Letter to the Editor

Lateral Flow Immunoassay for Quantitative Detection of Ractopamine in Swine Urine*

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A strip reader based lateral flow immunoassay (LFIA) was established for the rapid and quantitative detection of ractopamine (RAC) in swine urine. The ratio of the optical densities (ODs) of the test line (A_T) to that of the control line (A_C) was used to effectively minimize interference among strips and sample variations. The linear range for the quantitative detection of RAC was 0.2 ng/mL to 3.5 ng/mL with a median inhibitory concentration (IC_{50}) of 0.59±0.06 ng/mL. The limit of detection (LOD) of the LFIA was 0.13 ng/mL. The intra-assay recovery rates were 92.97%, 97.25%, and 107.41%, whereas the inter-assay rates were 80.07%, 108.17%, and 93.7%, respectively.

Ractopamine (RAC) misused in livestock production can increase the potential toxicological risks to humans. Many countries in the world, including China and most European countries, have forbidden the use of RAC as feed additives[1]. Various confirmation methods, including high-performance liquid chromatography[2], liquid chromatography plus mass spectrometry[3], have been used to monitor the illicit use of RAC, but these methods are unsuitable for routine screening because of the high cost of instruments, extensive clean-up procedures and the operation by trained people. Lateral flow immunoassay (LFIA) has the advantages of both chromatographic separation and immunoassay specificity. It is considered as an effective field test and has been widely used for quantitative detection of RAC residue in swine urine[4-5].

In the present study, we established a portable strip reader-based LFIA for the rapid quantitative detection of RAC residue in swine urine. The detection parameters, including interference from the urine matrix, the sample volume and the interpretation time, were optimized by analysing the dynamic curves of A_T, A_C, and the A_T/A_C ratio against incubation time. The specificity, reproducibility, and accuracy of the established LFIA were evaluated. The reliability of the new method was further compared with a commercial ELISA kit by analysing 48 real swine urine samples.

The RAC quantitative strip was produced as described previously[6] with some modification. Briefly, the colloidal gold probe was prepared by adding 1 mL anti-RAC mAb (15 μg/mL) to 10 mL colloidal gold solution. After being blocked with 1 mL of 10% BSA and centrifuged at 4500 g for 30 minutes, the colloidal gold probe was resuspended with 1 mL of PBS containing 5% sucrose, 2% fructose, 1% PEG 20 000, 1% BSA and 0.4% Tween-20[7], and then sprayed onto a treated conjugate pad at a density of 2.5 μL/cm. The BSA-RAC (0.3 mg/mL) and goat anti–mouse IgG (1 mg/mL) were dispensed onto the nitrocellulose (NC) membrane as test line (T) and control line (C) at a density of 0.8 μL/cm. The assembled strips were cut into pieces (4 mm) and then sealed in a plastic bag with desiccant gel.

The LFIA assay for RAC was based on a competitive immunoassay. The changes in ODs on both lines can indirectly reflect the dynamic process of antigens (on NC membrane) and colloidal gold probe (on conjugate pad) interaction[8]. In the present study, dynamic analysis was conducted for elaborating the effect of the interpretation time, loading sample volume, ionic strength and pH of urine on the stability and sensitivity of the quantitative LFIA. The dynamic curve was constructed as follows. After the sample was incubated for 1 min, the strip was scanned by using a commercial HG8 strip reader, which was provided by Shanghai Huguo Science Instrument Co., Ltd. The A_T and A_C were recorded every 30 seconds for 45 min.

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The immunoreaction between the colloidal gold probe and the BSA-RAC on the test line, as well as the donkey anti-mouse IgG on the control line were indicated by a curve by plotting the ODs against the incubation time.

As shown in Figure 1A, during the 45 min incubation time, the A_T and A_C increased sharply during the first 10 minutes, then increased slowly in the next 15 to 35 min and reached a stable value in the last 10 min (data not shown). However, the A_T/A_C ratio reached a stable phase 15 min after incubation and remained stable during the subsequent 30 min of observation time under 0 ng/mL to 1.5 ng/mL RAC-spiked concentration. Thus, the incubation for 15 min was necessary for the RAC LFIA quantitative analysis. The effects of sample volume on the sensitivity of LFIA shown in Figure 1B indicated that the A_T/A_C ratio increased by 7.93% and 7.66% at sample volumes of 85 and 100 μL, respectively. However, at a sample volume of 100 μL, the A_T/A_C ratio needed more time (20 min) to reach a stable phase. Thus, a sample volume of 85 μL was used for the following experiments. The effects of ionic strength on the consistency of A_T/A_C ratio are shown in Figure 1C. The results indicated that the A_T/A_C ratio remained relatively stable from 0.80±0.01 to 0.81±0.01 (P>0.05) under different NaCl concentrations although the values of A_T and A_C changed irregularly. To explore the effect of pH on the consistency of A_T/A_C ratio, the blank urine mixtures were adjusted respectively to final pH values of 5.0, 6.0, 7.0, 8.0, and 9.0. The stable A_T, A_C, and A_T/A_C ratio shown in Figure 1D indicated that pH markedly influenced the A_T and A_C. However, the A_T/A_C ratio remained relatively stable, ranging from 0.72±0.02 to 0.78±0.01 (P>0.05) when the pH varied from 6.0 to 9.0, whereas it declined to 0.41±0.03 at pH 5.0 (P<0.01) because the A_T decreased significantly.

