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Development of a colloidal gold-based lateral-flow immunoassay for the rapid detection of phenylethanolamine A in swine urine†

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Phenylethanolamine A (PEAA) is a newly emerged phenethanolamine member of the family of β-adrenergic agonists illegally used as feed additives for growth promotion. In this study, a highly sensitive and specific lateral-flow immunochromatographic assay (LFIA) using a colloidal gold-labeled monoclonal antibody was developed for the rapid detection of PEAA. The assay procedure could be accomplished within 10 min, and the result of this qualitative one-step assay was evaluated visually according to whether test lines appeared or not. When applied to the swine urine samples, the half maximal inhibitory concentration (IC_{50}), the limit of detection (LOD) and limit of quantification (LOQ) of the test strip under an optical density scanner were calculated to be 0.52 ± 0.11 ng mL^{-1}, 0.188 ng mL^{-1} and 0.263 ng mL^{-1}, respectively. The cut-off value of PEAA by the naked eye was 2.7 ng mL^{-1}. The specificity of the assay was evaluated by the measurement of cross-reactivity (CR) of the monoclonal antibody with PEAA, PEAA–NH_2 and 11 other β-adrenergic agonist compounds. Studies indicated that the monoclonal antibody was highly specific for PEAA and PEAA–NH_2, with negligible cross-reactivity with other β-adrenergic agonists including ractopamine (CR is 0.52%). To investigate accuracy and precision of the assay, swine urine samples were fortified with PEAA at different concentrations and analyzed by using the test strips with the scanner. Acceptable recovery rates of 92–102% and the intra- and inter-assay coefficients of variation (CV) of 8.70–17.65% were achieved. Parallel analysis of spiked swine urine samples with PEAA showed comparable results obtained from the lateral-flow test strip and LC-MS/MS. There was an acceptable correlation coefficient of 0.9789 between the two methods. Therefore, the described lateral-flow test strip could be used as a reliable, rapid and cost-effective on-site screening technique for the determination of PEAA residue in swine urine.

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1 Introduction

β-Adrenergic agonists are synthetic phenethanolamine compounds which enhance animal growth and increase feeding efficiency by inhibiting fat synthesis, stimulating lipolysis, increasing protein synthesis and carcass leanness.[1–4] However, the misuse of the growth promoters can lead to the excessive residues in edible meat or tissue. Consumption of the food contaminated by β-adrenergic agonists can cause acute intoxication of the cardiovascular system, nervous system and respiratory system and have adverse effects on human health.[5–7] There was an increasing concern of the hazards posed to human health by the presence of β-adrenergic agonist residues in animal tissues.[8] Therefore, β-adrenergic agonists, except ractopamine, which had been approved as a feed additive for swine and cattle in the United States and some other countries, are now banned as feed additives for growth promotion in food animals in China, the United States and most European countries. Recently a new β-adrenergic agonist named phenylethanolamine A appeared in China as the alternative for common β-adrenergic agonists in order to escape supervision.[9–12]

Phenylethanolamine A [PEAA, 2-(4-(nitrophenyl)butan-2-ylamino)-1-(4-methoxyphenyl)ethanol, $C_{19}H_{24}N_2O_4$, MW = 344.17, Fig. 1] was validated to be a phenethanolamine member of the family of β-adrenergic agonists. It has been prohibited from being used in animal feed and drinking water in China since 2010.[13] To reduce the potential risk of PEAA residues to human health and monitor the illegal use of PEAA, the Ministry of Agriculture of China issued a standard analytical method for the detection of PEAA in feed using high performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS) in 2010.[14] Recently some chromatographic analytical methods including liquid chromatography tandem mass spectrometry

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(LC-MS/MS) and high performance liquid chromatography (HPLC) have been developed for the detection of PEAA in biological and feed samples.[15–18] Although chromatographic analytical methods are accurate, they are expensive, time-consuming and require personnel with professional training to operate the sophisticated instruments. Therefore, there is an urgent need to develop sensitive, specific, rapid and low-cost screening methods for the detection of PEAA residues. The screening methods were often immunoassays, including enzyme-linked immunosorbent assay (ELISA), lateral-flow immunoassay (LFIA) and so on. ELISA and LFIA had been intensively applied for the detection of β-adrenergic agonists over the past twenty years.[19–42] In the last three years, the ELISA screening method based on the polyclonal and monoclonal antibodies was also developed for the detection of PEAA in urine, tissue and feed samples.[9–12]

The use of membrane based lateral-flow immunoassay tests for on-site screening provides a simple, low-cost, sensitive, specific and user-friendly alternative to expensive, laborious and time-consuming instrumental methods and more sophisticated immunoassay formats.[37–42] The primary aim of this paper was to develop a lateral-flow colloidal gold-based technique for the detection of PEAA residues in swine urine.

