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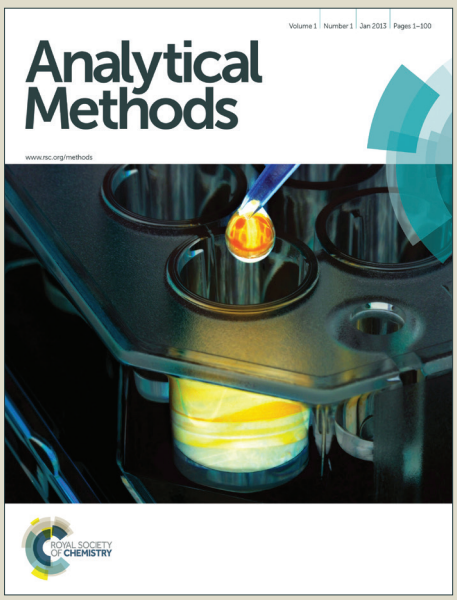

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ARTICLE TYPE

Monoclonal antibody-europium conjugate-based lateral flow time-resolved fluoroimmunoassay for quantitative determination of T-2 toxin in cereals and feed

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A method of lateral flow time-resolved fluoroimmunoassay (LF-TRFIA) was built up for rapid and ultrasensitive detection of T-2 toxin with TRFIA strips and an assorted portable TRFIA reader. Quantitative detection of T-2 toxin was realized by recording fluorescence intensities of the mAb-Eu (III) probes captured on the test line (T line) and control line (C line) of the assay strips. Quantitative standard curves for determining T-2 toxin in rice, maize, and feed were established by drawing a T/C ratio against the logarithmic concentration of T-2 toxin with the linear ranges of 0.125~200 ng/g for rice and maize and 0.25~200 ng/g for feed. The limit of detection (LOD) was 0.09 ng/g for rice and maize and 0.17 ng/g for feed. The recovery of the standard spike ranged from 94.2% to 111.0%, and the coefficient of variation (CV) was less than 15%. The results obtained by LF-TRFIA within 15 minutes showed a good correlation with the LC-MS/MS results, indicating that the LF-TRFIA method was reliable and suitable for rapid testing.

Introduction

T-2 toxin is a mycotoxin of type-A trichothecene mycotoxins, produced by various *Fusarium* species, such as *Fusarium tricincutum*, *Fusarium poae*, *Fusarium sporotrichiella*, *Fusarium sporotrichoides*, and *Fusarium moniliforme*. It is the most toxic trichothecene with toxophores of epoxy ring and double bond.[1-4] T-2 toxin has been evaluated as toxic, immunotoxic and haematotoxic. It can inhibit protein synthesis and DNA/RNA synthesis.[5-7] T-2 toxin appears mainly in cereals and cereal-based products, and can cause immunosuppressive effects and diseases as diarrhoea, skin irritation. Alimentary toxic aleukia， kashin-beck disease and keshan disease, which are three kinds of endemic diseases, are believed to be caused by T-2 toxin. Recently, several methods have been developed for the analysis of T-2 toxin, including confirmatory and fast assay approaches. Confirmatory approaches, such as gas chromatography(GC),[4] liquid chromatography(LC),[8-9] and liquid chromatography tandem mass spectrometry(LC-MS/MS)[10-11] require specific instrument and skilled operator, not suitable for out-lab detection. Fast assay methods are commonly based on test strip or sensor determinations, allowing a simple, fast and relatively inexpensive analysis of T-2 toxin.[12-16] Enzyme-linked immunosorbent assay (ELISA)[17] and colloidal gold immune chromatography test strips[18-19] are rapid methods using enzyme or colloidal gold as the labelling materials, respectively. In this paper, we use time-resolved fluorescence labelling material to develop fast quantitative determination of T-2 toxin, which obtains a higher sensitivity.

Fluorescence labelling detection has been widely used in the fields of life science, medical science, and immunology.[20] However, traditional fluorescence analysis is susceptible to nonspecific fluorescence.[21-22] Because organic-dye-based fluorescent markers can allow a narrow Stokes shifts (20~30 nm), the record of emission light can be affect by excitation light. In addition, because background fluorescence and scattered light almost cover the whole range of the fluorescence emission spectrum (350~600 nm), nonspecific fluorescence interference often occurs. Moreover, traditional organic fluorescent dyes are prone to photobleaching and quenching, leading to a decreased fluorescent intensity and limited sensitivity of the fluorescence labeling method. Time-resolved fluorescence analysis based on lanthanide used as a marker has more advantages than traditional fluorescence labeling.[23-27] *Time-resolved* means that fluorescence signals are recorded after attenuation in the fluorescence of the background material. The Stokes shift of lanthanide is larger (as over 150 nm) and fluorescence lifetime of the marker is higher than that of the background material (5~6 orders of magnitude). Therefore, time-resolved fluorescence analysis can eliminate the influence of various nonspecific fluorescence signals and achieve high sensitivity.

