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ORIGINAL PAPER

Semiquantitative immunochromatographic colorimetric biosensor for the detection of dexamethasone based on up-conversion fluorescent nanoparticles

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Abstract

A low-cost bifunctional immunochromatographic colorimetric biosensor was developed that can be read visually or by using an optical density scanner. Five test lines (T lines) coated with different antigens were set on a nitrocellulose (NC) membrane to indicate the concentration of analyte. This method was applied for the detection of dexamethasone. The corresponding detection range was 0.1–9 ng mL^{-1}, and the detection limit for dexamethasone in food supplements and cosmetic samples was 2.0 μg kg^{-1}. For visual inspection of the colour the quantitative relative error range between the proposed method and liquid chromatography was −62 to −25%, with a detection time of only 10 min. More accurate assay results were obtained by using an optical density scanner with the relative error range of −31 to 20%. The results indicated that the proposed method has the potential of application for rapid and efficient screening of dexamethasone in cosmetics and food supplements.

Keywords Dexamethasone · Quantification · Up-conversion luminescence nanoparticles · Immunochromatography · Colorimetric assay

Introduction

Immunochromatography has been used as a qualitative detection method for over 40 years. Quantitative immunochromatography has evolved as an important research topic in the scientific community, with various visible or optical density scanners introduced to the market till date [1–3]. However, a large number of consumers believe that 2000–20,000 USD for a scanner is too expensive. The overall mobility of these scanners is also limited due to their weight. As a consequence, the development of a quantitative immunochromatography detection method that does not require a matching scanner was put forward to help reduce the overall cost and facilitate on-site detection capabilities.

Regarding the development of cost-effective readout methods, there is a resurgence of interest for the use of simple visual techniques and direct visual inspection. In fact, some scholars believe that quantitative direct visual inspection immunoassays have the potential to revolutionize analytical science [4]. Panferov [5] and Serebrennikova [6] developed a bare eye–based semiquantitative colloidal gold immunochromatographic strip for the detection of potato virus X and procalcitonin, respectively. The test strip was coated with three antibodies as test lines (T lines) to form a double monoclonal antibody sandwich mode. However, this method has proven to be unsuitable for the immunoassay of small molecules. Measuring the dilution ratio when the sample reaches the cutoff value of the test strip via gradient dilution can be used to estimate the content of analyte, but this method has proven to be too time-consuming [7, 8]. Zhang developed a bare eye based–semiquantitative colloidal gold immunochromatographic strip for the detection of aflatoxin B1 [9, 10]. The test strip was coated with three of the same antigens as the T lines. When the labeled antibodies passed multiple T lines, the concentration decreased

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step by step. As a result, the combination between the antibody and T line was inhibited, while the concentration of aflatoxin B1 increased. However, the method required the use of an antibody with higher concentration. Therefore, its overall sensitivity was lower than that of a normal colloidal gold immunochromatographic strip featuring only one T line.

Competitive immunoassay describes a ternary system consisting of an analyte, coating antigen, and antibody. The structure and concentration of the coating antigen can influence the affinity of T lines for antibody [11]. Based on this fact, this study developed a new visual quantitative immunochromatographic colorimetric biosensor in which several immunochromatography assays with different sensitivities proceeded successively on a NC membrane. The assay involved multiple T lines coated with different antigens with different affinities on the immunochromatography NC membrane to exhibit binding effects between the labeled antibodies and the T lines at different analyte concentrations (Fig. 1). The coating antigens were ordered from the bottom up by their affinity from low to high. When the analyte concentration is low, the analyte can only fully inhibit a coating antigen with low affinity. As the combination of antibody and antigen is reversible, the analyte-antibody complex will be broken by another coating antigen with higher affinity, and then the antibody will conjugate with the coating antigen. Increasing the concentration of analyte will completely inhibit the coating antigen with higher affinity. The developed method was demonstrated to be simple, low-cost, and capable of providing the analyte concentrations directly. The strategy proposed in this paper is also universal. By adding T lines for different coating antigen structures and adjusting coating concentration, the majority of traditional competitive immunochromatography assays can be transformed to visual quantitative methods easily without affecting their sensitivity. Combining the advantages of lanthanide-doped up-conversion nanoparticles such as anti-bleaching and reduced background interferences [12, 13], a bifunctional immunochromatographic colorimetric biosensor was developed herein. The obtained results can be quantified visually or by using an optical density scanner for more accurate quantitative data.

Materials and methods

Materials and reagents

N-Hydroxysuccinimide (NHS) (purity 98%), 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide (EDC) (purity 98%), keyhole limpet hemocyanin (KLH), and bovine serum albumin (BSA) were purchased from Sigma-Aldrich (St. Louis, MO, USA). N-Monodesmethyl sibutramine (MS), N,N-didesmethyl sibutramine (DS), benzyl sibutramine, and homosibutramine were all purchased from TRC (Toronto, Canada). All chemicals were of analytical grade and used as received. A Milli-Q purification system (Millipore) was used for water purification. All other chemicals were of analytical or higher grade. Nitrocellulose (NC) membranes (CN140) were obtained from Sartorius (Göttingen, Germany). The real samples were verified using a liquid chromatography-tandem mass spectrometry system (LC-MS/MS) (Waters ACQUITY UPLC coupled to a Waters Xevo TQ-S) (Millford, MA, USA). The column used for the separation of DEX was a Waters ACQUITY BEH C_{18} (2.1 mm × 50 mm, 1.7 μm) (Millford, MA, USA). An enzyme-linked immunosorbent assay (ELISA) kit for DEX used for comparison was purchased from THIPH Co. Ltd. (Shenzhen, China). The antibody was purified using a CarboxyLink Coupling Plus Immobilization Kit (Pierce, Thermo Scientific), and the five coating antigens were prepared according to a method previously published by our group [14]. The hapten structure is shown in Fig. 2.

