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Advantages of time-resolved fluorescent nanobeads compared with fluorescent submicrospheres, quantum dots, and colloidal gold as label in lateral flow assays for detection of ractopamine

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ABSTRACT

Label selection is a critical factor for improving the sensitivity of lateral flow assay. Time-resolved fluorescent nanobeads, fluorescent submicrospheres, quantum dots, and colloidal gold-based lateral flow assay (TRFN-LFA, FM-LFA, QD-LFA, and CG-LFA) were first systematically compared for the quantitative detection of ractopamine in swine urine based on competitive format. The limits of detection (LOD) of TRFN-LFA, FM-LFA, QD-LFA, and CG-LFA were 7.2, 14.7, 23.6, and 40.1 pg/mL in swine urine samples, respectively. The sensitivity of TRFN-LFA was highest. In the quantitative determination of ractopamine (RAC) in swine urine samples, TRFN-LFA exhibited a wide linear range of 5 pg/mL to 2500 pg/mL with a reliable coefficient of correlation (R^2=0.9803). Relatively narrow linear ranges of 10–500 pg/mL (FM-LFA) and 25–2500 pg/mL (QD-LFA and CG-LFA) were acquired. Approximately 0.005 μg of anti-RAC poly antibody (pAb) was used in each TRFN-LFA test strip, whereas 0.02, 0.054, and 0.15 μg of pAb were used in each of the FM-LFA, QD-LFA, and CG-LFA test strips, respectively. In addition, TRFN-LFA required the least RAC-BSA antigens and exhibited the shortest detection time compared with the other lateral flow assays. Analysis of the RAC in swine urine samples showed that the result of TRFN-LFA was consistent with that of liquid chromatography-tandem mass spectrometry (LC-MS/MS) and a commercial enzyme-linked immunosorbent assay (ELISA) kit.

1. Introduction

Ractopamine, a phenylethanolamine with β-adrenergic agonist properties (Wang et al., 2015), is widely used as tocolytic, bronchodilator, and heart tonic in human and veterinary medicine (Ho et al., 2014). Recently, RAC has been illegally applied as nutrition-partitioning agent in the livestock industry to increase the efficiency of feed conversion and enhance the lean/fat ratio at a dose 5–10 times higher than the therapeutic dose (Gonzalez et al., 2010). Unfortunately, RAC residues might be a potential danger for consumers; their cardiovascular and central nervous systems are particularly at risk. Therefore, the Council Directive 96/22/EC of the European Union and Bulletin No. 176 of the Ministry of Agriculture of China prohibited the use of RAC as feed additives.

Many analytical methods have been developed for the confirmation or screening of the presence of RAC in various biological matrices. The conventional methods include high performance liquid chromatography (HPLC) (Du et al., 2014), gas chromatography coupled with mass spectrometry (GC-MS) (L. Wang et al., 2010), ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) (Dong et al., 2011), capillary electrophoresis with electrochemical detection (W. Wang et al., 2010), and ELISA (Bai et al., 2012; Pleadin et al., 2012). Although these methods are very sensitive, specific, and accurate, they require trained operators, complex sample pretreatment, and sophisticated instruments, which are not suitable for on-site monitoring. Lateral flow assay (LFA) is a one-step method with several advantages, such as rapidity, simplicity, specificity, and low-cost (Lourdes et al., 2015; Raeisossadati et al., 2016). Colloidal gold was most widely used as labels for qualitative (Zhang et al., 2009) or quantitative (A. Liu et al., 2015) detection of RAC with relatively high concentration.