Time-resolved fluoroimmunoassay (TRFIA) mostly relies on antigen-antibody reactions. The antibody is marked by fluorescent microspheres to make a tracer, which can then be used in immunoassay. In this method, fluorescence is launched as a signal instead of an enzyme in enzyme-linked immunosorbent assay (ELISA) or colloidal gold in a lateral flow test strip. During the past decades, the TRFIA method has been developed to detect a variety of substances.[28-31] Eu (III) is one of the lanthanide labels

that have been proven to give high sensitivity in time-resolved fluoroimmunoassay.[32-33] In this work, we reported the development of the LF-TRFIA method for quantitative determination of T-2 toxin in cereals and feed with a portable fluorescence reader and time-resolved immunochromatographic assay strips. The fluorescent microspheres introduced Eu (III) into polystyrene nanoparticles to form stable nanoparticles with desirable fluorescent properties. The carboxy modified on the surface of the polystyrene nanoparticles could be linked by amido groups from the antibody and formed a stable amide bond. With advantageous mAb-Eu (III) probes and an assorted reader, a rapid, sensitive, specific, and one-step strategy has been developed for T-2 toxin analysis.

Materials and methods

All experiments were performed in compliance with the relevant laws and institutional guidelines. The management committee of the experimental animal in Hubei Province has approved the experiments.

Reagents and instruments

T-2 toxin, HT-2 toxin, deoxynivalenol (DON), and T-2-bovine serum albumin conjugates (T-2-BSA) were purchased from aokin AG (Berlin, Germany). Aflatoxin B_1 (AFB_1), aflatoxin M_1 (AFM_1), ochratoxin A (OTA), zearalenone (ZEA), and Fumonisins B_1 (FB_1) were purchased from Sigma (San Francisco, USA). Polystyrene fluorescent microspheres were obtained from Shanghai Youni Biological Technology Inc. BSA was purchased from Roche Applied Science (Indianapolis, USA). 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) was obtained from Acros (Belgium). The 1% (solid content, W/V) Eu(III)-marked and COOH-modified monodisperse polystyrene nanoparticles were purchased from Shanghai uni-bio Biotechnology Co. Ltd. All the reagents were of analytical reagent grade or better. Water was obtained from a Milli-Q purification system (Millipore). Nitrocellulose (NC) membranes, glass fibers, and absorbent pads were purchased from the Millipore Corporation (Bedford, USA), and the silica gel SPE column was purchased from Shanghai Sanpont Co. Ltd.

XYZ3050 Biostrip Dispenser and CM 4000 Cutter from BioDot (Irvine, USA) were used to prepare test strips. A high-speed freezing centrifuge (CF16RX) was from Hitachi (Tokyo, Japan). An ultrasonic cleaner was purchased from Shanghai Hengqi Instruments & Apparatuses Co. Ltd.

The portable time-resolved fluorescence reader was composed of an optical system with an xenon lamp activated by pulses from a clock-pulse generator. The emission light was obtained using a side-window photomultiplier tube at a negative bias voltage. After the signals were processed using a rapid preamplifier-discriminator and pulse counter, the result was further delivered to the readout. It can be employed both on-site assay and in-house detection.

Conjugation of monoclonal antibodies and polystyrene fluorescent microspheres

The 1% (solid content, W/V) Eu(III)-marked and COOH-modified monodisperse polystyrene nanoparticles were used. A solution containing 200 μL microspheres was added into 800 μL 0.2 mol/L boric acid buffer (pH 8.18), and was mixed by using a vortex mixer. After sonication for 10 minutes, 40 μL 15 mg/mL fresh aqueous solution of EDC was added into the mixture, which was blended for 15 minutes. Then, the solution was centrifuged at 90 g at 10°C for 10 min. The supernatant including the rest of EDC was discarded, and 1 mL boric acid buffer containing 0.5% BSA was used to dissolve the precipitation. After sonication for 10 minutes, a certain amount of the anti-T-2 toxin monoclonal antibody was added. The mixture was rocked on a table concentrator overnight at 20°C. After that, the solution was centrifuged at 90 g at 10°C for 10 min, the supernatant including the rest of the monoclonal antibody was discarded, and the precipitation was dissolved with 0.5% aqueous solution of BSA to close the rest of the antibody-combining sites. After sonication for 10 minutes, the conjugates were rocked on the table concentrator for 2h at 250 g at room temperature. The monoclonal antibody-europium conjugate was stored at 4°C.

