Supplementary materials

Simultaneous detection of phenacetin and paracetamol by gold nanoparticle-based immunochromatographic test strip

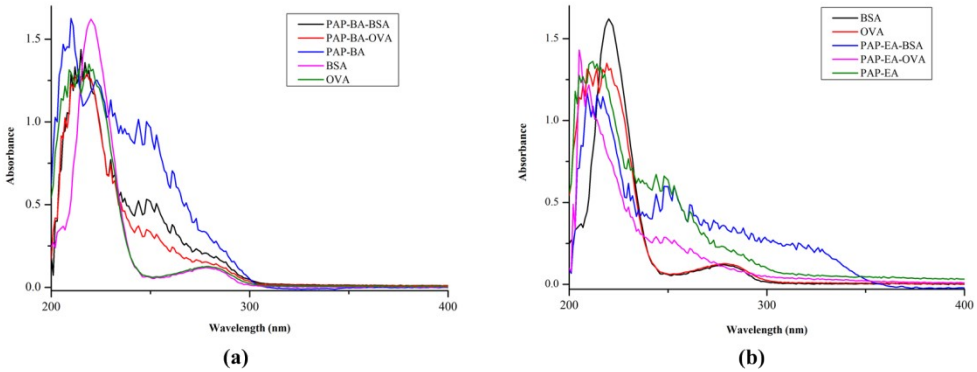


Figure S1. The UV-Vis spectrogram of fipronil antigens. (a) The UV-Vis spectrogram of PAP-BA-BSA and PAP-BA-ovalbumin; (b) The UV-Vis spectrogram of PAP-EA-BSA and PAP-EA-ovalbumin.

2. The antisera of immunized mice detected by *ic*ELISA.

Table S1. The antisera of immunized mice detected by *ic*ELISA.

Coating antigen	IC_{50} (ng mL^{-1})	
	Immunized by PAP-BA-BSA	Immunized by PAP-EA-BSA
PAP-BA-ovalbumin	151.6	252.1
PAP-EA-ovalbumin	56.7	567.3

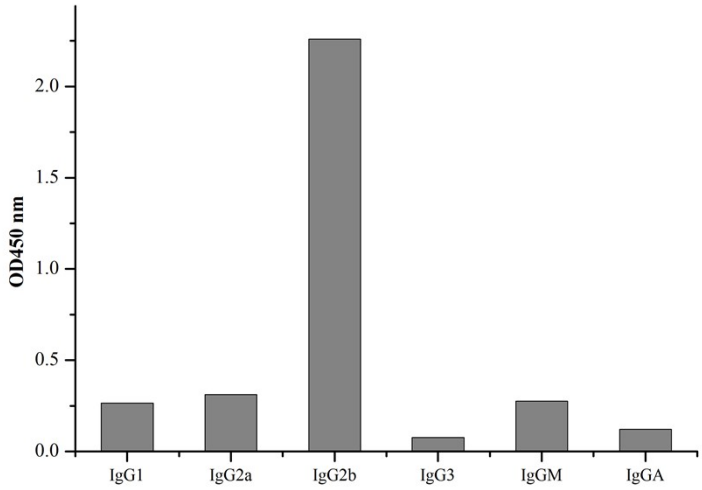


Figure S2. The subtype of anti-PNCT mAb detected by ELISA.

Table S2. Titer of purified anti-PNCTmAb detected by ELISA.

	Dilution of purified annti-PNCT mAb									
OD_{450}	4.0×10^3	8.0×10^3	1.6×10^4	3.2×10^4	6.4×10^4	1.3×10^5	2.6×10^5	5.1×10^5	1.0×10^6	Blank
	2.72	2.53	2.22	2.02	1.77	1.43	0.76	0.53	0.36	0.032

As shown in Table S1, the titer of purified anti-PNCT mAb was up to 1.02×10^6.