Instruments

An optical density scanner (Niutaier, China) was modified to function as an up-conversion luminescence reader by using a 300 mW, 980 nm laser instead of LED light as the excitation light source. The camera equipment used was a Huawei P20 mobile phone covered with a 540 nm ± 15 nm optical filter, and a 2 W 980 nm laser was used as the corresponding excitation light source. The XYZ3060 platform consisting of motion control with Biostrip Dispenser HGS102 and Airjet HGS102 (BioDot, USA) was used to construct T line and control line (C line) of the biosensor. Up-conversion fluorescence spectra were measured using an F-4500 fluorescence spectrophotometer (Hitachi, Japan) modified with a 2 W 980 nm laser. A Sigma 300 transmission electron microscope (TEM) (Carl Zeiss, Jena, Germany) was used for characterizing the lanthanide-doped up-conversion nanoparticles (UCNPs).

Synthesis of amino UCNPs

Amino UCNPs (130 nm) were prepared following a previous report [15] with some modifications. Briefly, 10-mmol NH_4F and 10-mmol NaOH were dissolved in 5 mL of water and 5 mL of ethylene glycol to form solution A. Then, 1 mmol of $Re(NO_3)_3$ (Re = Y, Yb, and Er at a molar ratio of 78:20:2) and 0.20 g of polyethyleneimine were dissolved in 3 mL of water and 4.5 mL of EG to form solution B. Solutions A and B were then mixed to prepare a homogenized solution at room temperature. The resulting mixture was then transferred to an autoclave, and the temperature was immediately increased to 180 °C. This temperature was maintained for 8 h, and the mixture was then allowed to cool to room temperature. The resulting nanoparticles were precipitated by addition of ethanol and washed three times with ethanol, followed by water washing. After centrifugation, the particles were redispersed in water for subsequent use. The NaYF4:Yb,Er UCNPs were

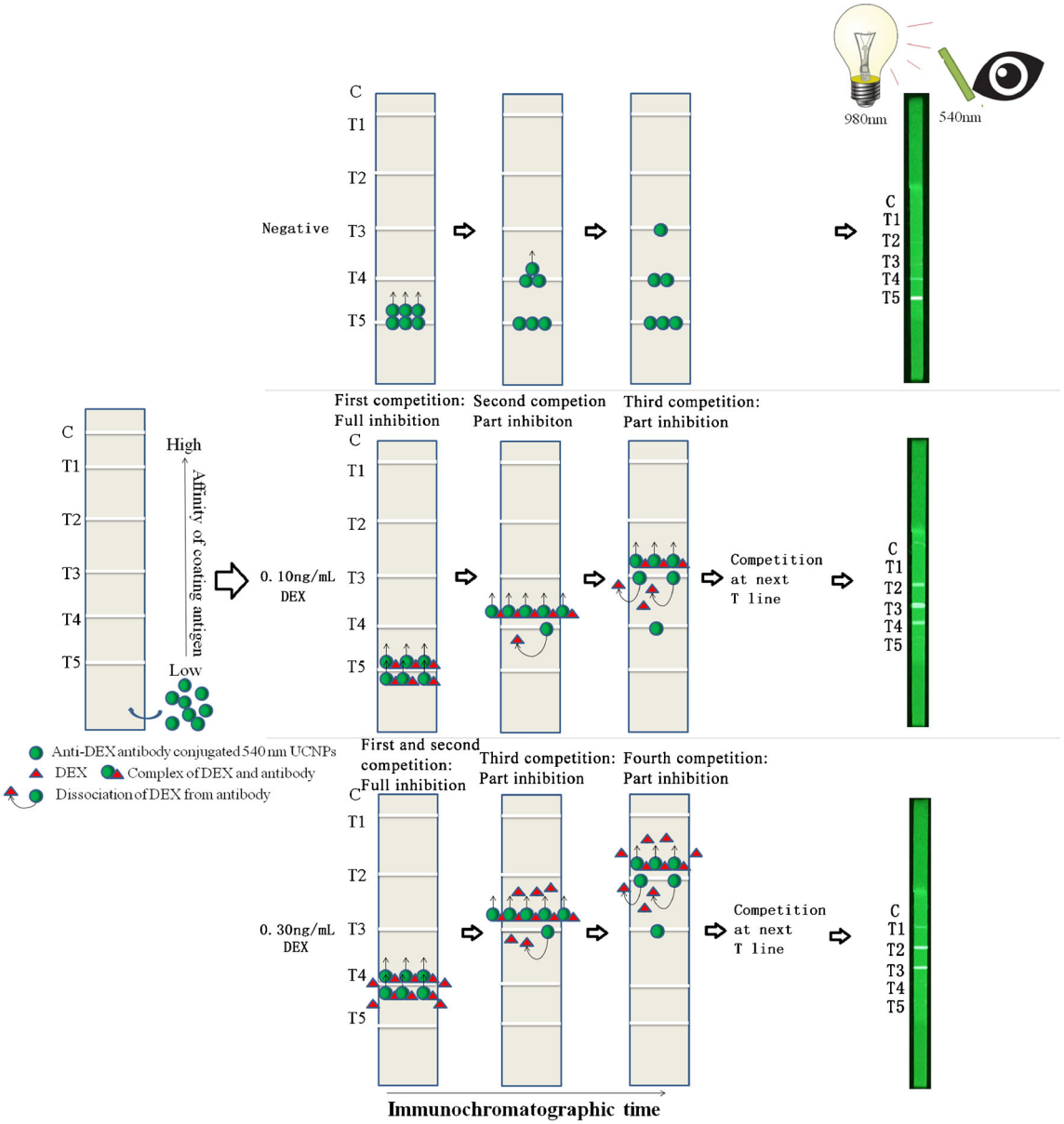

Fig. 1 Structure and testing principle of visual quantitative immunochromatographic colorimetric biosensor

coated with a 15-nm silica layer via microemulsion method [16] and modified with amino groups [17]. The form and optical properties of the amino UCNPs were then characterized by TEM and fluorescence spectrophotometry, respectively (Fig. 3).

