5–20 ng/g. The quantification of AFB1 in the corn samples were performed using the one-step cQLISA method and the ELISA method. The raw materials were grinded and prepared according to the following steps: the corn samples were grinded with a grinder and filtered with 20 mesh screens. 1 g of corn flour was added into 2 mL methanol–water solution (70:30, v/v), then oscillated on a vortex for 5 min, and centrifuged 6000 \times g for 1 min.

Results and discussion

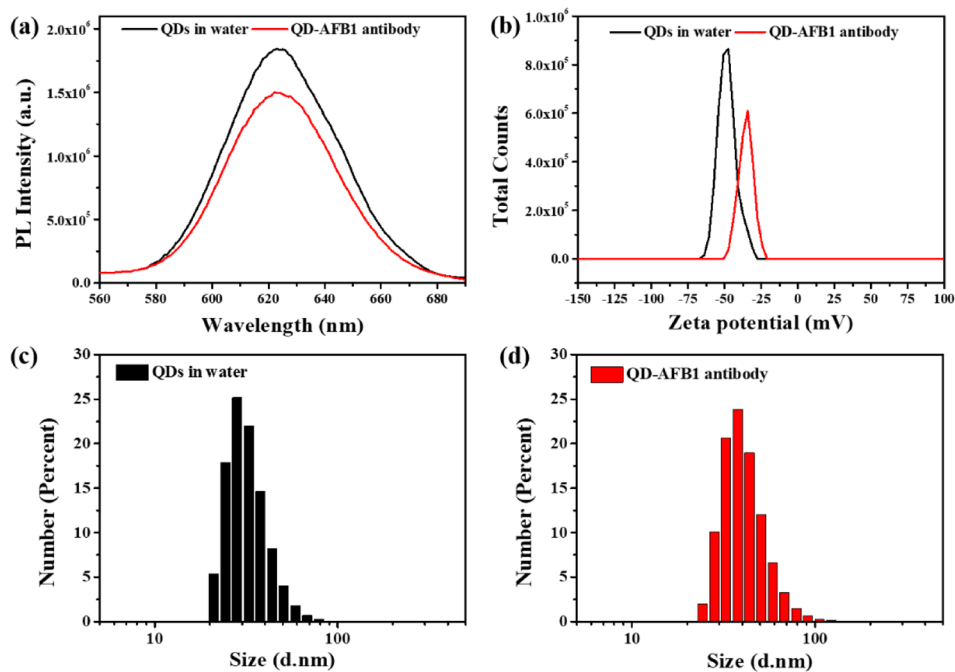
Characterization of aqueous QDs and the QD-AFB1 antibody

In this study, the high-quality hydrophobic CdSe/ZnS QDs (PL = 623 nm) were synthesized according to the strategy developed in previous research [36]. Fig. S1 (Supporting Information) shows the typical transmission electron microscopy (TEM) images of hydrophobic CdSe/ZnS QDs dispersing in hexane, which display good uniformity and dispersity with an average size of 15.08 nm. The biocompatible aqueous QDs were prepared successfully with the hydrophobic precursor by encapsulated with PMAH [37], increasing the average size to 15.36 nm (Fig. S1b, Supporting Information). Both hydrophobic and hydrophilic CdSe/ZnS QDs showed high fluorescence intensity and high QYs (92% and 88%, respectively). As shown in Fig. S2 (Supporting Information), the PL intensity of corresponding aqueous QDs dropped slightly compared with the hydrophobic QDs, suggesting the maintenance of fluorescent ability of the nanomaterial through the modification [34, 38]. Besides, the photographs clearly showed that the hydrophobic CdSe/ZnS QDs were completely transferred from the organic phase to the aqueous phase, and both hydrophobic QDs and corresponding hydrophilic QDs were well dispersed.

One of the key factors determines the performance of cQLISA method is the stability of the label material [39]. Therefore, the PL intensity was measured under different physiological conditions to verify the stability of optical properties of the aqueous QDs. As shown in Fig. S3a (Supporting Information), the aqueous QDs had good pH stability in acidic-to-alkaline environments (pH 2–14) with the PL intensity remained above 75%. The photostability test was carried out by illuminating continuously the aqueous QDs with a 365 nm ultraviolet lamp (32 W), and the PL intensity still reached 83% after 7 days (Fig. S3b, Supporting Information). The thermostability test was conducted at 60 °C for seven days, result in less than 10% PL intensity loss (Fig. S3c, Supporting Information). The above results suggested that the aqueous QDs had good photostability and thermostability in various relevant conditions.

