Analytical Methods

Electronic Supplementary Information

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

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1. Materials and methods

M135 and M180 NC membrane from Millipore (Bedford, MA, USA), CN140 NC membrane from Sartorius (Goettingen, Germany), PuraBind R AE99 NC membrane from Whatman (Maidstone, Kent, England) and BioTrace NT NC membrane from PALL (Saint Germain-en-Laye, France) were used.

1.1 Optimal concentrations of anti-PEAA mAb and coating antigen

Several parameters were optimized to develop the sensitive LFIA method. Firstly, the concentrations of anti-PEAA mAb labeled by colloidal gold and PEAA-OVA conjugate were determined using a checkerboard assay similar to the competitive ELISA. Briefly, 1 mL of anti-PEAA mAb at the serial concentrations of 0.25, .0.5, 1.0 and 2.0 mg mL^{-1} were labeled by colloidal gold, respectively, and then dispensed on the conjugated pad. The PEAA-OVA conjugate at the serial concentrations of 0.15, 0.3, 0.6 and 1.2 mg mL^{-1} were spotted onto the membrane to form the test lines, respectively. The performance of test lines was investigated with a BioDot TSR3000 Membrane Strip Reader by using the blank and spiked swine urine samples with PEAA at 1.0 ng mL^{-1}. All measurements were repeated 6 times. The relative optical density (ROD) of the blank, spiked swine urine samples (ROD_0 and ROD_1) and the inhibition ratio (IR) of 1.0 ng mL^{-1} were calculated.

$$IR\ (\%) = (1- ROD_1/ ROD_0) \times 100\%$$

The better combinations of anti-PEAA mAb and and PEAA-OVA conjugate were chosen to characterized by IC_{50} value with the PEAA standard concentration range of 0.033-8.1 ng mL^{-1} using strip reader.

1.2 Selection the optimum membrane

M135 and M180 NC membrane from Millipore (Bedford, MA, USA), CN140 NC membrane from Sartorius (Goettingen, Germany), PuraBind R AE99 NC membrane from Whatman (Maidstone, Kent, England) and BioTrace NT NC membrane from PALL (Saint Germain-en-Laye, France) were used. The measurements were also repeated 6 times and the inhibition ratios (IR) of 1.0 ng mL^{-1} were calculated.

1.3 Selection of key regeants

For the LFIA method, the stable, blocking and surface active agents, such as sucrose, BSA and Tween 20, respectively, were key reagents. The optimal concentrations of these reagents were determined by using the blank and spiked swine urine samples with PEAA at 1.0 ng mL^{-1}. The measurements were also repeated 6 times and the inhibition ratios (IR) of 1.0 ng mL^{-1} were calculated.

2. Results and discussion

2.1 Optimal concentrations of anti-PEAA mAb and coating antigen

As the primary factors, the optimum concentrations of anti-PEAA mAb labeled by colloidal gold and PEAA-OVA conjugate were used to improve the sensitivity of the LFIA. According to the checkerboard titration, the optimum concentrations were those that resulted in the maximum IR value and the lowest antibody and coating antigen concentrations. The 4 better concentration combinations of anti-PEAA mAb and PEAA-OVA were 1.0 and 0.6 mg ml^{-1}, 1.0 and 0.3 mg ml^{-1}, 1.0 and 1.2 mg ml^{-1}, 0.5 and 0.6 mg ml^{-1}(Table S1). These better combinations were characterized by IC_{50} value with the PEAA standard concentration range of 0.033-8.1 ng mL^{-1} using strip

reader. The results were shown in Fig S1, the IC_{50} values were calculated to be 0.55 ± 0.15, 1.21 ± 0.13, 1.15 ± 0.11 and 1.23 ± 0.22 ng mL^{-1}. Therefore, the optimal concentrations of anti-PEAA mAb and PEAA-OVA were selected to be 1.0 mg ml^{-1} and 0.6 mg ml^{-1}.

Table S1 Optimal concentrations of anti-PEAA mAb and PEAA-OVA

PEAA-OVA (mg mL^{-1})	**anti-PEAA mAb (mg mL^{-1})**					
	0.25			**0.50**		
	ROD_0	**ROD_1**	**IR (%)**	**ROD_0**	**ROD_1**	**IR (%)**
0.15	100±12	59 ± 9	41.00± 9.00	215±17	134 ± 11	37.67± 5.12
0.30	93±6	57 ±11	38.71± 11.11	551±33	278 ± 23	49.55± 4.17
0.60	242±15	128 ±11	47.11± 4.55	**666±23**	**294 ± 23**	**55.86± 3.45**
1.20	410±21	227 ±17	44.63± 4.15	1295±56	625 ± 34	51.74± 2.63

PEAA-OVA (mg mL^{-1})	**anti-PEAA mAb (mg mL^{-1})**					
	1.00			**2.00**		
	ROD_0	**ROD_1**	**IR (%)**	**ROD_0**	**ROD_1**	**IR (%)**
0.15	276±11	169 ± 9	38.77± 3.26	377±25	206 ± 9	45.36± 2.39
0.30	**607±28**	**252 ± 15**	**58.48± 2.47**	909±21	522 ± 25	42.57± 2.75
0.60	**721±26**	**259 ± 29**	**64.08± 4.02**	1301±62	797± 83	38.74± 6.38
1.20	**1415±74**	**695 ± 59**	**50.89± 4.17**	1506±67	914 ± 76	39.31± 5.05

Note: ROD_0: ROD value of blank swine urine sample; ROD_1: ROD value of spiked urine sample (1 ng mL^{-1} PEAA); IR: inhibition ratio of 1 ng mL^{-1}, IR (%) =(1-ROD1/ROD0)%.