Preparation of UCNP-labeled anti-DEX

DEX–agarose-based conjugates were prepared using a CarboxyLink Coupling Plus Immobilization Kit and following the instructions provided by the manufacturer. The ascites solution was diluted to 5 mg mL^{-1} (protein concentration) using phosphate-buffered saline (PBS) (0.01 M, pH 7.4). Approximately 2 mL of the ascites solution and 2 mL of DEX–agarose-based conjugate were then added to a spin column. The column was incubated for 60 min at room temperature while rocking to ensure binding, followed by centrifugation. The agarose-based conjugate was washed with 2 mL of 0.01 M pH 7.4 PBS and centrifuged thrice (this latter step was repeated a total of three times). Approximately 2 mL of 2 mg mL^{-1} EDC NHS–activated UCNPs (prepared according to Wang's method [18]) was added to the spin column containing the agarose-based conjugate. The UCNPs were then added in excess for complete labeling of the IgG molecules. Subsequently, 10 μL of 5-M sodium cyanoborohydride was added to the reaction and incubated for 2 h at room temperature while rocking to ensure successful binding. The reaction mixture was then centrifuged. The resin was washed with

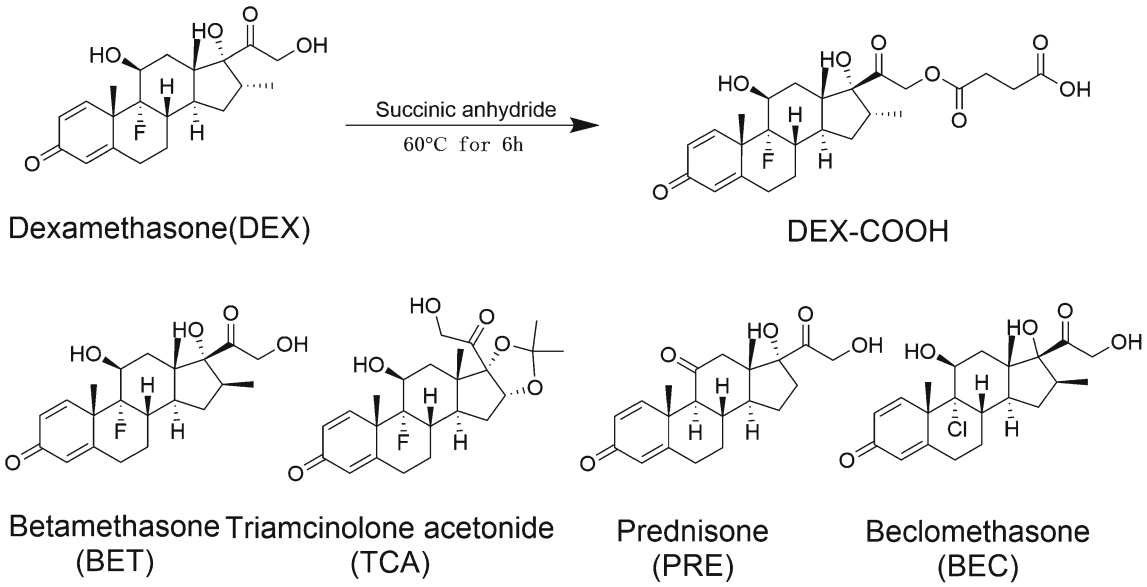

Fig. 2 Synthetic route for antigen production

2 mL of 0.01 M pH 7.4 PBS, and the reaction mixture was centrifuged. This step was repeated thrice for complete removal of unbound UCNPs. The agarose mixture was then eluted with 2 mL of cold elution buffer (0.1-M glycine•HCl at pH 3.0) and placed in a centrifuge tube containing 100 μL of buffer (1 M PBS, pH 9.0). After centrifugation, the resulting precipitate was resuspended in PBS (0.015 M, pH 7.4 containing 1% (w v) PVP K-40, 1% (w v) PEG 40000, 1% (w v) trehalose, and 2% (w v) BSA).

Preparation of fluorescent biosensor

The structure of the immunochromatographic colorimetric biosensor is shown in Fig. 1a. Six parallel lines of coated antigen (corresponding type and concentration shown in Fig. 4) and goat anti-mouse IgG were drawn on the NC membrane. These parallel lines were then denoted as C line, T line 1, T line 2, T line 3, T line 4, and T line 5 (top to bottom). After vacuum drying at 37 °C for 12 h and at room temperature for another 12 h, the absorbent paper and NC membrane were attached to the PVC soleplate. These immunochromatographic colorimetric biosensors were then vacuum dried at room temperature, and 2 μg of the sibutramine antibody-fluorescent microsphere conjugate (measured by mass of fluorescent microspheres) was added, followed by freeze drying.