The above aqueous QDs were used as fluorescent tags for the construction of AFB1 antibody labeled probes. The fluorescence spectra of aqueous QDs presented a narrow and symmetrical fluorescence peak ($\lambda_{\text{ex}} = 365$ nm, PL = 623 nm) with full width at half maximum of 40 nm (Fig. 2a). After AFB1 antibody was conjugated to QDs, the peaks position and shape of the QD-AFB1 antibody probe was similar to those of the aqueous QDs, indicating

Fig. 2 Fluorescence spectra (a) and zeta potential (b) of the aqueous QDs and the QD-antibody, dynamic light scattering of the aqueous QDs (c), and dynamic light scattering of the QD-antibody (d)



that the QD-AFB1 antibody probes had good optical properties. Meanwhile, its fluorescence intensity achieved 80.5% of aqueous QDs, and the minor loss of fluorescence was likely due to the centrifuge separation during the coupling process. The results in Fig. 2b showed that the zeta potential of aqueous QDs and the QD-AFB1 antibody probe shifted from -48.3 mV to -34.1 mV, which was considered to be stable (the value was above $+30$ mV or below -30 mV) in solution [40]. As shown in Fig. 2c, d, the hydrodynamic size of aqueous QDs was 51.01 nm, and it increased to 70.42 nm after the conjugation with AFB1 antibody successfully.

Optimization of the experimental conditions

To improve the performance of AFB1 quantitative detection technique, a series of cQLISA experimental conditions were considered to have an important influence on the detection results, including the blocking buffer, the probe dilution buffer, the optimum reaction time, and a checkerboard titration experiment.

The selection of blocking buffer

Different concentrations of BSA (0, 0.25 wt%, 0.5 wt%, 1 wt%, 2 wt%, and 5 wt%) were added into PBS buffer used as blocking solution to evaluate the effectiveness of cQLISA for the detection of AFB1. The BSA agent is commonly utilized to block the uncovered sites on the microplate surface, and can also be used in ELISA system to eliminate non-specific binding [41, 42]. As the AFB1 standard samples

with seven times dilution by PBS buffer were determined by the established one-step cQLISA, the actual half maximal inhibitory concentration (IC₅₀) was provided through calculation. As shown in Fig. S4a (Supporting Information), the result of AFB1 detection in tests had the IC₅₀ = 4.01 ng/mL when no blocking was used. In Fig. S4b–f (Supporting Information), a series of concentrations of BSA (0.25–5 wt%) in PBS buffer (0.01 M, pH 7.4) as blocking solution to block uncombined binding sites. As shown in Fig. S4 (Supporting Information), the appropriate concentrations of BSA as the blocking agent could reduce the nonspecific adsorption, thereby the IC₅₀ was lower and the sensitivity of detection was improved under a certain concentration of BSA. At last, the 1 wt% BSA in PBS buffer was used as the optimal blocking buffer of the cQLISA, and the IC₅₀ was 1.92 ng/mL.

The selection of probe dilution buffer

Different versions of probe dilution buffers were tested, including 5 mM BS buffer (pH 8.0), 5 mM Hepes buffer (pH 8.0), 5 mM Tris buffer (pH 8.0), and 5 mM PBS buffer (pH 8.0) contain 0.5 wt% BSA (Fig. S5a–d Supporting Information). By analyzing the four different conditions, the fluorescence intensity (B/B₀) declined with the increasing concentration of AFB1 antigens, the lowest IC₅₀ (1.89 ng/mL) was obtained in 5 mM Tris buffer in Fig. S5c (Supporting Information). Subsequently, different concentrations of BSA (0.1–1 wt%) contained in 5 mM Tris buffer were also evaluated in Fig. S5e–g (Supporting Information). For the better result in Fig. S5f (Supporting Information), 0.2 wt% BSA in 5 mM Tris buffer was selected as the probe dilution buffer.