Preparation of monoclonal antibody against T-2

The preparation of monoclonal antibody against T-2 was followed the similar strategy, including the antigen preparation, immunization, and monoclonal antibody development, as reported previously.[34-35] First, the immunogen (T-2-BSA) was synthesized as previously described using a typical EDC-NHS coupling method.[36] Second, in the initial immunization, 1 mg mL^{-1} of T-2-BSA conjugate in PBS was emulsified with an equal volume of Freund's complete adjuvant, followed by being injected multiple-site subcutaneously into 6-week-old female Balb/c mice. In subsequent injections, the same dosage of T-2-BSA was emulsified with an equal volume of Freund's incomplete adjuvant. Three intraperitoneal injections were carried out after the first immunization with an interval of 4 weeks. The booster injection three days before cell fusion was carried out with 2-fold dosage of antigen without emulsification with adjuvant. Third, fusion between SP2/0 myeloma and spleen cells were performed using hybridoma technique as previously described.[37] The resulting hybridoma clones were propagated, then one part of cells were cryopreserved in freezing solution and stored in liquid nitrogen, another part of cells were injected intraperitoneally into Balb/c mice. Finally, the ascitic fluids were purified by the caprylic acid–ammonium method as described previously. [38]

Fabrication of the immunochromatographic assay strip

The immunochromatographic assay strip was composed of a sample pad (13 mm), nitrocellulose membrane (25 mm), absorption pad (17 mm), and a backing card, as shown in Fig. 1. The sample pad was made from glass fibers, treated with blocking buffer, and completely dried at 37°C overnight. Nitrocellulose membranes were spotted using XYZ3050 Biostrip Dispenser (BioDot) with the optimal amounts of T-2–BSA for the test line and rabbit anti-mouse IgG for the control line, leaving a 9 mm space between the two lines, which were dried for 2h at 37°C. T-2–BSA and rabbit anti-mouse IgG were dissolved in 0.01 mol/L pH 7.4 PBS containing 1% BSA beforehand. The sample pad, NC membrane, and absorption pad were stuck on the plastic backing board sequentially with a 1~2 mm overlap. The master card was cut into 4.5 mm wide strips using a CM 4000 Cutter (BioDot). The strips were then sealed in a plastic strip cartridge with desiccant gel and stored at 4°C. In many papers, a

conjugated pad was a part of the strip. In this work, a sample vial containing both the conjugate and the sample extract was used instead.

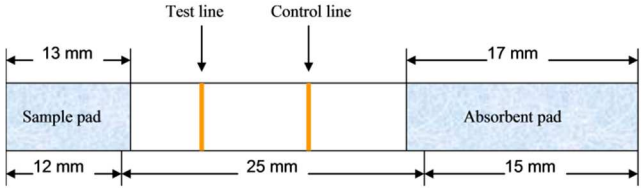

Fig.1 Constructional detail of the immunochromatographic assay strip

The LF-TRFIA method

The LF-TRFIA method was realized with the help of immunochromatographic assay strips and a portable fluorescence reader. The assay was performed in a sample vial by mixing 100 µL sample extracts and 100 µL mAb-Eu(III) probes which were diluted in the analysis buffer. The sample vial was incubated at 37°C for 12 min. The strip was inserted into the sample vial for absorption of the mixture by the pad in a capillary migration process, as shown in Fig. 2(A). The indirect competitive immunoassay was performed on the strip, as shown in Fig. 2(B). If the sample was without T-2 toxin, the mAb-Eu (III) probes reached the T line and C line and reacted with the T-2–BSA and rabbit anti-mouse IgG. If the sample was with a little amount of T-2 toxin, the amount of mAb-Eu (III) probes reacting with the T-2–BSA decreased. If the sample was with excess T-2 toxin, the mAb-Eu (III) probes reacted with the toxin and there was no probe reacting with the T-2–BSA. The fluorescence signals of the test line and control line were invisible unless using UV light. The fluorescence signal intensities of the test line and control line in the first and third cases recorded by the portable time-resolved fluorescence reader were shown in Fig. 2(C). The optimal excitation wavelength and emission wavelength for europium were 365 nm and 613 nm, respectively. The light of the portable time-resolved fluorescence reader was just set at 365 nm, and the reader was used to measure the fluorescence of 613 nm with time delay. The reader scanned the two bands (test line and control line), collected fluorescence signals, produced two peaks, and calculated calculus values of the two peak areas in 15s. Standard curves for the samples could be put into the reader. Then, the machine could read other strips for this kind of samples and present the results directly.