2 Materials and methods

2.1 Reagents

Bovine serum albumin (BSA), goat anti-mouse IgG, Tween 20, PEG-20000, gold(III) chloride trihydrate (ACS reagent), polyvinyl alcohol, sodium azide, EDTA and sucrose (no. S9378) were purchased from Sigma Co. (St. Louis, MO, USA). PEAA was supplied by Hangzhou DNA Sci-Tech Co. (Hangzhou, China). Ractopamine, formoterol, clenbuterol, salbutamol, terbutaline, cimaterol, cimbuterol, clorprenaline, bambuterol, tulobuterol and zilpaterol were purchased from Dr. Ehrenstorfer Co. (Augsburg, Germany). HPLC-grade formic acid, methanol and acetonitrile were purchased from Merck (Darmstadt, Germany). Hi-Flow Plus 180 membrane from Millipore (Bedford, MA, USA), conjugate pad grade 8964 and absorbent pad type 133 from Pall (Saint Germain-en-Laye, France), and glass fiber grade F075-17

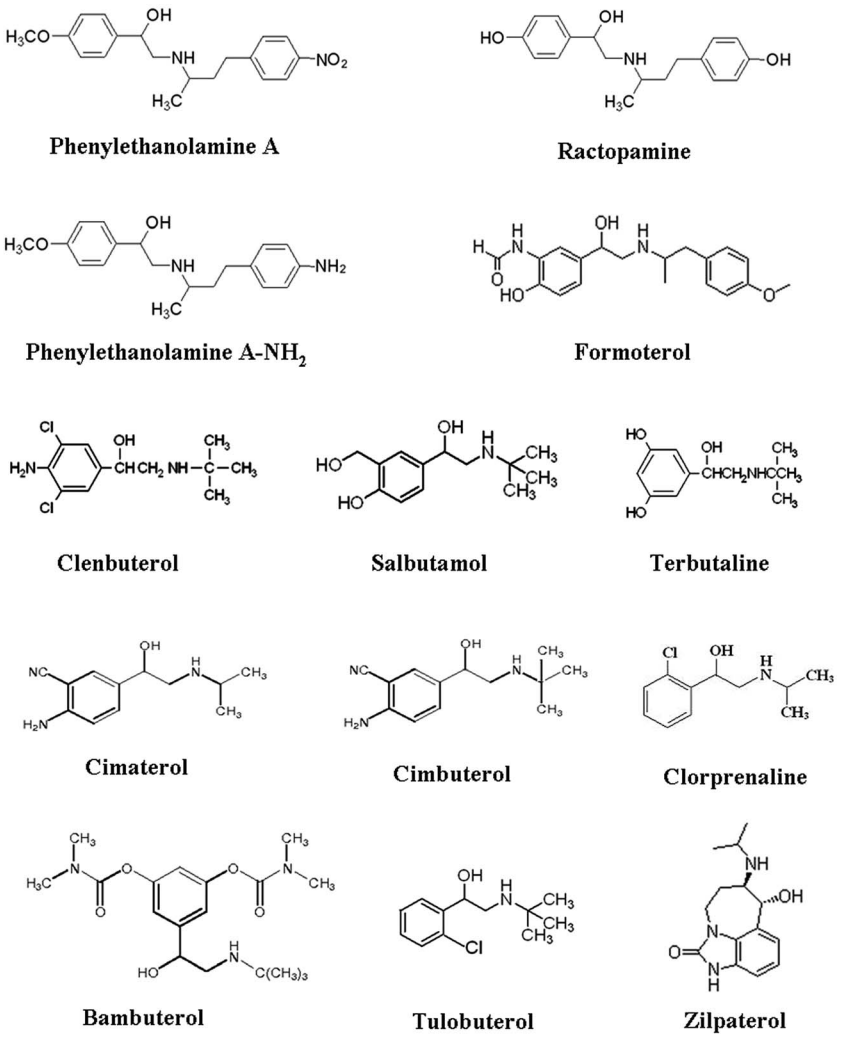

Fig. 1 Chemical structures of PEAA, PEAA–NH_2 and other β-adrenergic agonists used in this study.

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from Whatman (Maidstone, Kent, England) were used. Ultrapure water was generated from a NANO pure system (Thermo, USA).

2.2 Monoclonal antibody to PEAA and coating antigen

Monoclonal antibody 2H8 (IgG2a/κ) specific for PEAA was obtained by immunizing mice with PEAA–BSA as described previously.[11] The monoclonal antibody was purified from ascites using the protein A affinity column (Amersham Biosciences, Uppsala, Sweden) according to the manufacturer's manual. The coating antigen of the PEAA–OVA conjugate was prepared by the diazotization method as described previously.[11] Experimental procedures were carried out strictly in accordance with the "Administrative Rules for Laboratory Animals in Zhejiang Province" (2009), and were approved by Animal Care and Use Committee of Hangzhou Normal University (Hangzhou, China). All efforts were made to minimize the animals' suffering and to reduce the number of animals used.