Performance of the UCNP-ICA

The liquid sample (100 μL) was added to the reaction cup and mixed thoroughly. The resulting mixture was then transferred

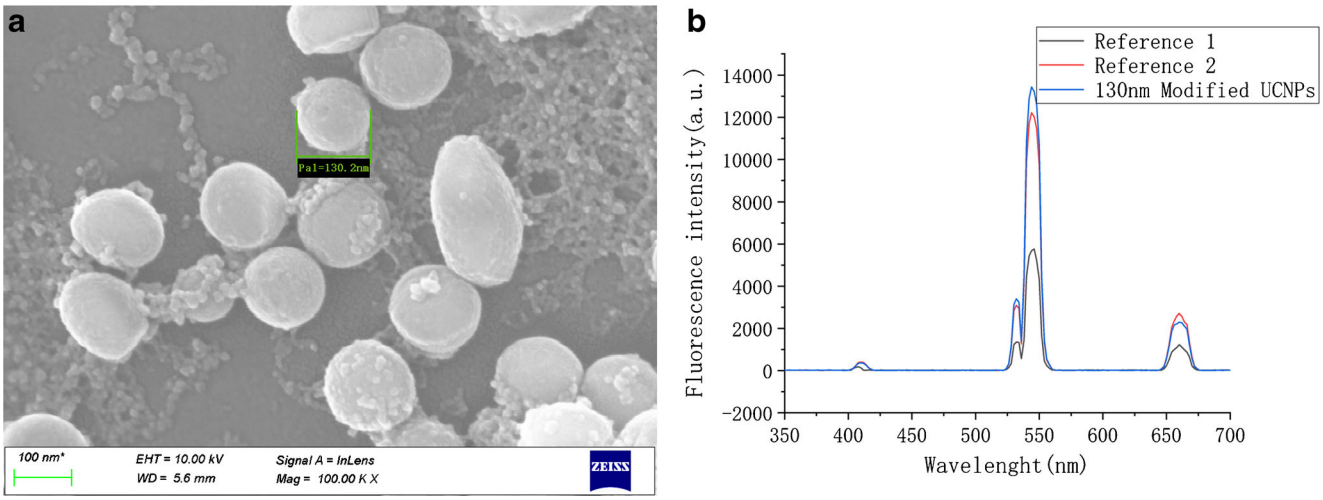

Fig. 3 TEM image and fluorescence scanning graph, showing the preparation of UCNPs. **a** TEM image of 130-nm UCNPs, **b** comparison of the fluorescence intensity for the three UCNPs. Reference [1] shows commercial amino UCNPs from Fluonano Biotech Co. Ltd. (Hefei, China), reference [2] shows 165-nm amino UCNPs prepared by a previously reported method [15]

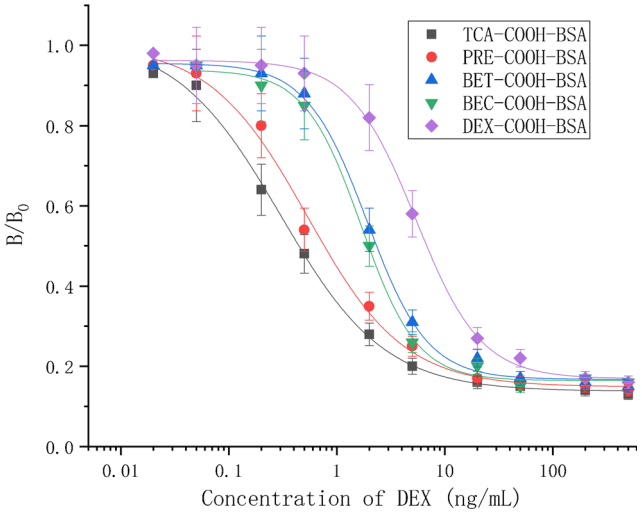

Fig. 4 ELISA standard curve with various coating antigens to detect DEX ($n = 6$)

to the biosensor. Results were obtained after chromatography for 10 min. The result was negative when line 5 was visible. After standard DEX solution at different concentrations was placed in the reaction cup, the concentrations at which T lines 1, 2, 3, 4, and 5 disappeared were recorded and denoted as the marker reference concentrations. The concentration at which T line 5 disappeared indicated the sensitivity of the test paper. At the equilibrium time of immunochromatography, the color of T line 3 and the C line no longer deepened.

The biosensor can also be read using a fluorescence scan reader. The integral area of the T line (FI_T) within a fixed peak width was used to express the fluorescence intensity of each line. T3 line fluorescence intensity was detected and used to develop a standard curve (standard curve 1) when the DEX concentration was 0.10, 0.20, 0.30, 0.50, and 0.90 ng mL^{-1}, respectively. T1 line fluorescence intensity was detected and used to develop another standard curve (standard curve 2) when the DEX concentration was 0.90, 20, 30, 50, and 9.0 ng mL^{-1}, respectively. When the T3 line was visible, the standard curve 1 was generated and used to calculate the concentration of DEX. When the T3 line was invisible but the T1 line was visible, the standard curve 2 was used to calculate the concentration of DEX. When all the T lines were invisible and the C line was visible, it was deemed necessary to dilute the sample another 50 times.

Detection of DEX in real samples

Cosmetics with skin whitening and acne treatment functions as well as food supplements designed to increase bone density were purchased online. The samples (1 g each) were weighed out and then placed in a polystyrene centrifuge tube. Then, 19 mL of water was added and the resulting mixture was vortexed for 1 min. After centrifugation at 1000 g for 1 min, 100 μL of the supernatant was used for UCNP-ICA. If the four T lines all disappeared, the supernatant required an additional dilution of 50 times. The performance of the UCNP-ICA was analyzed as described in the "Performance of the UCNP-ICA" section. A commercial ELISA kit for DEX was used for method comparison. LC-MS/MS was then used for the cosmetics and food supplement samples to further verify the data reported by Van den Hauwe [19] and Gao [20], respectively.