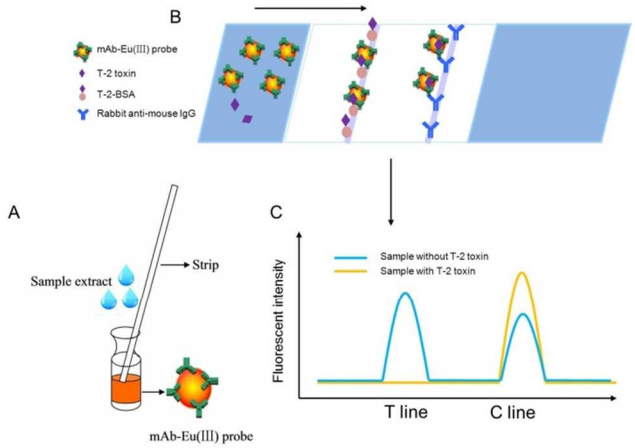

Fig.2 Schematic sketch of the LF-TRFIA method

Sample preparation

The preparation of cereal samples for LF-TRFIA was made as follows: 50 g of the blank cereal sample was extracted with 100 mL methanol/water (70:30, v/v) and stirred by mini-size Joyoung Soymilk Maker for 2 min. After filtered with double-filter paper, 1 mL of the extract was diluted with 4 mL analysis buffer. The diluted extract was filtered by 0.45 µm filter membrane. Then, the final solution could be detected with the strips. The difference between cereal sample preparation and feed sample preparation was that 25 g of the blank feed sample was extracted with 100 mL methanol/water (70:30, v/v) containing 4% NaCl and before dilution the filtrate was purified by silica gel SPE column. The T-2 toxin standard solution was spiked into the blank extract. The naturally-contaminated cereal and feed samples were prepared in the same procedure as the above procedure without T-2 toxin spiking.

Establishing standard curves

Along with the gradually-increased T-2 toxin concentrations in the samples, the fluorescence signal of the T line was dimed and that of the C line almost remained the same. The change in the fluorescence signal of the T line could make a qualitative or semiquantitative determination. In this work, standard curves of the T line and C line were investigated to make a quantitative determination.

To establish standard curves, blank rice, maize, and feed samples with T-2 toxin undetectable by LC-MS/MS were fortified with appropriate amounts of the standard T-2 toxin solution. The T-2 toxin concentrations of the spiked samples were from the blank level to the cut-off level, including 0, 0.125, 0.25, 0.5, 1, 2, 5, 10, 20, 40, 50, 80, 100, 200, 400, and 500 ng/g. To obtain the standard curve, $R_{T/C(T\text{-}2)}$ was recorded as the ratio of fluorescent intensities on test line and control line, and a natural logarithm T-2 concentration, thus providing $R_{T/C(T\text{-}2)} = b \ln c_{T\text{-}2} + a$. In this work, four standard curves including the analysis buffer, rice, maize, and feed were established and the matrix effects were compared. According to a state standard of China (GB/T 5009-2003, entitled "Methods of food hygienic analysis-Physical and chemical section-General principles"), The LOD was calculated as this equation LOD = Ks/b, in which *s* means triplex value of standard deviation from the recorded values of 20 blank samples, *b* means the slope of standard curve, K is usually

defined as 3. The linear range was obtained from standard recovery experiments.

Evaluation of the method

To evaluate a method, specificity, accuracy, repeatability, reproducibility, and stability were considered. Specificity was evaluated by investigating cross-reactivities with other mycotoxins including HT-2, DON, AFB_1, AFM_1, OTA, ZEA, and FB_1, some of which were similar to T-2 toxin in the molecular structure. The spiked cereal and feed samples were used to evaluate the accuracy of this method. Each sample was detected by five strips, and the repeats were used to calculate the coefficient of variation (CV) to evaluate the repeatability. Reproducibility was evaluated on the performance of the strips at different days. Stability was examined by treating the strips with toxin-free analysis buffer. These strips and mAb-Eu (III) probes were stored for 0~6 months. The naturally contaminated cereal and feed samples were detected with this method and LC-MS/MS for application and validation.

Results and discussion

Optimization of the amount of the antibody for conjugation

The antibody was conjugated with fluorescence microspheres by covalent interactions. The amount of the antibody could affect coupling efficiency and sensitivity. Serial amounts (4 μL; 8 μL; 12 μL; 16 μL; 24 μL; 48 μL) of 2.7 mg/mL antibody were used in the conjugation process. The amount of the antibody in the supernatant after centrifugation could be detected by ELISA based on the same antibody with a known concentration. Then, the coupling ratio could be calculated. The result was that the coupling ratios of all the six conjugates were greater than 95%. To finally confirm the optimal conjugation, the conjugates were diluted by the analysis buffer several times and detected by the strips. The 0, 20, and 100 ng/g T-2 toxin spiked analysis buffer were used on the conjugates. As shown in Fig. 3, the third conjugate outperformed others. The T line had a clear distinction between the negative solution and the positive solution, and the best one had distinct sensitivity.