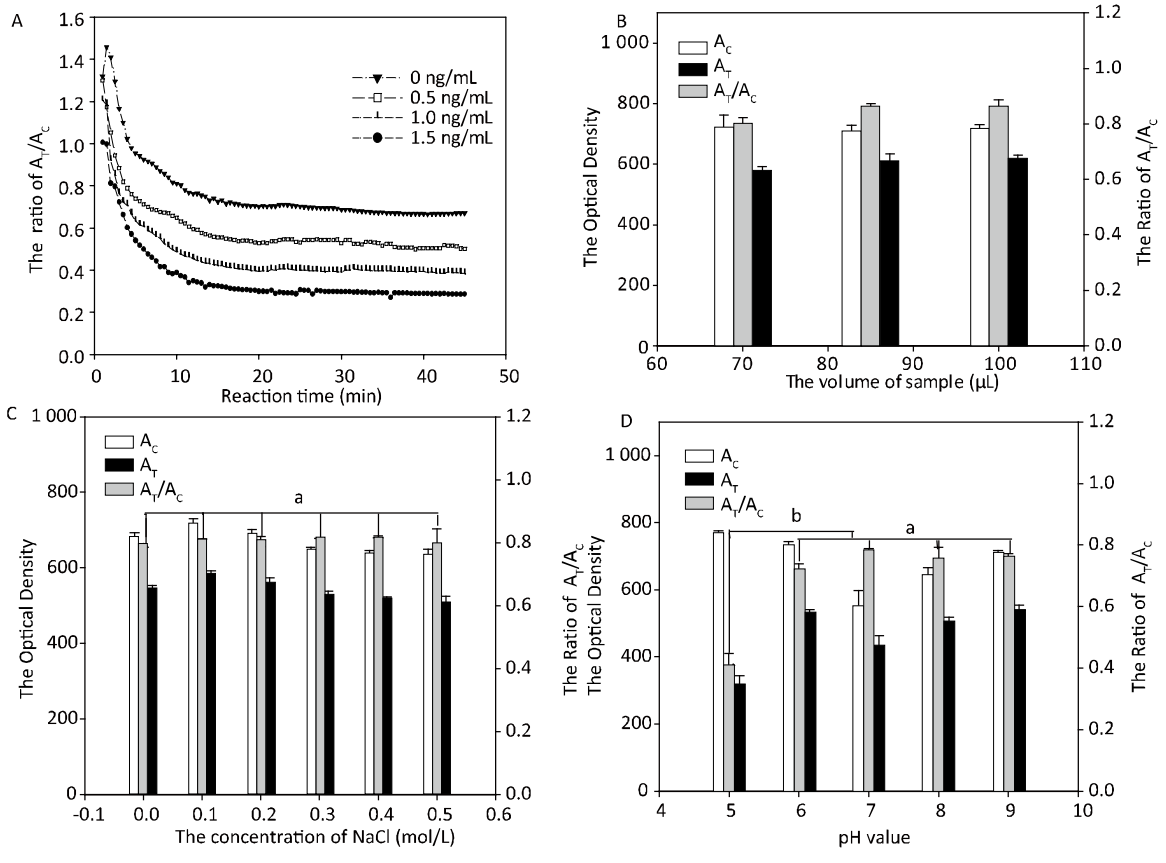

Figure 1. Optimizing the LFIA assay. (A) Immunoreaction dynamics of A_T/A_C ratio at different RAC concentrations. (B) Effect of sample volume on the A_T, A_C, and A_T/A_C ratio. (C) Effect of the ionic strength of urine samples on the A_T, A_C, and A_T/A_C ratio. (D) Effect of the pH value in urine samples on the A_T, A_C, and A_T/A_C ratio. The letter a in Figure 1C and Figure 1D indicates that the means are not significantly different (one-way ANOVA, P>0.05). The letter b in figure 1D indicates that the means are significantly different (one-way ANOVA P<0.01).