Results and discussion

Evaluation of coating antigen

The coating hapten structure is closely related to the sensitivity of an immunoassay [21]. Glucocorticoids represent a large drug family with similar structural features and can be derived from the carboxyl site using a simple synthetic method. Thus, the structure-function relationship between the hapten structure and the immune binding site has to be studied first. The dilution factor of the antibody with an absorbance of about 1.0 at 450 nm at a coating concentration of 1.0 mg L^{-1} was used to express the affinity of the antibody to the coating antigen. The observed dilution factors of the antibody for TCA-COOH-BSA, PRE-COOH-BSA, BET-COOH-BSA, BEC-COOH-BSA, and DEX-COOH-BSA were 2×10^4, 4×10^4, 4×10^4, 4×10^4, and 8×10^4, respectively. DEX-COOH-BSA is a homologous coating antigen. The difference between this species and BET-COOH-BSA is the optical isomerism at the five-membered ring near the linkage. The difference in affinity indicated that the four rings present in DEX were indeed attached to the antibody. Furthermore, previously conducted studies by our group have shown that the antibody can only combine with glucocorticoids having a cyclohexa-2,5-dien-1-one structure, including BEC, PRE, and TCA. Indeed, the cyclohexa-2,5-dien-1-one scaffold is a key structure in determining the affinity of the DEX antibody [14]. Competitive ELISA standard curves are shown in Fig. 4. The corresponding IC_{50} values for ELISA assay with coating antigens derived from TCA-COOH-BSA, PRE-COOH-BSA, BET-COOH-BSA, BEC-COOH-BSA, and DEX-COOH-BSA were 0.35 ng mL^{-1}, 0.78 ng mL^{-1}, 2.0 ng mL^{-1}, 1.3 ng mL^{-1}, and 5.4 ng mL^{-1}, respectively. These results indicate that it is feasible to use different coating antigens with varying sensitivities to produce useful visual immunochromatographic results.

Selection of immunochromatographic system

Based on the uniformity of the key structural features and the differences in the other structural parts, it is feasible but inconvenient to design a series of coating antigens to prepare several biosensors with different sensitivities. Setting several T lines with different sensitivities in one biosensor

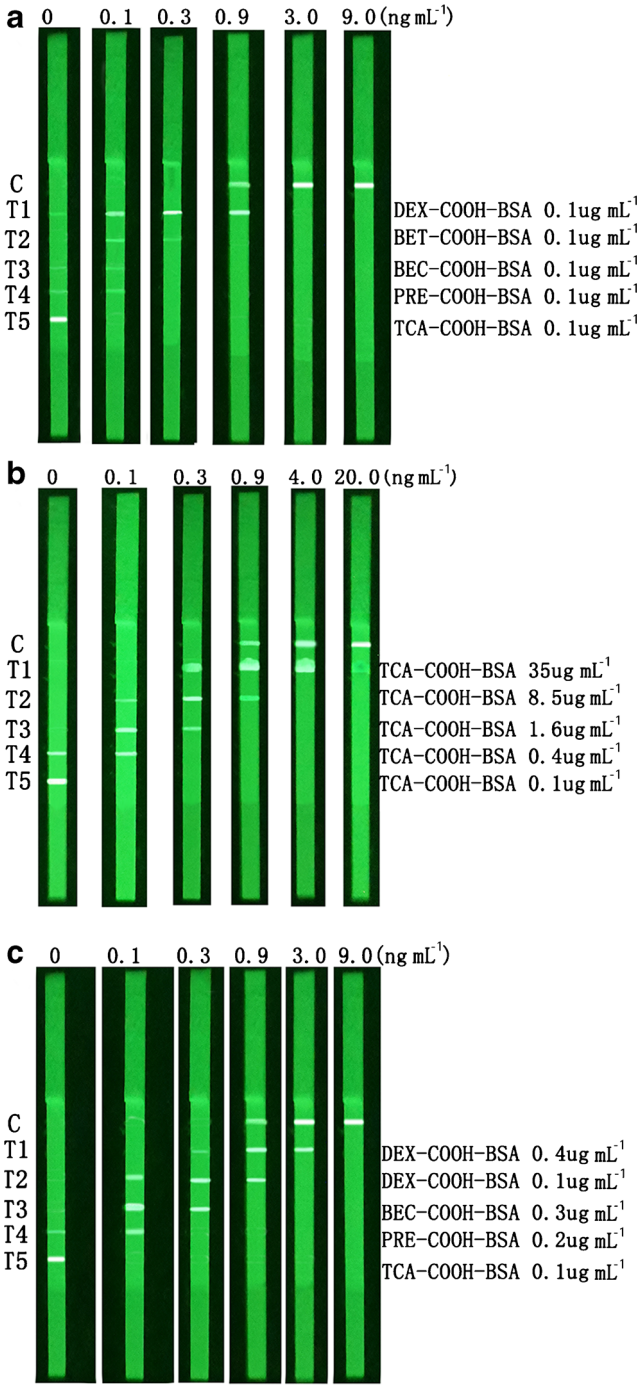

Fig. 5 Visual quantification up-conversion luminescence immunochromatographic system. **a** Coating with various coating antigens at the same concentration. **b** Coating with a single antigen at various concentrations. **c** Coating of a series of heterogonous and homologous antigens at different concentrations

may decrease the cost, and the test can be performed in an easy fashion. The five coating antigens were coated at identical concentrations on the NC membrane, but they did not generate a visual gradient (Fig. 5a). In the same immunochromatographic system, the approximate affinity was difficult to determine visually.