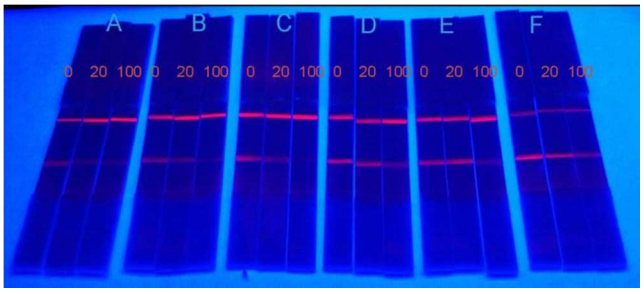

Fig.3 Optimization of the amount of the antibody for conjugation (A: 2.7 mg/mL, 4 μL; B: 2.7 mg/mL, 8 μL; C: 2.7 mg/mL, 12 μL; D: 2.7 mg/mL, 16 μL; E: 2.7 mg/mL, 24 μL; F: 2.7 mg/mL, 48 μL)

Optimization of the strip

In general, analytical performance of strips is affected by many parameters such as the type and pore size of the membrane, blocking buffer, analysis buffer, and immunoreagent amount. These parameters were evaluated in this paper.

Three kinds of NC membrane (Millipore HF095, Millipore HF135, and Millipore HF180) were used to prepare strips to find which one was the optimal membrane. The chromatography speed, sensitivity, and band width were compared. Chromatography on the Millipore HF180 NC membrane was slow while it was very fast on the Millipore HF095 NC membrane. A fast speed, however, was not good for competitive reaction. The bands on the Millipore HF095 NC membrane were thicker, which was not good for the recording by the reader. The speed and bands on Millipore HF135 were modest and the sensitivity was the best in the comparison experiments, the data of which was not shown in this paper.

Three kinds of blocking buffer (0.01 mol/L pH 7.4 PBS+2% BSA+2.5% sucrose+0.02% NaN_3; 0.01 mol/L pH 7.4 PBS+2% BSA+0.1% TrionX-100+0.3% PVPK30+2.5% sucrose+0.02% NaN_3; 2.9% Na_2HPO_4+0.3% NaH_2PO_4+1% Tween-20+1% PVPK30+0.25% EDTA+0.5% BSA+0.02% NaN_3) were evaluated to determine which one had the best effect for the sample pad. All of these three types of buffer could work well as fluorescent microspheres could move up and there was no residual on the sample pad. The third one had better sensitivity in the comparison experiments. The results indicated that 2.9% Na_2HPO_4+0.3% NaH_2PO_4+1% Tween-20+1% PVPK30+0.25% EDTA+0.5% BSA+0.02% NaN_3 was chosen as the optimal blocking buffer for the sample pad.

Three kinds of analysis buffer (water; 0.4% Tween-20; 1% sucrose+0.5% BSA+2.5% Tween-20) were employed for the strips. 1% sucrose+0.5% BSA+2.5% Tween-20 was found to be the best one for the strips. As a surfactant, Tween-20 could improve hydrophilicity and wettability, reduce the surface tension of the liquid, increase the movement speed, prevent non-specific binding, and ensure an adequate reaction and low residues. Sucrose and BSA played the role of stabilizer in the analysis buffer.

The amount of the fluorescent nano-polystyrene-mAb probe in one reaction, antigen on the test line, and rabbit anti-mouse IgG on the control line could directly influence the final luminosity of the strips. The mAb-Eu (III) probe solution was diluted to different concentrations (25-fold, 50-fold, 100-fold, and 200-fold) for further testing. The concentrations of T-2–BSA and rabbit anti-mouse IgG were 0.25 mg/mL and 0.5 mg/mL, respectively. The dispenser rate for drawing two lines was set to a series of amount: 0.3 μL/cm, 0.4 μL/cm, 0.5 μL/cm, 0.6 μL/cm, and 0.7 μL/cm. The immunoreagent amounts were evaluated as the "checkerboard titration" in ELISA by trial and error. Finally, the optimal dilution times of the fluorescence microsphere was 50-fold, the optimal spraying rates of T-2–BSA and rabbit anti-mouse IgG used for the test and control lines were both 0.4 μL/cm. The values of the two lines read from the reader were above 5000 and the ratio of the T line to the C line (T/C) was around 1~2. Under these conditions, the method showed good linearity and sensitivity.