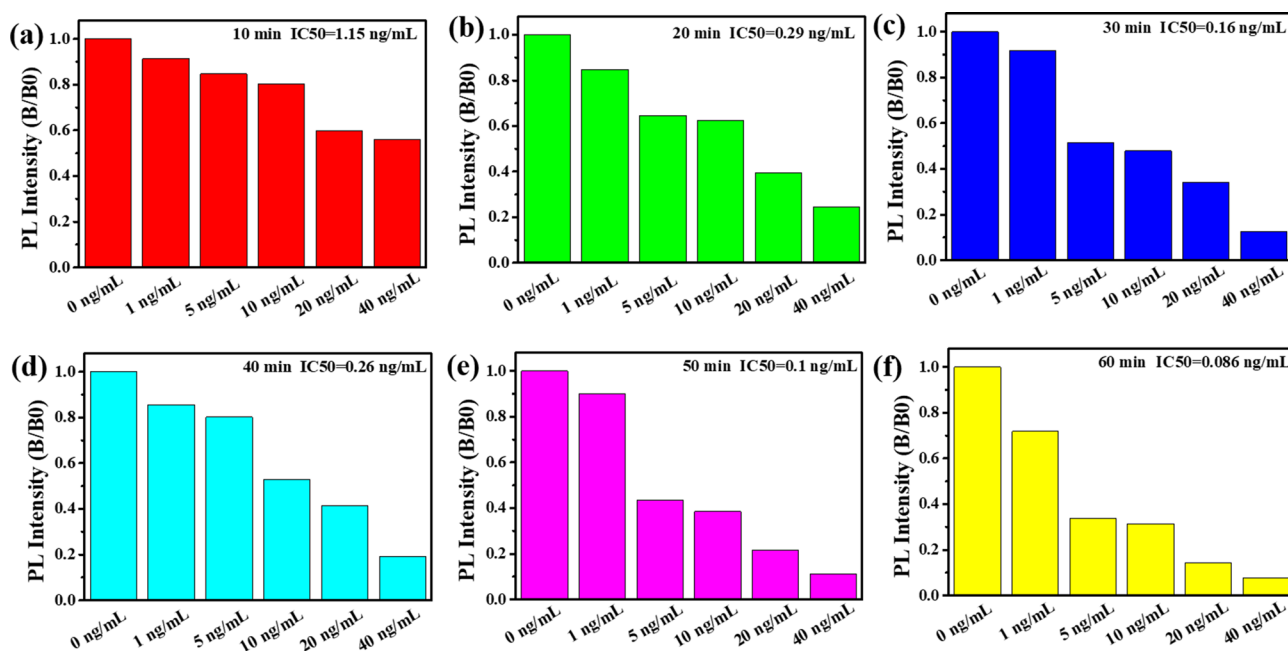


Fig. 3 Evaluation of PL intensity for QD-probes of different incubating time

The fluorescence intensity (B/B₀) was also influenced by the Tris buffer with different ionic strengths. From Fig. S5i–l (Supporting Information), the lowest IC₅₀ (1.16 ng/mL) was found when 20 mM Tris containing 0.2 wt% BSA was used as probe dilution buffer. Therefore, 20 mM Tris (pH 8.0) containing 0.2 wt% BSA was chosen as the favorable probe dilution buffer for the succeeding experiment.

Checkerboard titration

In the competitive immunoassay method, the appropriate antigen coating concentration and the amount of probe have strong influence on the experiment. To confirm the concentration of coating antigen (AFB1-BSA) and the dilution ratio of the QD-AFB1 antibody probe, a checkerboard titration experiment proceeded. Firstly, the AFB1-BSA antigens were diluted to different concentrations gradient (6.2, 1.55, 0.775, and 0.517 μg/mL) by CB buffer, and fixed in the corresponding wells. Subsequently, a series of concentrations of AFB1 antigens (0, 10, 20, and 40 ng/mL) and the QD-AFB1 antibody probe (1:1,000, 1:2,000, 1:4,000, and 1:8,000) were added into the corresponding wells. There is an inverse correlation between the fluorescence intensity at the bottom of the microplate and the AFB1 standard antigens only when the amount of QD-AFB1 antibody probe concentration is appropriate. The results shown in Table S1 presented that the fluorescence intensity obviously declined with the increasing concentrations of AFB1 antigens when the diluted ratio of the QD-AFB1 antibody probe was 1:1000 and the AFB1-BSA antigen was 0.775 μg/mL.