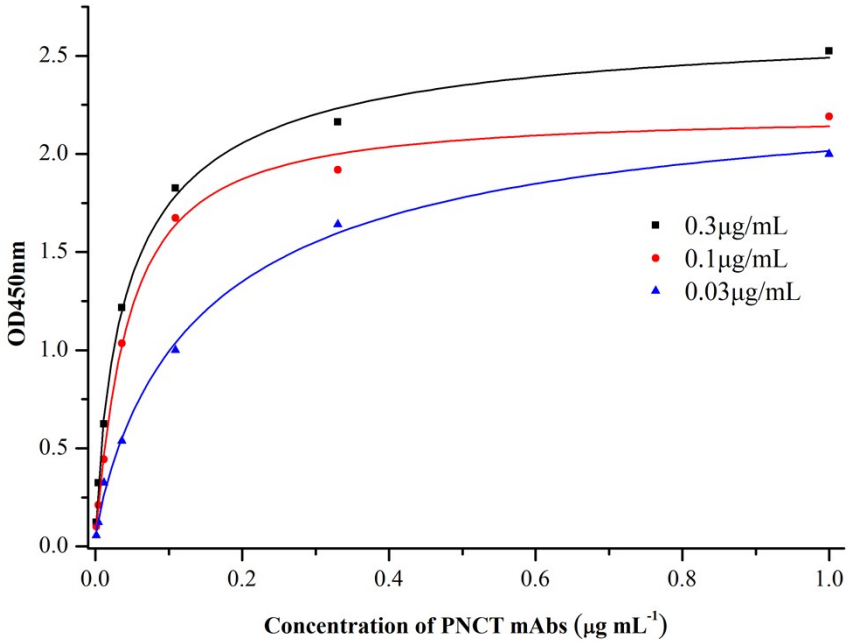


Figure S3. Affinity curve for anti-PNCT mAb.
The average affinity constant was calculated to be 5.32×10^8 L mol^{-1}.

Table S3. The cross-reactivity of anti-PNCT mAb with PAP determined by *ic*ELISA.

Competitive analogues	IC_{50} (ng mL^{-1})	CR (%)
PNCT	3.51	100
PAP	34.75	10.1

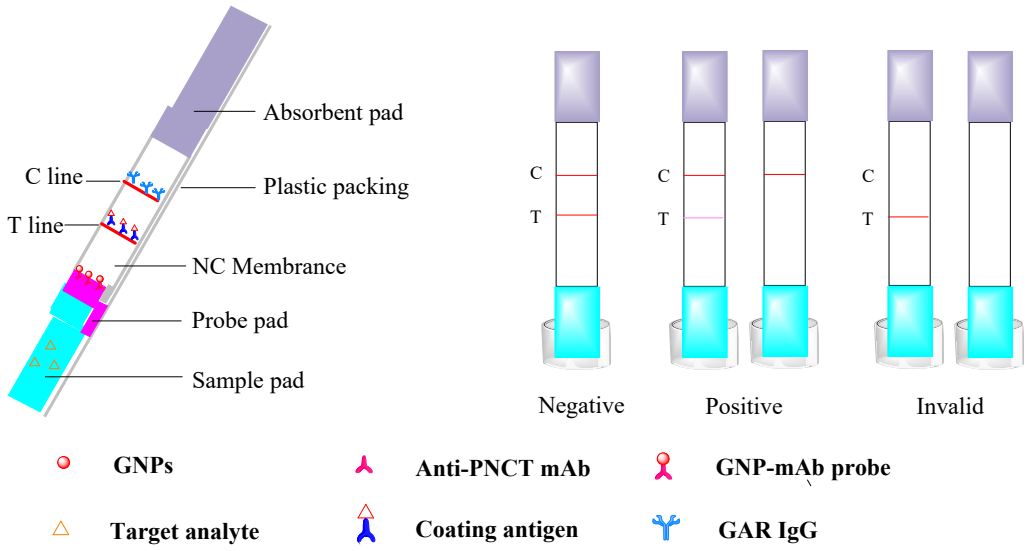


Figure S4. Schematic diagrams and the test principle of the LFI strip.