Optimization of the test process

In the test process, the sample volume, reaction volume, reaction time, and reaction temperature were under observation as influencing factors. In this work, the sample extract was mixed with the same volume of the mAb-Eu (III) probe that had been diluted for 50 times. For an adequate reaction, 200 μL was

enough. Therefore, 100 μL sample extract and 100 μL diluted mAb-Eu (III) probe were used in a detection procedure. To obtain the optimal reaction time, a triple-experiment was conduct with an increasing stop-reaction time of 30 s, 1 min, 2 min, 3 min, 4 min, 5 min, 6 min, 7 min, 8 min, 9 min, 10 min, and 11 min, respectively. Immediately after the stop-time, the fluorescent signals of the T line and C line were recorded. Results found, after a 7-min reaction, the fluorescence signals of T line and C line became similar and remained stable for several minutes. The results and variation trend were shown in Fig. 4, discovering that the optimal reaction time for the test process was 7 min. Since 37°C was the optimum temperature for an antigen-antibody reaction, a constant temperature incubator set at 37°C was used in the test process.

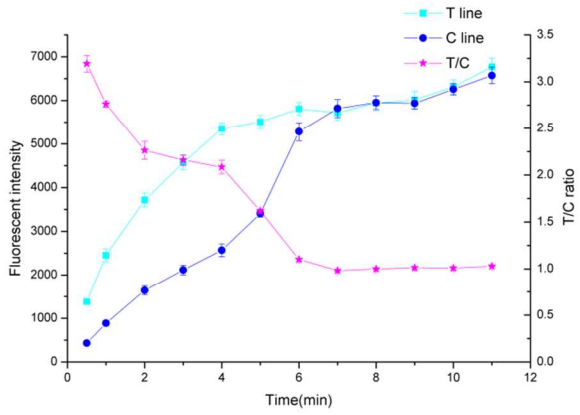

Fig.4 Temporal evolution of the fluorescent intensity of immunochromatographic reaction on the test strip

Establishing standard curves

Based on the above optimized detection conditions, a series of spiked standard solutions at different T-2 toxin concentrations were analyzed by the strips to establish standard curves. Each concentration was examined in triplicate. Fig. 5 showed four standard curves for the analysis of buffer, rice, maize, and feed. And the LOD of this method was calculated for each kind of sample. The results were shown in Table 1. A matrix effect, which depended on the complexity of the sample matrix, existed in the process. The feed matrix was more complex than that of rice and maize. A more complex sample matrix led to a smaller absolute value of the slope of a standard curve and a lower sensitive method. All of the four standard curves gave good absolute values of the slopes. The absolute values of the slopes of the standard curves for rice and maize were close to that of the analysis buffer and higher than that of the feed. The curvilinear equation, linear range, correlation coefficient (R^2), and LOD were shown in Table 1. The typical responses of the strips for T-2 toxin with different concentrations were shown in Fig. 6.

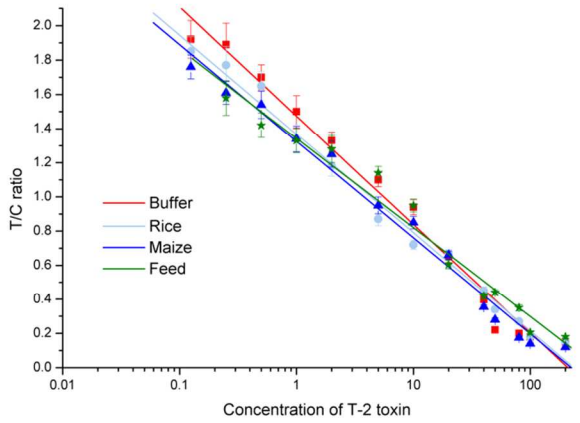

Fig.5 Four standard curves for the analysis of buffer, rice, maize, and feed

Table 1 Standard curves for the analysis of buffer, rice, maize, and feed

Sample	Linear equation	R^2	Linear range for the test solution (ng/g)	LOD (ng/g)	Dilution times	Dynamic range for samples (ng/g)	LOD (ng/g)
Buffer	y=1.474-0.6348*log(x)	0.9915	0.125~200	0.08	—	—	—
Rice	y=1.3677-0.5764*log(x)	0.9946	0.125~200	0.09	10	1.25~2000	0.9
Maize	y=1.3258-0.5641*log(x)	0.9929	0.125~200	0.09	10	1.25~2000	0.9
Feed	y=1.3427-0.5219*log(x)	0.9850	0.25~200	0.17	20	5~4000	3.4