2.3 Preparation of standard solutions and swine urine samples

Standard solutions of PEAA, PEAA derivative (PEAA–NH_2) and other β-adrenergic agonists were prepared by diluting stock solutions of these compounds (1 mg mL^{-1}, in methanol, store at −20 °C). PEAA and derivative stock solutions were diluted in normal swine urine, which was determined to be the negative content of β-adrenergic agonists by LC-MS/MS, at 0, 0.03, 0.10, 0.30, 0.90, 2.70, and 8.10 ng mL^{-1} and other β-adrenergic agonists of ractopamine, formoterol, clenbuterol, salbutamol, terbutaline, cimaterol, cimbuterol, clorprenaline, bambuterol, tulobuterol and zilpaterol at 10, 25, 50, 100, 250, 500, 1000, 2000, 4000, and 8000 ng mL^{-1}.

Swine urine samples were collected manually in glass vials and stored at −20 °C from several local small farms where swine with mix of genders such as Duroc, Landrace and Yorkshire were bred and fed in Zhejiang Province, China.

2.4 Instruments

Centrifugation was carried out with a Megafuge 11R centrifuge (Thermo, USA). UV-vis data were recorded on a UV-4802S spectrophotometer (Unico, China). The conjugate pads and membrane were spotted on them by using a Quanti 3000 Biojet attached to a XYZ Bioatrip Dispenser (Bio-Dot, CA, USA). The prepared master card was cut into 3.8 mm wide strips using a CM 4000 Cutter (Bio-Dot, CA, USA). The test lines were scanned with a BioDot TSR3000 Membrane Strip Reader (BioDot, CA, USA).

2.5 Colloidal gold-based lateral-flow immunoassay

2.5.1 Preparation of colloidal gold labeled monoclonal antibody. Colloidal gold with an average diameter of 40 nm was prepared by controlled reduction of gold chloride with 1% sodium citrate according to the procedure described by Hayat.[43] Briefly, 100 mL of 0.2% gold chloride trihydrate solution in super purified water was heated to boil, and then 1.5 mL of 1% sodium citrate solution was added while stirring. After the color changed from light yellow to brilliant red, the solution was boiled for another 5 min, and then cooled to and stored at room temperature with 0.05% sodium azide added.

The colloidal gold labeled monoclonal antibody against PEAA (2H8) was prepared as described by Yokota *et al.* with some modification.[44] Briefly, 1 mL of anti-PEAA mAb at an optimum concentration of 1 mg mL^{-1} was incubated with 10 mL of colloidal gold solution (pH 8.9) for 30 min at room temperature. Blocking with 1 mL 10% BSA solution in 0.02 M sodium borate buffer (pH 8.9) at room temperature for another 10 min, the mixture was centrifuged at 4 °C, 20 000×*g* for 30 min and then the labeled anti-PEAA mAb was washed by repeated centrifugation (20 000×*g*) with 1% BSA in 0.02 M sodium borate buffer (pH 8.9) at 4 °C for 30 min. The precipitates were resuspended with 1 mL PBS (0.05 M, pH 7.4) containing 1% BSA and 0.05% sodium azide and stored at 4 °C for use.

2.5.2 Preparation of the conjugate pad. The conjugate pad (300 × 8 mm) was dispensed with 300 μL of the optimum mixture of colloidal gold labeled anti-PEAA mAb (300 μL) diluted with 700 μL PBS containing 5.0% (w/v) sucrose, 5.0% (w/v) BSA, 0.8% (w/v) NaCl, 0.1% (w/v) EDTA, 0.3% (v/v) Tween 20 and 0.05% (w/v) sodium azide by using a Quanti 3000 Biojet attached to an XYZ Bioatrip Dispenser. After dispensing, the pad was dried at 37 °C for 2 h and then stored in a desiccator at room temperature.

2.5.3 Preparation of the membrane. Test and control lines were spotted on the Hi-Flow Plus 180 membrane (300 × 25 mm) using a Quanti 3000 Biojet attached to an XYZ Bioatrip Dispenser (Bio-Dot, CA, USA). The test line was separately coated with a PEAA–OVA conjugate at the bottom of the membrane. Goat anti-mouse IgG was dispensed on the top of the membrane as the control line. The distance between the lines was 70 mm. The PEAA–OVA conjugate and goat anti-mouse IgG were separately diluted in PBS containing 5% methanol (v/v) to the optimum concentration of 0.6 and 1.2 mg mL^{-1}, respectively, and applied in the form of dots at 50 dots per μL per cm to form the test and control lines. After drying at 37 °C for 60 min, the membrane was blocked with PBS (0.05 M, pH 7.4) containing 1% (w/v) casein at room temperature for another 60 min. Then the membrane was dried at 37 °C for 2 h, vacuum-packaged in a plastic bag containing silica as the moisture absorbent and stored under dry conditions at room temperature for use.