Optimization of incubation time

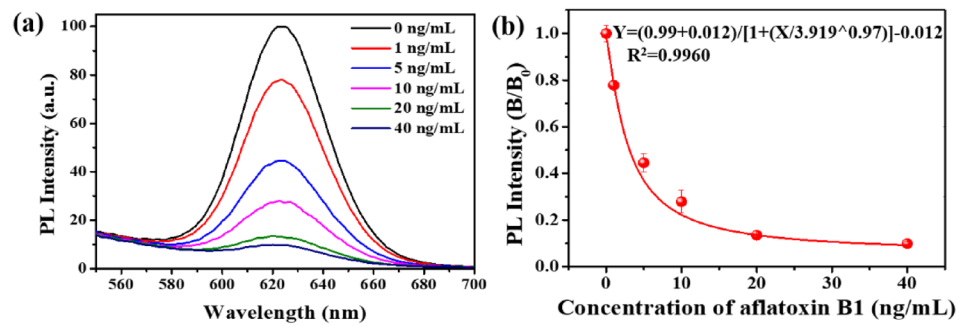
A series of concentrations of AFB1 antigens and the QD-AFB1 antibody probes were added into the microplate, and the mixture was incubated in one step. To optimize the fluorescence developing time, different times (10 min, 20 min, 30 min, 40 min, 50 min, and 60 min) were chosen as the incubation time. As shown in Fig. 3, the combination of the added AFB1 antigens and the QD-AFB1 antibody probes were insufficient when the reaction time was short. With the increase of incubation time, the combination of the added AFB1 antigens and the QD-AFB1 antibody probes were sufficient, and there were less QD-AFB1 antibody probes that could bind the AFB1-BSA antigens to reduce the fluorescence intensity (B/B₀), especially the high-concentration antigens. As shown in Fig. 3f, the fluorescence intensity (B/B₀) obviously declined with the increasing concentrations of AFB1 antigens, and the 60 min was chosen as the optimum incubation time.

Calibration curves of cQLISA method and methodology evaluation

Calibration curves of cQLISA method

Under optimal conditions, the calibration curve of the one-step cQLISA sensor was established by plotting the fluorescence intensity (B/B₀) against the various concentrations of the AFB1 standard samples. As shown in Fig. 4a, the fluorescence intensity declined gradually with the increase

Fig. 4 Fluorescence spectra (a) and calibration curve of AFB1 standard antigens (b) by cQLISA method (n = 3)



of AFB1 concentrations. The calibration curve in Fig. 4b showed a good linear relationship range from 1 to 40 ng/mL, and the best linear fit of the B/B₀ (Y) against logistic of AFB1 concentration (X) was $Y = (0.99 + 0.012) / [1 + (X/3.919^{0.97})] - 0.012$ (n = 3) with a high coefficient of determination ($R^2 = 0.9960$). The sensitivity of cQLISA was evaluated by the values of IC₅₀ and limit of detection (LOD). The LOD was defined as the concentration of AFB1 when the inhibition rate is 10% [43–45]. By calculation, the IC₅₀ of this cQLISA assay was 542 pg/mL and LOD was 56 pg/mL when AFB1 standard samples were diluted 7 times in PBS buffer, respectively. It illustrated a one-step detection method based on CdSe/ZnS QDs not only achieved the quantification detection of AFB1 antigens but also owned high sensitivity.

ELISA for quantification of AFB1

The commercial ELISA kit was purchased from Helica as a reference platform. According to the detailed steps provided in the instructions (see the experiment section for details), we tested some of the listed AFB1 standards. The AFB1 samples were tested in this platform and the calibration curve showed a good linear relationship range from 1 to 20 ng/mL (Fig. 5). The linear regression equation was $Y = (0.99 - 0.017) / [1 + (X/2.41^{1.53})] + 0.017$ (n = 3) with $R^2 = 0.9916$. According to the calculation, the actual IC₅₀ was 509 pg/mL and LOD was 113 pg/mL, respectively. Compared with the commercial AFB1 ELISA method (Helica), the sensitivity of the one-step cQLISA method increased by twofold, and the operation steps were simplified, which could reduce the operation steps and human error.

The accuracy and precision

To evaluate the accuracy of the cQLISA method, a series of different concentration corn samples were measured. The AFB1 standard antigens were added to the negative corn sample with final concentrations of 1, 5, 10, 20, and

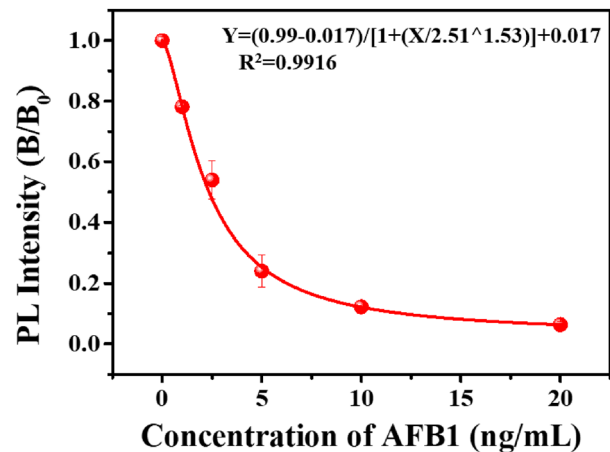


Fig. 5 Calibration curve of AFB1 standard antigens by ELISA method (n = 3)

40 ng/mL, which was comparable to previous studies and roughly covers the AFB1 range of contaminated corn samples in the market [46, 47]. Subsequently, the corn samples were extracted and measured. The comparison between the detected results from the cQLISA method and the real values was calculated and listed in Table 1. The recovery rate of current cQLISA assay for AFB1 was within the range of 87.01% to 114.7%, and the standard deviation was less than 15%, which was also comparable with ordinary ELISA method that was more complicated in terms of operation [23, 24]. These results suggested that the established cQLISA method for AFB1 detection had good accuracy.