[a] Footnote text

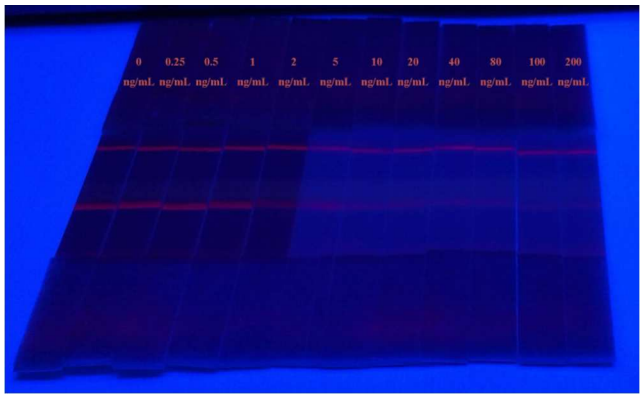

Fig.6 Change of the fluorescent bands of the strips for detecting T-2 toxin with different concentrations

Specificity of the method

To confirm specificity of the test strip, cross-reactivity was taken into consideration. The analysis buffer was doped with T-2 toxin and its competitors including HT-2, DON, AFB_1, AFM_1, OTA, ZEA, and FB_1, which were presented at the concentrations of 500 ng/mL, 500 ng/mL, 20 ng/mL, 20 ng/mL, 200 ng/mL, 100 ng/mL, and 500 ng/mL, respectively. The cross-reactivity results showed that the luminescence of the test lines was the same with that of the pure analysis buffer. It indicated that the mAb-Eu (III) probe-based strips had no cross-reactivity with other mycotoxins.

Accuracy, repeatability, reproducibility, and stability of the method

To further evaluate the accuracy and reproducibility of the newly-developed technique, spiked blank samples for rice, maize, and feed at four concentrations of 10 ng/g, 50 ng/g, 100 ng/g, and 500 ng/g were investigated for five repeats at each concentration using the strips. As indicated in Table 2, the recovery results of this method were in conformity with the spiked T-2 toxin concentrations. The recovery was in the range from 94.2% to 111.0%. For the repeatability, the CV was calculated via an intra-assay from the average value of the five specific strips for each concentration to evaluate the intra-assay variations. Result found that a considerable CV of 2.3%-13.7%, suggesting a good repeatability. For its reproducibility, an inter-assay experiment by using these strips in five different days was conducted in the same procedure. It was found to be a CV of 2.3%-14.8%, implying excellent reproducibility. Both intra-assay and inter-assay CVs decreased with the increase of T-2 toxin concentration.

Thus, the developed LF-TRFIA method was of good repeatability and reproducibility. During the stability examination, the mAb-Eu (III) probe was homogeneous and steady. The intensities of the fluorescence bands in 6 months were slightly weaker than those in the first day, which had no influence on the detection. Then, this developed LF-TRFIA method was still usable after six-month storage.

Table 2 Recovery analysis of LF-TRFIA for rice, maize, and feed

Sample		Spiked T-2 toxin (ng/g)	Result[a] (ng/g)	Recovery (%)	CV (%)
Rice	Inner-assay[b] (n=5)	10	10.9±1.1	109.0±10.7	9.8
		50	51.2±2.3	102.4±4.6	4.4
		100	98.4±4.6	98.4±4.6	4.7
		500	492.2±11.4	98.4±2.3	2.3
	Intra-assay[c] (n=5)	10	11.1±1.1	111.0±10.7	9.7
		50	52.0±2.2	104.0±4.3	4.1
		100	97.0±5.7	97.0±5.7	5.9
		500	491.2±13.0	98.2±2.6	2.6
Maize	Inner-assay (n=5)	10	10.7±1.5	107.0±14.5	13.5
		50	50.4±2.8	100.8±5.6	5.5
		100	97.0±5.7	97.0±5.7	5.9
		500	491.0±11.6	98.2±2.3	2.4
	Intra-assay (n=5)	10	10.5±1.2	105.0±12.5	11.9
		50	50.2±2.9	100.4±5.8	5.8
		100	95.0±7.9	95.0±7.9	8.3
		500	490.8±11.4	98.16±2.3	2.3
Feed	Inner-assay (n=5)	10	10.3±1.4	103.0±14.1	13.7
		50	48.6±3.5	97.2±7.0	7.2
		100	95.0±6.0	95.0±6.0	6.3
		500	489.0±14.0	97.8±2.8	2.9
	Intra-assay (n=5)	10	10.2±1.5	101.8±15.1	14.8
		50	48.2±3.9	96.4±7.9	8.2
		100	94.2±6.4	94.2±6.4	6.8
		500	487.0±15.3	97.4±3.1	3.1

[a] The report data is the mean±SD.
[b] The assays are carried out in five replicates in the same day.
[c] The assays are carried out in five different days.