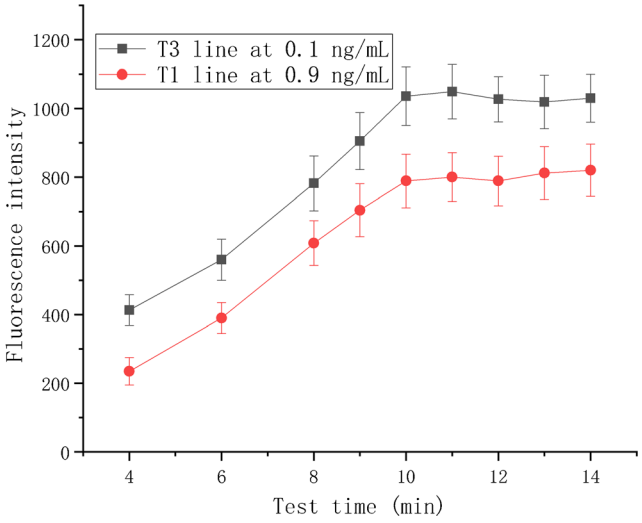

Fig. 6 Test time optimization for UCNP-ICA to detect DEX ($n = 6$)

In a competitive immunoassay, the coating antigen can inhibit the combination of antibody to analytes. Therefore, increasing the concentration of the coating antigen is another way to adjust the overall sensitivity. Another visual immunochromatographic reaction type was constructed herein using different concentrations of coating antigens (Fig. 5b). TCA-COOH-BSA with concentrations of 0.10 μg mL^{-1}, 0.40 μg mL^{-1}, 1.6 μg mL^{-1}, and 8.5 μg mL^{-1} resulted in a clear color gradient. However, higher concentrations of coating antigen led to an antigen overload on the NC membrane, resulting in a T line dispersion.

Thus, combining these two features, i.e., using low-affinity heterogeneous coating antigens to construct high-sensitive T lines and using homologous coating antigens with different concentrations to construct low sensitive T lines, has proven to be appropriate (Fig. 5c). The results showed that the chromatic variation between the different DEX concentrations was significant and further indicated that generating more T lines to increase the detection range was indeed possible.

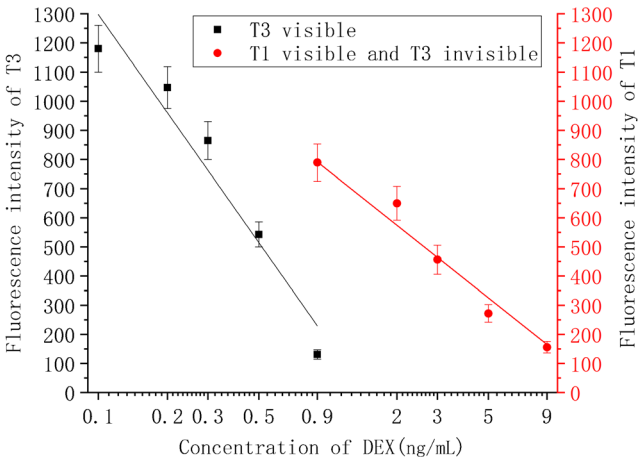

Fig. 7 Standard curve determined for DEX using UCNP-ICA ($n = 6$)

Table 1 DEX detection in health-care foods and cosmetics using three methods ($n = 6$)

Sample	Immunochromatographic assay			
	Visual quantification (mg/kg)	Scanning quantification (mg/kg)	ELISA (mg/kg)	LC-MS/MS (mg/kg)
Tablet1	0.9	1.8 ± 0.2	2.2 ± 0.2	1.5 ± 0.1
Tablet2	0.9	1.5 ± 0.2	1.0 ± 0.1	1.3 ± 0.1
Capsule1	0.3	0.45 ± 0.06	0.94 ± 0.11	0.52 ± 0.02
Capsule2	3	3.1 ± 0.4	4.9 ± 0.5	4.5 ± 0.2
Cream 1	0.3	0.54 ± 0.07	0.68 ± 0.08	0.42 ± 0.2
Cream 2	0.9	1.3 ± 0.2	2.4 ± 0.3	1.9 ± 0.1
Cream 3	0.9	1.2 ± 0.1	2.9 ± 0.2	2.2 ± 0.1
Cream 4	3	5.1 ± 0.7	8.8 ± 0.8	6.9 ± 0.3
Cream 5	0.3	0.55 ± 0.07	0.72 ± 0.07	0.45 ± 0.02
Cream 6	3	6.5 ± 0.8	10 ± 1	7.9 ± 0.3
Mask 1	0.3	0.53 ± 0.07	0.88 ± 0.07	0.77 ± 0.03
Mask 2	0.9	1.8 ± 0.2	2.0 ± 0.2	2.3 ± 0.1
Mask 3	0.9	1.5 ± 0.2	1.0 ± 0.1	1.3 ± 0.1
Essence 1	3	4.3 ± 0.6	5.2 ± 0.4	4.5 ± 0.2
Essence 2	15	30 ± 4	30 ± 2	38 ± 2

Quantitative determination

The visual quantitative immunochromatographic colorimetric biosensor can be read by using an optical density scanner for obtaining more accurate quantitative data. The UCNP-ICA reaction time is another important parameter affecting the accuracy of quantification. Figure 6 shows the responses of UCNP-ICA towards 0.1 ng mL^{-1} DEX at line 3 and 0.9 ng mL^{-1} DEX at line 1 for different time periods, respectively. The fluorescence intensity of UCNP-ICA was collected using an up-conversion luminescence reader. The fluorescent signal intensity initially increased sharply and then became constant. Considering time and signal intensity stability, 10 min was selected as a suitable time period for subsequent experiments.

As the biosensor output semiquantitative values are based on the chromogenic condition of the T line, the concentration of coating antigen is lower than the concentration of coating antigen of colorimetric method, which leads to the narrow corresponding detection range of single T line. Thus, two T lines were used to develop quantitative method. Figure 5c shows the optical intensity of the T3 line that varied inversely with a DEX concentration between 0.1 ng mL^{-1} and 0.9 ng mL^{-1}. The optical intensity of the T1 line varied inversely, with DEX concentrations ranging between 0.9 ng mL^{-1} and 9 ng mL^{-1}. Thus, an optical density scanner was used to capture the UCNP-ICA responses and two standard curves were generated (Fig. 7). When the T3 line was visible, the standard curve, $y = 763–626 \times \lg x$ ($R^2 = 0.94$), was used for quantification. When the T3 line was invisible but the T1 and C lines were visible, the second standard curve, $y = 178–1119 \times \lg x$ ($R^2 = 0.97$), was used for quantification.