2.5.4 Preparation of the sample pad and absorbent pad. Glass fiber grade F075-17 from Whatman (Maidstone, Kent, England) was used as the sample pad. The sample pad (300 × 20 mm) was saturated with sodium borate buffer (0.02 M, pH 9.2) containing 2.0% (w/v) sucrose, 1.0% (w/v) BSA, 0.8% (w/v) NaCl, 0.2%(w/v) polyvinyl alcohol, 1.0%(w/v) PEG20000 and 0.05% (w/v) sodium azide at room temperature for 30 min. Then the sample pad was dried at 37 °C for 2 h and stored as described above. The absorbent pad was cut into 300 × 30 mm for use.

2.5.5 Assembly of the test strip. On a plastic baking plate (300 × 80 mm), the conjugate pad was attached to the bottom of the membrane with 1–2 mm overlapping on the membrane, and then the sample pad was attached to the bottom of the conjugate pad in a similar manner. The absorbent pad was attached

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to the top of the membrane with 1–2 mm overlapping on the membrane also. The prepared master card was cut into 3.8 mm width strips using a CM 4000 Cutter (Bio-Dot, CA, USA). The strips were then sealed in an aluminum foil bag containing desiccant gel and stored under dry conditions at room temperature until use.

2.5.6 Assay procedure and principle. The principle of test strips was illustrated as in Fig. 2. The test strips were inserted into 80–100 μL of standard or swine urine samples for 20 s and then put flatwise to allow the liquid to migrate. The specific colloidal gold-labeled anti-PEAA mAb, which was redissolved from the conjugate pad, reacted with PEAA (if it was present in the urine samples). In the mean while, excess of colloidal gold-labeled anti-PEAA mAb was trapped by the PEAA–OVA immobilized on the membrane forming a red test line and further trapped by the goat anti-mouse IgG antibody forming the control line while the whole complex was migrating along the membrane. After 10 min, the test result was evaluated visually or the test line was scanned with a BioDot TSR3000 Membrane Strip Reader (BioDot, CA, USA).[37,40] The G/peak and $G/D \times$ area of the relative optical (ROD) decreased as the PEAA concentration in the standard samples increased. The concentration of PEAA and the ROD (%) produced a sigmoidal dose–response curve that fits to a four-parameter logistic curve pattern indicating the classical competition. The negative test resulted in two red lines (test and control lines). The more PEAA present in the sample, the weaker appeared the test line. The positive sample gave only one red line (the control line). If no control line was present, the test was considered to be invalid.

2.5.7 Immunochromatographic time of the test strip. The test strips were inserted into 80–100 μL of the blank swine urine sample (0 ng mL^{-1}) and spiked swine urine samples with PEAA at concentrations of 0.15, 0.30, and 0.45 ng mL^{-1} for 20 s and put flatwise to allow the liquid to migrate for 2–16 min. And then, the test line of every strip was investigated with a BioDot TSR3000 Membrane Strip Reader.

2.5.8 Sensitivity and specificity of the test strip. The test strip of PEAA was based on the competitive principle, the inverse relationship between concentrations of PEAA in the sample and development of red color on the test lines. Therefore, the sensitivity of the test strip should be determined by testing the PEAA standard samples. The relative optical densities (ROD) decreased as the PEAA concentration in the standard samples increased. Similar to the ELISA assay, the half maximal inhibitory concentration (IC_{50}) with the strip was quantitatively defined as the amount of PEAA in the standard samples that caused 50% decrease of the ROD than that produced by the 0 ng mL^{-1} sample in the present study. By using the strip reader, the sensitivity of the test strip was characterized by the IC_{50} value with the PEAA standard concentration range of 0.033–8.1 ng mL^{-1} under optimized conditions.

To evaluate the specificity of the test strip, cross-reactivity (CR) experiments were conducted by measuring the IC_{50} values of PEAA, PEAA–NH_2 and the 11 other β-agonist compounds (ractopamine, formoterol, clenbuterol, salbutamol, terbutaline, cimaterol, cimbuterol, clorprenaline, bambuterol, tulobuterol and zilpaterol) as competitors. As a quality control, the PEAA calibration curve was generated in every experiment. The CR values were obtained by calculating the ratio of IC_{50} values produced by the competitors and PEAA using the following equation:

$$\text{CR}\ (\%) = (\text{IC}_{50}\ \text{of PEAA})/(\text{IC}_{50}\ \text{of competitors}) \times 100\%.$$

2.5.9 Fortification experiment of the test strip. The colloidal gold immunoassay validation was carried out using the limit of detection (LOD), the limit of quantification (LOQ),