Determination of AFB1 in real corn samples

The performance of the one-step cQLISA method for AFB1 was evaluated with a commercial ELISA kit by analyzing 20 AFB1 real contaminated corn samples using protocol specified by each platform (Fig. 6). The linear regression equation constructed with the AFB1 concentrations generated by commercial ELISA and cQLISA was $Y = 0.93X + 0.7$ with a high consistency ($R^2 = 0.942$). This

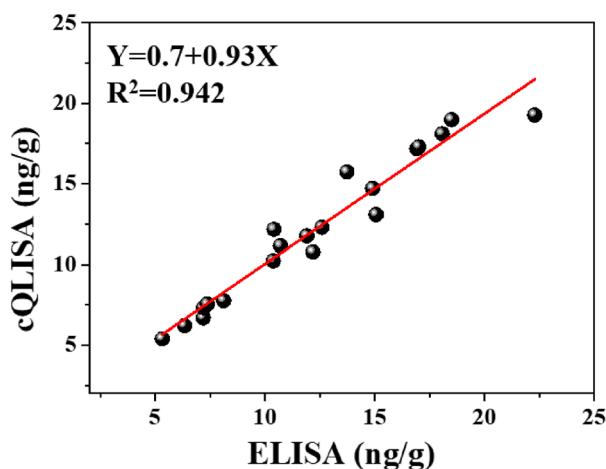
Table 1 Detection of corn samples at different concentrations (N=6)

Sample (n=6)	Spiked value ^b (ng/mL)	Test value (ng/mL)	Real spiked value ^c (ng/mL)	Recovery (%)	CV (%)
Basic sample ^a	0	0.92			9.1
P1	1	1.86	0.95	95.2	6.0
P2	5	6.65	5.73	114.7	5.2
P3	10	12.12	11.20	112.1	14.3
P4	20	21.76	20.84	104.2	11.6
P5	40	35.72	33.80	87.0	14.3

^aBasic sample: The negative corn flour samples

^bSpiked value: Concentration of AFB1 added into the negative corn flour corn samples

^cReal spiked value: The spiked value subtracting the background of basic sample

**Fig. 6** Comparison of results for AFB1 in real contaminated corn samples by cQLISA (y axis) and ELISA (x axis)

result indicated that the newly developed cQLISA method had excellent concordance with the commercial ELISA method and could be used for detection of AFB1 in real corn samples with less experimental operation and shorter time (within 60 min). The microplates of coated AFB1-BSA antigens and the QD-antibody probes were preserved for 7 days at 4 °C, and the PL intensity of this cQLISA was measured for every day. As shown in Fig. S6, the PL intensity almost unchanged at 4 °C after 7 days storage, which exhibited the satisfactory stability of this cQLISA for 0 ng/mL or 40 ng/mL of AFB1 (n = 3). The reliable performance of the cQLISA could particularly benefit the screening test of AFB1 in food sample when large amount of corns was being harvested and a less ideal environment or technique support is available.

Conclusion

In this work, a rapid one-step quantitative QD-labeled immunosorbent assay for the accurate detection of AFB1 was developed, aiming at improving the sensitivity and convenience of application in different conditions through the food collection and manufacture process. The water-soluble QDs prepared by our group showed high fluorescence and QY, contributed to the improvement of key performance. The detection range of current method was 1 to 40 ng/mL with a good linear regression ($R^2 = 0.996$), and the IC₅₀ and LOD for AFB1 sample were 542 pg/mL and 56 pg/mL. The proposed one-step cQLISA presented a high accuracy and stability for detection of the AFB1 contaminated corn samples, and demonstrated a twofold increase in sensitivity compared with the commercial ELISA kits. The reduction of operation steps and reaction time could benefit the large-scale food sample testing in harvest seasons with less trained technicians. In future studies, effort should be paid to improve the pre-coating and maintenance of the test kit to adapt to a more flexible testing environment such as manufacture sites and field of food collection.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s11694-021-01223-y>.

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Declarations

Conflict of interest Yanbing Lv, Yifan Yang, Ruili Wu, Yanxia Xu, Jinjie Li, Ning Li, Huaibin Shen, Yujuan Chai, and Lin Song Li declare that they have no conflict of interest.

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