Sample testing

DEX is a potent and long-acting glucocorticoid frequently used in the therapy of rheumatic, allergic, and dermatologic symptoms [22, 23]. However, DEX is also illegally used in cosmetics to whiten skin and treat acne. In food supplements, DEX is usually illegally added to increase bone density [24–26]. Herein, 50 cosmetic samples with skin whitening and acne treatment functions as well as 50 food supplement samples designed to increase bone density were purchased online and analyzed using the proposed visual quantification up-conversion luminescence immunochromatography semiquantitative method. Simultaneously, the samples were analyzed using LC-MS/MS. In 85 of the samples, no DEX was detected and no false positive results were observed. However, DEX was detected in 11 cosmetics and 4 food supplement samples, i.e., 22% and 8% of the screened samples, respectively. This finding indicates that the illegal addition of DEX to cosmetics and food supplements sold online is rather common and therefore requires urgent restriction and control. Qualitatively, the results of visual colorimetric immunochromatography and LC-MS/MS were also consistent. The quantitative results are shown in Table 1. A suitable positive correlation between the visual colorimetric immunochromatography results and those obtained by a national standard method was found, with relative error ranging from −62 to −25%. Using an up-conversion luminescence reader resulted in a decrease in the relative error.

The relative error of −31 to 20% was close to the levels of ELISA, i.e., −13 to 62%. The results also showed that visual colorimetric immunochromatography can meet the requirements of rapid qualitative DEX detection. In addition, Table 1 indicates that the addition range of DEX to the actual samples spanned three orders of magnitude. The samples were prone to exceed the quantitative linear range of the instrument when liquid chromatography-mass spectrometry was used for detection, requiring multiple dilutions. In contrast, visual colorimetric immunochromatography can provide a quantitative reference, rendering it possible to dilute the samples within the quantitative curve of the instrument. This may add another layer of convenience when further confirming the visual results with LC-MS/MS.

Conclusions

This work presents a novel bifunctional quantitative immunochromatographic biosensor, which allows the results to be read by bare eye or an up-conversion luminescence reader. The proposed method used multi-test lines coated with a series of heterogonous and homologous antigens with varying concentrations. The detection limit of this method for actual samples was 2 μg kg^{-1}, and no false positive or false negative qualitative values were identified. Furthermore, the quantitative results exhibited a certain reference significance. Hence, this assay can provide an easy, fast, and low-cost method for potential application in on-site quantitative determinations.

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Compliance with ethical standards

Conflict of interest The author(s) declare that they have no competing interests.

References

1. Tang XQ, Li PW, Zhang ZW, Zhang Q, Guo JN, Zhang W (2017) An ultrasensitive gray-imaging-based quantitative immunochromatographic detection method for fumonisin B1 in agricultural products. Food Control 80:333–340. https://doi.org/10.1016/j.foodcont.2017.05.013
2. Wu SS, Zhu FF, Hu LM, Xia J, Xu GM, Liu DF, Guo Q, Luo K, Lai WH (2017) Development of a competitive immunochromatographic assay for the sensitive detection of amantadine in chicken muscle. Food Chem 232:770–776. https://doi.org/10.1016/j.foodchem.2017.04.058
3. Li BB, Song JZ, Chen JL, Ma L, Li XX, Li JT, Guan M (2018) Novel immunochromatography assay based on background fluorescence quenching for the sensitive determination of serum cystatin C. Anal Lett 52(8):1340–1351. https://doi.org/10.1080/00032719.2018.1538232
4. Mahmud G, Campbell CJ, Bishop KJM, Komarova YA, Chaga O, Soh S, Huda S, Kandere-Grzybowska K, Grzybowski BA (2009) Directing cell motions on micropatterned ratchets. Nat Phys 5(8):606–612. https://doi.org/10.1038/nphys1306
5. Panferov VG, Safenkova IV, Zherdev AV, Dzantiev BB (2017) Setting up the cut-off level of a sensitive barcode lateral flow assay with magnetic nanoparticles. Talanta 164:69–76. https://doi.org/10.1016/j.talanta.2016.11.025
6. Serebrennikova KV, Samsonova JV, Osipov AP (2019) A semi-quantitative rapid multi-range gradient lateral flow immunoassay for procalcitonin. Mikrochim Acta 186(7):423. https://doi.org/10.1007/s00604-019-3550-2
7. Fu XX, Xie RS, Wang J, Chen XJ, Wang XH, Sun WB, Meng JJ, Lai DW, Zhou LG, Wang BM (2017) Development of colloidal gold-based lateral flow immunoassay for rapid qualitative and semi-quantitative analysis of ustiloxins a and B in rice samples. Toxins 9(3):79. https://doi.org/10.3390/toxins9030079
8. Molinelli A, Grossalber K, Führer M, Baumgartner S, Sulyok M, Krska R (2008) Development of qualitative and semiquantitative immunoassay-based rapid strip tests for the detection of T-2 toxin in wheat and oat. J Agr Food Chem 56(8):2589–2594. https://doi.org/10.1021/jf800393j
9. Zhang DH, Li PW, Zhang Q, Li R, Zhang W, Ding XX, Li CM (2012) A naked-eye based strategy for semiquantitative immunochromatographic assay. Anal Chim Acta 740:74–79. https://doi.org/10.1016/j.aca.2012.06.015
10. Zhang DH, Li PW, Liu W, Zhao L, Zhang Q, Zhang W, Ding XX, Wang JL (2013) Development of a detector-free semiquantitative immunochromatographic assay with major aflatoxins as target analytes. Sensors Actuat B-Chem 185:432–437. https://doi.org/10.1016/j.snb.2013.05.034
11. Liu CM, Dou XW, Zhang L, Kong WJ, Wu L, Duan YP, Yang MH (2018) Development of a broad-specificity antibody-based immunoassay for triazines in ginger and the quantitative structure-activity relationship study of cross-reactive molecules by molecular modeling. Anal Chim Acta 1012:90–99. https://doi.org/10.1016/j.aca.2018.01.042
12. Farka Z, Juřík T, Kovář D, Trnková L, Skládal P (2017) Nanoparticle-based immunochemical biosensors and assays: recent advances and challenges. Chem Rev 117(15):9973–10042. https://doi.org/10.1021/acs.chemrev.7b00037
13. Hu GS, Sheng W, Zhang Y, Wu XN, Wang S (2015) A novel and sensitive fluorescence immunoassay for the detection of fluoroquinolones in animal-derived foods using upconversion nanoparticles as labels. Anal Bioanal Chem 407(28):8487–8496. https://doi.org/10.1007/s00216-015-8996-4
14. Zhang SW, Yao TQ, Wang SF, Feng RH, Chen LQ, Zhu V, Hu GP, Zhang H, Yang GW (2018) Development of an upconversion luminescence nanoparticles–based immunochromatographic assay for the rapid detection of dexamethasone in animal tissue. Food Anal Methods 12(3):752–760. https://doi.org/10.1007/s12161-018-01411-5
15. Zhang SW, Yao TQ, Wang SF, Feng RH, Chen LQ, Zhu V, Hu GP, Zhang H, Yang GW (2019) Upconversion luminescence nanoparticles-based immunochromatographic assay for quantitative detection of triamcinolone acetonide in cosmetics. Spectrochim Acta A Mol Biomol Spectrosc 214:302–308. https://doi.org/10.1016/j.saa.2019.02.053
16. Liu CH, Wang H, Li X, Chen DP (2009) Monodisperse, size-tunable and highly efficient β-NaYF4:Yb,Er(Tm) up-conversion luminescent nanospheres: controllable synthesis and their surface