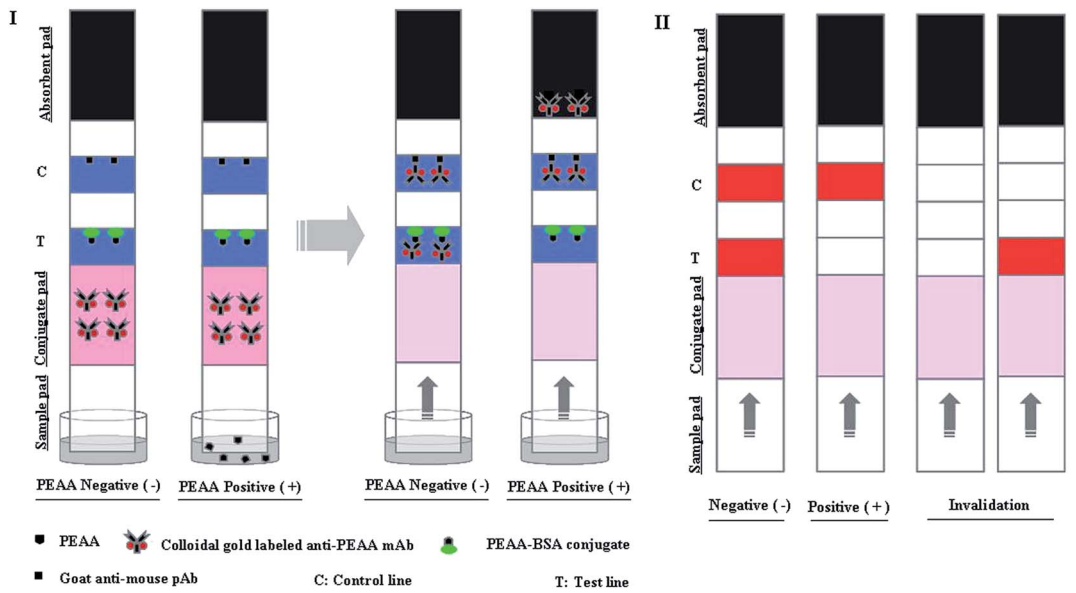

Fig. 2 A schematic diagram of the colloidal gold-based immunochromatographic assay for detection of PEAA. I: model of samples tested. II: model of visual result.

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the recovery (%) of the fortified PEAA and coefficients of variation (CVs). The 20 blank swine urine samples, obtained by 20 different animals and certified as free of PEAA using liquid chromatography tandem mass spectrometry (LC-MS/MS) previously, were analyzed in 6 replicates for PEAA by using the test strips with the scanner. The concentrations of PEAA in the blank samples were calculated according to the standard curve (0, 0.033, 0.1, 0.3, 0.9, 2.7, and 8.1 ng mL^{-1}), as well as the mean value for 20 blank urine samples. The LOD and LOQ were calculated as the mean of the measured content of blank different samples ($n = 20$) plus three standard deviations (mean + 3SD) and six standard deviations (mean + 6SD) (Commission Decision 87/410/EEC), respectively.[25,40]

To test accuracy and precision of the strip, the blank swine urine samples were spiked with PEAA at concentrations of 0.25, 0.50, 1.00, and 2.00 ng mL^{-1} and analyzed in 6 replicates by using the test strips with the scanner. Sample recoveries were determined from the standard curve and calculated according to the following equation: recovery rate (%) = measured concentration/fortified concentration × 100%. The precision of the test strip was analyzed by repeated determination of the intra- and inter-assay CVs of the spiked samples at the PEAA concentrations of 0.25, 0.50, 1.00, and 2.00 ng mL^{-1}. Intra-assay variation was calculated as the mean value of six replicates on one single day. Inter-assay variation was determined by analyzing six replicates carried out on three different days.

2.6 LC-MS/MS analysis of PEAA in swine urine

In parallel with the strip tests, LC-MS/MS analysis of PEAA was performed with a Shimadzu HPLC instrument (Shimadzu; Kyoto, Japan) and a Micromass Quattro Premier XE system (Waters; Manchester, UK) equipped with an electrospray ionization (ESI) source in this study. Chromatographic separations were performed on an Acquity BEH C_{18} column (100 mm × 2.1 mm, 1.7 μm) maintained at 30 °C. Solvent A (0.1% formic acid) and solvent B (acetonitrile) constituted the mobile phase. The gradient program was set as follows: 0–1.0 min, 5% B; 1.1–3.0 min, a linear gradient from 5% to 80% B; 3.1–5.0 min, 80% B; 5.1–6.0 min, 5% B. The flow rate during the whole process was 0.30 mL min^{-1} and the injection volume was 10 μL.