To meet the requirement of sensitive detection, more quantitative LFA has been developed recently using various labels (Quesada-Gonzalez and Merkoci, 2015; Shan et al., 2015). Fluorescence labeling detection has been widely used in the fields of diagnostics (Yang et al., 2015), environment, and food (D. Liu et al., 2015). Traditional organic

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fluorescent dyes are prone to quenching and photobleaching, leading to a decreased fluorescence intensity and limited sensitivity of the FM-LFA (Zhang et al., 2015). During the past decades, the development of semiconductor quantum dot has attracted intensive attention in detection area. Compared with organic fluorophores, quantum dots (QD) are new fluorescent labels with great potential (Morales-Narváez et al., 2015). QDs have been widely used to improve the detection sensitivity of LFA because they have narrow emission spectra, broad excitation range, and highly fluorescent quantum yields (Moralesnarváez et al., 2015; Xu et al., 2014). Time-resolved fluoroimmunoassay based on lanthanide (such as Eu(III)) label has many unique advantages compared with traditional fluorescence analysis (Majdinasab et al., 2015; Tang et al., 2015). Time-resolved refers to the distinguishing of useful fluorescence signal from nonspecific background fluorescence with the help of the delay time. Time-resolved fluoroimmunoassay using the lanthanide labels, which possess larger Stokes shift (over 150 nm) and longer fluorescence lifetime (up to 1 ms), can greatly reduce interference of nonspecific fluorescence signals, and thus acquire high sensitivity (Juntunen et al., 2012).

In our study, RAC was used as a model target. TRFN, FM, QD, and CG were used as labels in TRFN-LFA, FM-LFA, QD-LFA, and CG-LFA for the quantitative detection of RAC. The reaction dynamics, sensitivity, linear range, accuracy and precision, specificity, and reliability of four LFAs were compared. To our knowledge, this is the first study on the systematic and comprehensive comparison of the four nanoparticles as LFA labels.

2. Materials and methods

2.1. Materials and equipment

Time-resolved fluorescent nanobeads (TRFN, 1%, solid content, w/v; Carboxylate-modified Eu (III)-chelate-doped polystyrene nanobeads; excitation=365 nm, emission=615 nm) were purchased from Nanjing Microdetection Biotech Co., Ltd. (Nanjing, China). The fluorescein isothiocyanate fluorescent submicrospheres (FM, 1%, solid content, w/v; excitation=470 nm, emission=525 nm) were obtained from Merck company (Darmstadt, Germany). Quantum dots (QD, 1.0 mg/mL, w/v; Carboxylate-modified CdSSe/ZnS core/shell nanocrystals with amphiphilic polymer coating; excitation=365 nm, emission=610 nm) were acquired from the Hangzhou Najing Technology Co., Ltd. (Hangzhou, China). The colloidal gold (CG, 20 nm) was prepared in our laboratory (Li et al., 2013). Nitrocellulose membrane was supplied by Millipore (Bendford, MA, USA). Polyvinylchloride (PVC) backing pad, absorbent pad, filter pad, sample pad, and conjugate pad were purchased from Shanghai Kinbio Tech. Co., Ltd. (Shanghai, China). RAC-BSA conjugate antigen and commercial ELISA kits were provided by Wuxi Zodoboer Biotech. Co., Ltd. (Wuxi, China). Bovine serum albumin (BSA), N-(3-dimethylaminopropyl)-Nethylcarbodimide hydrochloride (EDC·HCl), and N-hydroxy-sulfo-succinmide (sulfo-NHS) were acquired from Sigma (St. Louis, MO, USA). The rabbit anti-RAC pAb was obtained from Suzhou Arend Bio-Tech Co., Ltd. (Suzhou, China). The goat anti-rabbit IgG was purchased from Meridian Life Science, Inc. (Memphis, TN, USA). Ractopamine (RAC), clenbuterol (CLB), salbutamol (SAL), bambuterol (BAM), mabuterol (MAB), penbutolol (PEN), terbutaline (TER), tulobutezol (TUL), cimbuterol (CIB), cimaterol (CIA) and brombuterol (BRO) were obtained from Dr. Ehrenstorfer, GmbH (Augsburg, Germany). Swine urine samples were provided by Guohong Swine Breed Farm (Nanchang, China). All solvents and other chemicals were of analytical reagent grade.