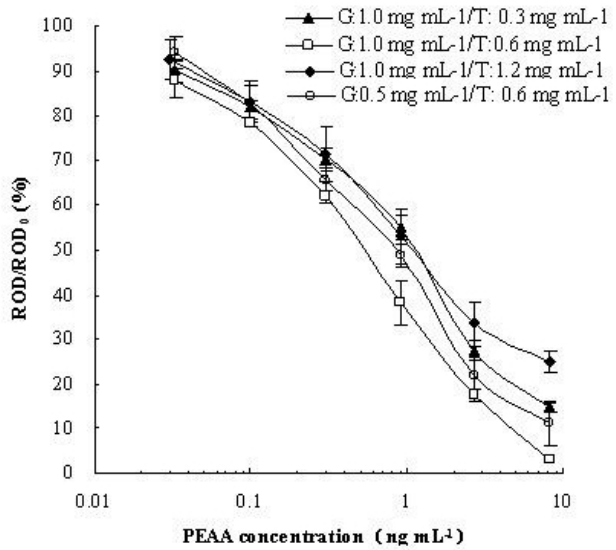

Fig. S1 Standard curves for concentration combinations of anti-PEAA mAb and PEAA-OVA.

2.2 Optimal operating conditions of LFIA

For the LFIA method, NC membrane was one of the important carrier materials. In this previously study, 5 kinds of NC membrane were chosen to compare, and the results were shown in Fig. S2 A. The LFIA strip used M180 NC membrane achieved the maximal sensitivity and the minimal background interference. Therefore, Millipore M180 NC membrane was selected in the follow-up experiments.

In addition, the different concentrations of stable, blocking and surface active key agents, such as sucrose (1.25, 2.5, 5.0 10.0%), BSA (1.25, 2.5, 5.0 10.0%) and Tween 20 (0.15, 0.3, 0.6 1.2%), respectively, were investigated, and the results were shown in Fig. S2 B - D. 5% sucrose, 5% BSA and 0.3% Tween 20 were selected for the LFIA in this study.

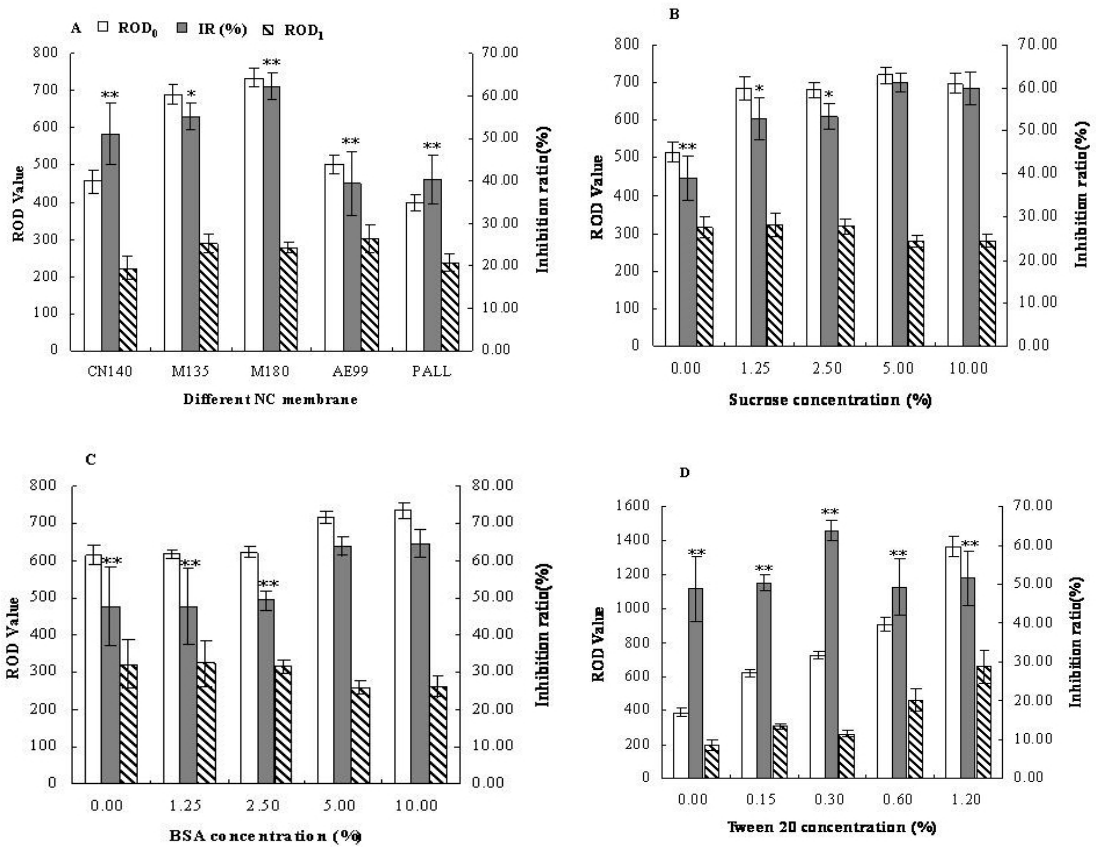

Fig. S2 Suitable operating conditions of PEAA LFIA method: (A) selection of NC membrane, (B) the optimum concentration of sucrose, (C) the optimum concentration of BSA, (D) the optimum concentration of tween 20.