Mass spectrometric detection was conducted on a Micromass Quattro Premier XE system (Waters; Manchester, UK) equipped with an electrospray ionization (ESI) source. Positive mode and multiple reaction monitoring (MRM) were selected for the detection experiment. The parameters were set as follows: capillary voltage, 3000 V; source temperature, 150 °C; desolvation temperature, 400 °C; cone gas (N_2) flow rate, 60 L h^{-1}; desolvation gas (N_2) flow rate, 750 L h^{-1}; collision cell pressure, 4 × 10^3 mbar. The selected MRM transitions for PEAA were m/z 345.3–327.0 and 345.3–150.0 with a dwell time of 250 ms. The transition chosen for quantification was 345.3–150.0. The optimized collision energies for the transitions of 345.3–327.0 and 345.3–150.0 were 20 eV and 33 eV, respectively.

Comparison was made using linear regression analysis with the line modeled having a zero intercept. The resulting correlation coefficients served as measures of assay variability between the test strip and LC-MS/MS method, whereas slopes of the correlations served as indicators of differences in assay responsiveness.

3 Results and discussion

3.1 Optimization of colloidal gold immunoassay for PEAA

The colloidal gold based and competitive immunoassay was developed as a rapid visual qualitative test which gave a simple yes/no response to the levels of the target analyte. Therefore, the optimal conditions for the negative test which gave the most intensely red colored test line and the smallest amount of PEAA that resulted in no red color development at the test line should be studied. In addition, the difference between positive and negative samples should be easily distinguished within a reasonably short immunochromatographic time. For these purposes, the optimal condition experiments for the lateral-flow assay for PEAA were tested similar to the "checkerboard titration" in competitive ELISA (shown in the ESI†). Using urine samples spiked with PEAA at 0–8.1 ng mL^{-1}, the optimal conditions were selected for the further experiments under the following conditions: PEAA–OVA conjugate and goat anti-mouse IgG concentrations of 0.6 and 1.2 mg mL^{-1}, respectively, forming the test and control lines, 300 μL of the mixture of colloidal gold labeled anti-PEAA mAb (300 μL) diluted with 700 μL PBS dispensing on the conjugate pad.

In accordance with the above optimal conditions, the performance of test lines was investigated with a BioDot TSR3000 Membrane Strip Reader to test the immunochromatographic time by using a blank swine urine sample and spiked urine samples with PEAA at 0.15, 0.30 and 0.45 ng mL^{-1}. The relative optical density (ROD) increased simultaneously for 10 min, and did not increase obviously after 10 min (Fig. 3). The results showed after 10 min, almost all colloidal gold-labeled antibodies would bind to the PEAA–OVA and goat anti-mouse IgG coated on the nitrocellulose membrane, if the PEAA levels in the urine samples are negative or below the particular level. At the immunochromatographic time of 10 min, the difference between positive and negative samples could be also easily

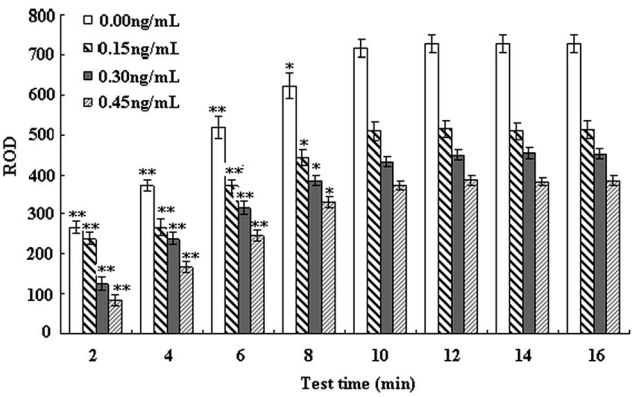

Fig. 3 The mean relative optical density (ROD) of the PEAA at 0, 0.15, 0.30 and 0.45 ng mL^{-1} standards at different times with test strips ($n = 6$, at 25 °C).

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distinguished by the naked eye. So the immunochromatographic time of 10 min was selected for further experiments.

3.2 Sensitivity of the test strip

The sensitivity of the test strip was determined by testing the spiked urine samples with PEAA at 0–8.1 ng mL^{-1}. Scanned with the BioDot TSR3000 Membrane Strip Reader, the relative optical densities (RODs) decreased as the PEAA concentrations in the urine samples increased. The relationship between the concentrations of PEAA and the ROD/ROD_0 (%) showed the sigmoidal dose–response curves which fit to a four-parameter logistic curve pattern indicating the classical competition (Fig. 4). In the present study, the IC_{50} of PEAA was calculated to be 0.52 ± 0.11 ng mL^{-1}. As compared to previous reports on the detection of PEAA using ELISA, the IC_{50} of PEAA in this assay was at the similar level.[9–12]

The colloidal gold immunoassay was studied as a rapid visual qualitative test which gave a simple yes or no response to the levels of the target analytes. The cut-off value by the naked eye was defined here as the amount of PEAA in the standard samples that resulted in no red color development at the test lines. In accordance with visual evaluation, the cut-off value of PEAA was about 2.7 ng mL^{-1} (Fig. 5).