A XYZ-3050 Platform was purchased from BioDot (Irvine, CA). An automatic programmable cutter was supplied from Hangzhou Fenghang Technology Co., Ltd. (Hangzhou, China). The TRFN , FM, QD, and CG portable test strip readers were obtained from Suzhou Helmen Precision Instrument Co., Ltd. (Suzhou, China). The LEDs in TRFN, FM, and QD portable reader served as the excitation sources at 365 ± 20, 470 ± 20, and 365 ± 20 nm, respectively. The detection wavelengths of TRFN, FM, and QD portable reader were 615 ± 20, 525 ± 20, and 610 ± 20 nm, respectively. The wavelength of measurement for the CG nanoparticle was 525 ± 20 nm. Work current and optical power of four LEDs were 30 mA and 5 mW. The delayed time-resolved mode of TRFN portable reader was done with a 200-μs delay time and a 400-μs counting window.

The four labels and label-pAb probes were characterized with a particle size analyzer (Malvern Instruments Ltd., Worcestershire, U.K.). The size distributions and surface morphologies of the four labels were determined using a high-resolution transmission electron microscope (JEOL JEM 2100, Tokyo, Japan) and a high-resolution scanning electron microscope (Hitachi S-4800, Tokyo, Japan). The excitation and emission spectra of the three fluorescent labels were recorded with a Hitachi F-4500 fluorescence spectrophotometer (Tokyo, Japan). The UV–vis absorption spectra of colloidal gold were obtained with a UV–vis spectrophotometer (Shimadzu, UV-2300, Japan).

2.2. Preparation of four labeled-pAb probes

2.2.1. Preparation of TRFN-pAb probe

The time-resolved fluorescent nanobeads labeled anti-RAC pAb (TRFN-pAb) probe was prepared as previously reported with some modifications (Juntunen et al., 2012). TRFN (10 mg/mL, w/v) at 5.0 μL was added into 0.5 mL of 0.05 M boric acid buffer, and the suspension was mixed with a vortex mixer. Then, 5.0 μL of fresh EDC solution (0.5 mg/mL) was added into the mixture, which was stirred for 15 min. Next, 50 μL of anti-RAC pAb solution was added dropwise to the suspension. The solution was incubated at room temperature for 2 h and then blocked with 50 μL of blocking buffer containing BSA (10%, w/v) and Tween-20 (1%, v/v) for 2 h. The mixture was centrifuged at 14,000 rpm at 4 °C for 20 min. The precipitate was suspended in 0.1 mL of boric acid buffer (0.05 M, pH 7.4) with 0.2% BSA and 0.5% Tween-20.

2.2.2. Preparation of FM-pAb probe

The fluorescent submicrospheres labeled anti-RAC pAb (FM-pAb) probe was prepared according to our previous report (Deng et al., 2014). Briefly, 5.0 μL of FM (10 mg/mL, w/v) was dissolved in 1 mL of 0.02 M phosphate buffer. Then, 100 μL of anti-RAC pAb solution was added dropwise to the suspension. An 8.5 μL EDC solution (0.5 mg/mL) was added to the mixture and then stirred. The solution was then incubated at room temperature for 2 h and blocked with 100 μL of BSA (10%, w/v) for 30 min. The mixture was centrifuged at 12,000 rpm at 4 °C for 10 min. The precipitate was suspended in 0.1 mL of phosphate buffer (0.02 M, pH 7.4) with 1% BSA, 1% Tween-20, 5% sucrose, 3% trehalose, and 0.1% sodium azide.

2.2.3. Preparation of QD-pAb probe

The quantum dots labeled anti-RAC pAb (QD-pAb) probe was prepared according to a previous protocol with some modifications (Li et al., 2010). A total of 10.0 μL of quantum dots (1 mg/mL, w/v) was added into 0.2 mL of 0.05 M boric acid buffer. Then, 5.0 μL of fresh EDC solution (0.5 mg/mL) and 2.5 μL of fresh NHSS solution (0.125 mg/mL) were added into the solution and stirred for 30 min. Next, 50 μL of anti-RAC pAb solution was added dropwise to the suspension. The solution was incubated at room temperature for 1 h and blocked with 28.5 μL of NH_2-Glu (1%, w/v) for 45 min. The solution was adjusted to pH 4.5 with 1.0 μL of 1.0 M HCl. Finally, the mixture was centrifuged at 19,000 rpm at 4 °C for 10 min, and the precipitate was suspended in 0.1 mL of phosphate buffered saline (PBS 0.01 M, pH 7.4) containing 20% glycerin.