modifications. J Mater Chem 19(21):3546–3553. https://doi.org/10.1039/b820254k
17. Wang M, Mi CC, Wang WX, Liu CH, Wu YF, Xu ZR, Mao CB, Xu SK (2009) Immunolabeling and NIR-Excited Fluorescent Imaging of HeLa Cells by Using NaYF4:Yb,Er Upconversion Nanoparticles. ACS Nano 3(6):1580–1586. https://doi.org/10.1021/nn900491j
18. Wang PL, Wang RG, Zhang W, Su XO, Luo HF (2016) Novel fabrication of immunochromatographic assay based on up conversion phosphors for sensitive detection of clenbuterol. Biosens Bioelectron 77:866–870. https://doi.org/10.1016/j.bios.2015.10.053
19. Van den Hauwe O, Perez JC, Claereboudt J, Peteghem CV (2001) Simultaneous determination of betamethasone and dexamethasone residues in bovine liver by liquid chromatography/tandem mass spectrometry. Rapid Commun Mass Sp 15(11):857–861. https://doi.org/10.1002/rcm.308
20. Gao JM, Cao J, Ding H (2018) Uncertainty evaluation for the determination of dexamethasone in cosmetics by ultra performance liquid chromatography-tandem mass spectrometry. China Pharmacist 21(3):539–542,546. https://doi.org/10.3969/j.issn.1008-049X.2018.03.051
21. Zhu D, Mu Y, Li Q, Pang X, Wang X, Liu Y, Liu Y, Chen G, Chen H (2016) A novel method for artificial antigen synthesis and preparation of a polyclonal antibody for the sensitive determination of leucomalachite green in fish samples by enzyme-linked immunoassay. Anal Methods 32:6236–6243. https://doi.org/10.1039/C6AY01564F
22. Chu CC, Hsing CH, Shieh JP, Chien CC, Ho CM, Wang JJ (2014) The cellular mechanisms of the antiemetic action of dexamethasone and related glucocorticoids against vomiting. Eur J Pharmacol 722:48–54. https://doi.org/10.1016/j.ejphar.2013.10.008
23. Sami M, Mohri M, Seifi HA (2015) Effects of dexamethasone and insulin alone or in combination on energy and protein metabolism indicators and milk production in dairy cows in early lactation - a randomized controlled trial. PLoS One 10(9):e0139276. https://doi.org/10.1371/journal.pone.0139276
24. Barbera S, Tarantola M, Sala G, Nebbia C (2018) Canonical discriminant analysis and meat quality analysis as complementary tools to detect the illicit use of dexamethasone as a growth promoter in Friesian bulls. Vet J 235:54–59. https://doi.org/10.1016/j.tvjl.2018.03.006
25. Kim H, Jang M, Park R, Jo D, Choi I, Choe J, Oh WK, Park J (2018) Conessine treatment reduces dexamethasone-induced muscle atrophy by regulating MuRF1 and Atrogin-1 expression. J Microbiol Biotechnol 28(4):520–526. https://doi.org/10.4014/jmb.1711.11009
26. Liu M, Li XY, Li JJ, Wu ZY, Wang F, Liu L, Tan XC, Lei FH (2017) Selective separation and determination of glucocorticoids in cosmetics using dual-template magnetic molecularly imprinted polymers and HPLC. J Colloid Interface Sci 504:124–133. https://doi.org/10.1016/j.jcis.2017.05.041