In this work, the LOD for PEAA in swine urine samples was 0.188 ng mL^{-1} and the LOQ was 0.263 ng mL^{-1}, which is comparable with that of detection of PEAA using both the LC-MS/MS and ELISA method.[9–12,16] The LOD and LOQ of the present study could meet the requirement of rapid screening detection for PEAA residues in swine urine samples.

3.3 Specificity of the test strip

The cross reactivity of the PEAA test strip with PEAA derivative ($PEAA–NH_2$) and 11 other β-adrenergic agonist compounds was examined at 25 °C. The IC_{50} and the cross-reactivities of the test strip to PEAA, $PEAA–NH_2$ and other β-adrenergic agonists were analyzed with the four parameter logistic equation and are shown in Table 1. The result showed that the anti-PEAA mAb was highly specific to PEAA and its derivative $PEAA–NH_2$ with an IC_{50} of 0.49 ± 0.09 ng mL^{-1} (106%) ($n = 6$). No cross-reactivity of anti-PEAA mAb to other tested β-adrenergic agonists except ractopamine for concentrations up to 100 ng mL^{-1} was observed. The specificity will reduce the possibility of false positive results.

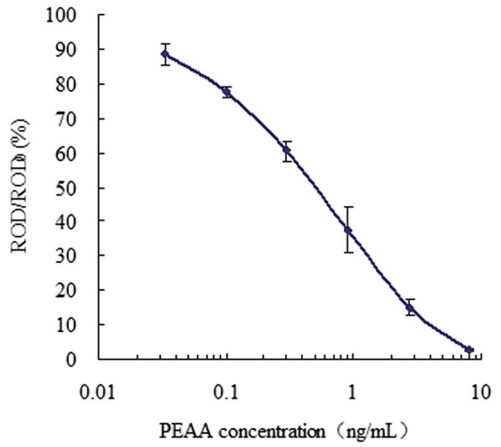

Fig. 4 Standard curves for PEAA quantitation with test strips and strip reader ($n = 10$, at 25 °C). ROD represents the mean relative optical density of PEAA standards and ROD_0 is the mean relative optical density at 0 ng mL^{-1}. The concentrations of PEAA standards solution are 0.033, 0.1, 0.3, 0.9, 2.7 and 8.1 ng mL^{-1}, respectively.

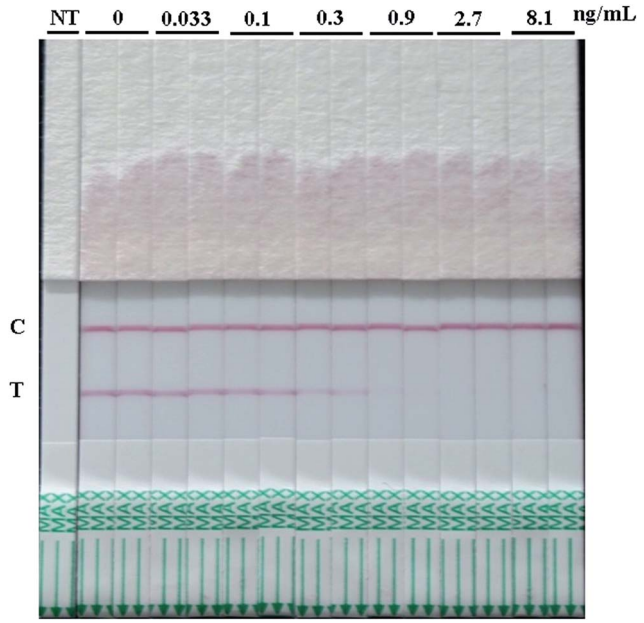

Fig. 5 Colloidal gold-based lateral-flow immunoassay for detection of PEAA in spiked swine urine samples (at 25 °C). The upper line is the control line (C) and the bottom line is the PEAA test line (T), respectively. NT is the not tested strip. The urine samples were spiked with PEAA at 0, 0.033, 0.1, 0.3, 0.9, 2.7 and 8.1 ng mL^{-1}, respectively.

3.4 Validation

LC-MS/MS analysis, which was considered as one of the confirmatory methods for identification and quantification of

Table 1 Cross-reactivity of the PEAA test strip with β-adrenergic agonists ($n = 6$, at 25 °C)

Competitors	IC_{50} (ng mL^{-1})	Cross-reactivity (%)
Phenylethanolamine A	0.52 ± 0.11	100
Phenylethanolamine A derivative	0.49 ± 0.09	106
Ractopamine	100	0.52
Formoterol	>500	<0.1
Clenbuterol	>500	<0.1
Salbutamol	>500	<0.1
Bambuterol	>500	<0.1
Cimaterol	>500	<0.1
Cimbuterol	>500	<0.1
Clorprenaline	>500	<0.1
Terbutaline	>500	<0.1
Tulobuterol	>500	<0.1
Zilpaterol	>500	<0.1