Application and validation

Twenty samples including rice, maize, and feed were bought from the supermarket and farm product market. These samples were investigated using this developed LF-TRFIA method with LC-MS/MS for reference. The results by the two methods were presented in Table 3. As listed in Table 3, the results of the LF-TRFIA method were in good agreement with the reference LC-MS/MS method. Fig. 7 showed good consistence of the two methods. With good performance, this newly-developed method could be used in T-2 toxin analysis in cereals and feed.

Table 3 Comparison of the LF-TRFIA and LC-MS/MS methods

Sample	Results by LF-TRFIA (ng/g)	Results by LC-MS/MS (ng/g)	Relative error (%)
rice-1	9.93±0.47	9.52±0.33	4.31
rice-2	10.45±1.26	9.87±0.41	5.88
rice-3	3.88±0.40	3.64±0.21	6.59
rice-4	3.85±0.46	3.33±0.16	15.62
rice-5	2.86±0.33	2.54±0.17	12.60
rice-6	ND	ND	—
maize-1	12.56±1.09	10.87±0.37	15.55
maize-2	11.03±0.87	11.72±0.43	5.89

maize-3	5.92±0.55	5.78±0.25	2.42
maize-4	ND	ND	—
maize-5	6.85±0.50	6.72±0.31	1.93
maize-6	22.13±2.32	24.20±0.68	8.55
maize-7	3.75±0.38	3.88±0.28	3.35
maize-8	14.66±1.08	15.32±0.60	4.31
pig feed	27.68±1.87	25.87±1.09	7.00
cow feed	ND	ND	—
rabbit feed	ND	ND	—
chicken feed	9.25±0.60	8.34±0.52	10.91
mouse feed	ND	ND	—
cat feed	6.15±0.65	6.72±0.39	8.48

Note: *ND* means *not detected*.

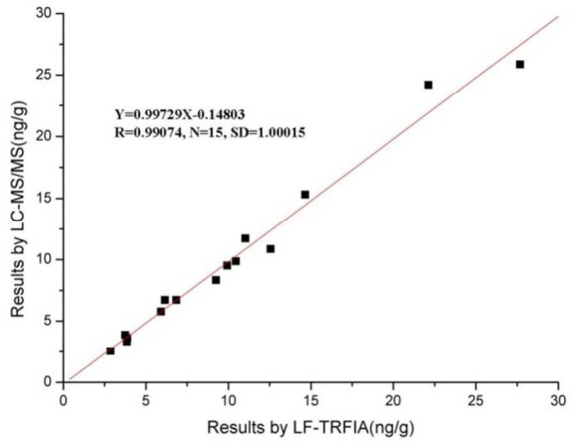

Fig.7 Correlation of the results obtained by LF-TRFIA and LC-MS/MS

Conclusions

In conclusion, we have developed a highly sensitive and rapid method for analysis of T-2 toxin in cereals and feed by using time-resolved fluorescence immunochromatographic assay strips and an assorted portable fluorescence reader. In this paper, the linear range of the LF-TRFIA method was 0.125~200 ng/g for rice and maize and 0.25~200 ng/g for feed. The LOD was 0.09 ng/g for rice and maize and 0.17 ng/g for feed. The recovery of the standard spike ranged from 94.2% to 111.0%, and the CV was less than 15%. The LF-TRFIA method was reliable for obtaining good recovery and low CV. When compared with LC-MS/MS, LF-TRFIA was relatively accurate. Generally speaking, the sensitivity of the mAb-Eu (III) probe-based strip method was better than that of the colloidal gold-based strip method and comparable to that of the ELISA method. In addition, the linear range of LF-TRFIA was wider than that of ELISA when using the same monoclonal antibody. These indicated LF-TRFIA was an advanced method for detection.

The fluorescent microspheres in this work were monodisperse and homogeneous. As the nucleus, the europium compound was surrounded by organic matters. The fluorescent property of these microspheres was stable and worked well in detection. A sample vial was used instead of the conjugated pad to contain both the conjugate and the sample extract. It was good for probe release and easy to control. Sometimes if the machine for preparing strips was not on the same condition, then the spraying amount might be different. Repeatability might be influenced greatly by the amount of the antigen on the test line and rabbit anti-mouse IgG on the control line. Therefore, the same batch of strips should be used in an experiment including establishing standard curves and test for practical samples. Fluorescent microspheres of different kinds and lengths of the link arm had been synthesized, which may have better sensitivity. It is worth studying in future.

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