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Table 2 The recoveries, intra- and inter-assay variations of urine samples spiked with PEAA ($n = 6$, at 25 °C)

	Intra-assay variation			Inter-assay variation		
Fortified concentration (ng mL^{-1})	Measured ±SD (ng mL^{-1})	Recovery (%)	CV (%)	Measured ±SD (ng mL^{-1})	Recovery (%)	CV (%)
0.25	0.23 ± 0.02	92.00	8.70	0.24 ± 0.04	96.00	16.67
0.50	0.48 ± 0.05	96.00	10.42	0.51 ± 0.09	102.00	17.65
1.00	0.93 ± 0.10	93.00	10.75	0.99 ± 0.15	99.00	15.15
2.00	1.85 ± 0.18	92.50	9.73	1.90 ± 0.24	95.00	12.63

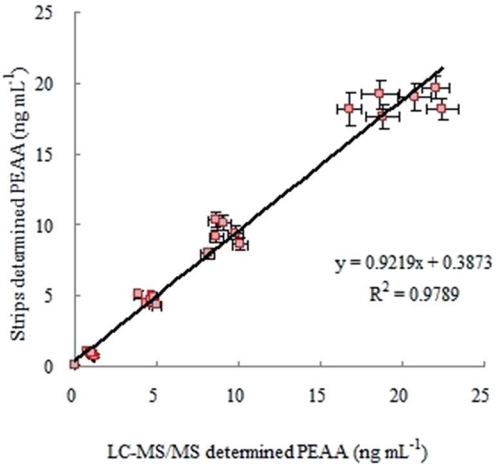

Fig. 6 Correlation between test strips ($n = 6$) and LC-MS/MS ($n = 6$) for the detection of PEAA in spiked swine urine samples.

β-adrenergic agonists, was performed to quantify the amount of PEAA in spiked urine samples with PEAA at concentrations of 1, 5, 10 and 20 ng mL^{-1} and parallel with the strip tests. The spiked urine samples were diluted 1 : 10 and then analyzed by using the test strip with the scanner.

The results of accuracy and precision for the strip are shown in Table 2, the average recoveries ranged from 92% to 102%, the coefficients of variation (CVs) ranged from 8.70% to 10.75% for intra-assay and 12.63% to 17.65 for inter-assay. The results indicated that recoveries within 25% of theoretical values and coefficients of variation below 20% were acceptable for screening detection of PEAA residues in swine urine.

The values showed in figure were the average of six repeated tests. The correlation coefficient (R^2) for the test strip analysis and the LC-MS/MS analysis of PEAA in swine urine samples was 0.9789, indicating an acceptable agreement between the two methods for the detection of PEAA (Fig. 6). The slope of the correlation was 0.9219, indicating that the quantitative results of PEAA by LC-MS/MS were little greater than the detected results by the test strip method (Fig. 6). The results suggested that the test strip method based colloidal gold based lateral flow immunoassay was reliable for the PEAA detection in swine urine samples, meanwhile, the method offered advantages of sample preparation and high throughput.

4 Conclusions

In the present study, we established a colloidal gold-based lateral-flow immunoassay for the rapid detection of PEAA in swine urine by using a monoclonal antibody produced with the immunogen PEAA–BSA conjugate. The assay could be accomplished within 10 min without the need for any sample preparation. By scanning the relative optical density (ROD) of the test lines, this test strip format assay could be quantitatively analyzed in accordance with the mathematical model of RPNA (Qian and Bau, 2004).[45] Similar to the competitive ELISA, the IC_{50}, sensitivity, specificity, LOD, LOQ, accuracy and precision of the PEAA test strip were easily calculated and analyzed. In the present study, the IC_{50}, LOD and LOQ of the test strip under an optical density scanner were calculated to be 0.52 ± 0.11, 0.188 and 0.263 ng mL^{-1}, respectively. As compared to the previous reports on the detection of PEAA using ELISA, the IC_{50} of PEAA in this assay was at the similar level.[9–12] The results from visual evaluation of the lateral-flow tests of spiked swine urine samples showed that the cut-off value of PEAA was 2.7 ng mL^{-1}. The developed assay showed excellent specificity for the PEAA measurements, because the monoclonal antibody was highly specific for PEAA and PEAA–NH_2, with negligible cross-reactivity with other β-adrenergic agonists. Acceptable recovery rates of 92–102% and the intra- and inter-assay coefficients of variation (CVs) of 8.70–17.65% were achieved. The results with test strips and LC-MS/MS analysis for detection of PEAA in spiked swine urine samples proved the reliability of the immunoassay. In conclusion, the described colloidal gold-based lateral-flow immunoassay format could be used for rapid and cost-effective screening of PEAA residues in swine urine samples.

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