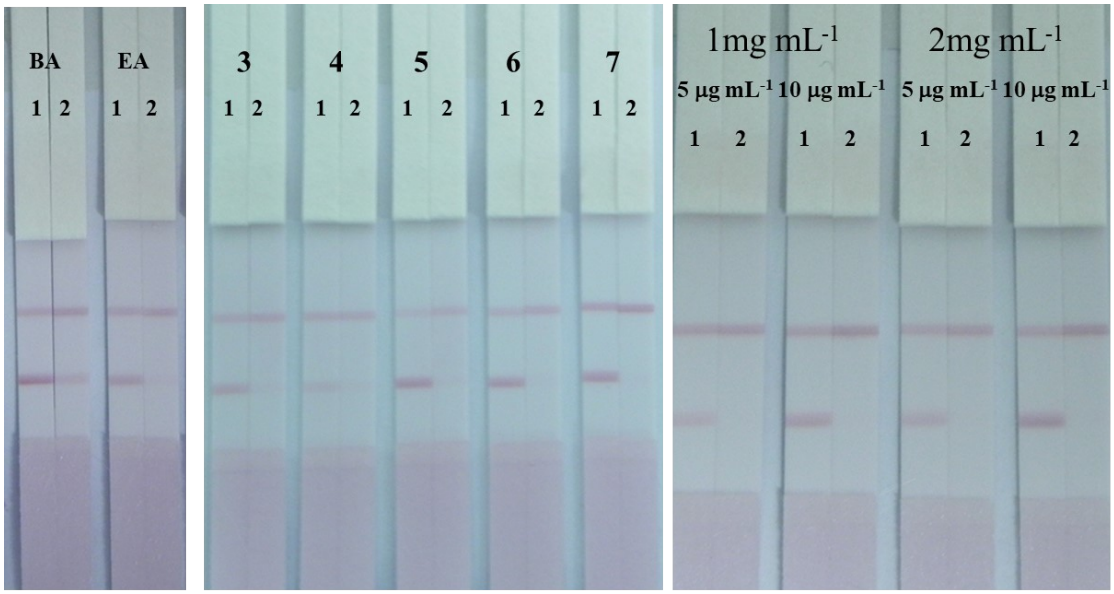


Figure S5. The optimization of LFI strip. (a) Optimization of two coating antigen: BA, EA represent the coating antigen PAP-BA-ovalbumin and PAP-BA-ovalbumin; (b) Optimization of the GNPs-mAb probe resuspension. 3= 0.02 M PBS containing 5% trehalose, 1% BSA and 0.05% NaN_3, 4= BSA, 5= Tween-20, 6= triton X-100, and 7= ON-870. 1, 2 represent the PNCT concentration at 0 and 50 ng mL^{-1}, respectively; (c) Optimization of the concentration of coating antigen in PBS at 1.0 and 2.0 mg mL^{-1}, and optimization of the concentration of the GNPs-mAb at 5.0 and 10 μg mL^{-1}.

Table S4. Chromatogram condition for the analysis of PNCT and PAP by HPLC.

Instrument conditions	HPLC Waters 1525EF system		
Spectrum transmission electron microscope Column	a reversed-phase C18 column (Agilent Zorbax Eclipse Plus) (4.6 ×150 mm, 5 μm)		
	Column temperature: 25 ℃		
Mobile Phase	A: acetonitrile, 30%		
	B: water, 70%		
Detection wavelength	250 nm		
Gradient Profile	Time (min)	Percentage A (%)	Percentage B (%)
	0	30	70
	10	30	70
Injection Volume	10 μL		
Flow velocity	1.0 mL min^{-1}		

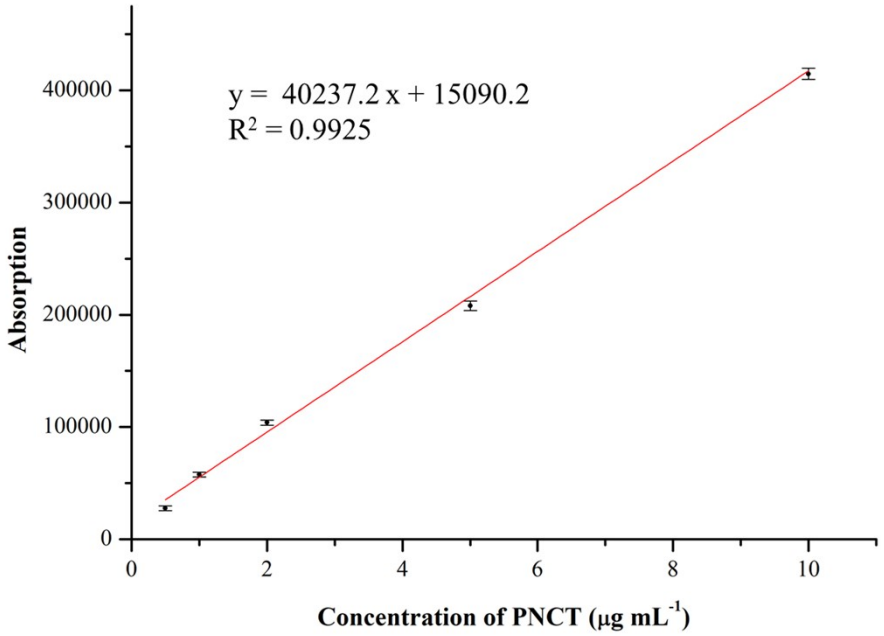


Figure S6. The regression equation of PNCT in acetonitrile by HPLC.

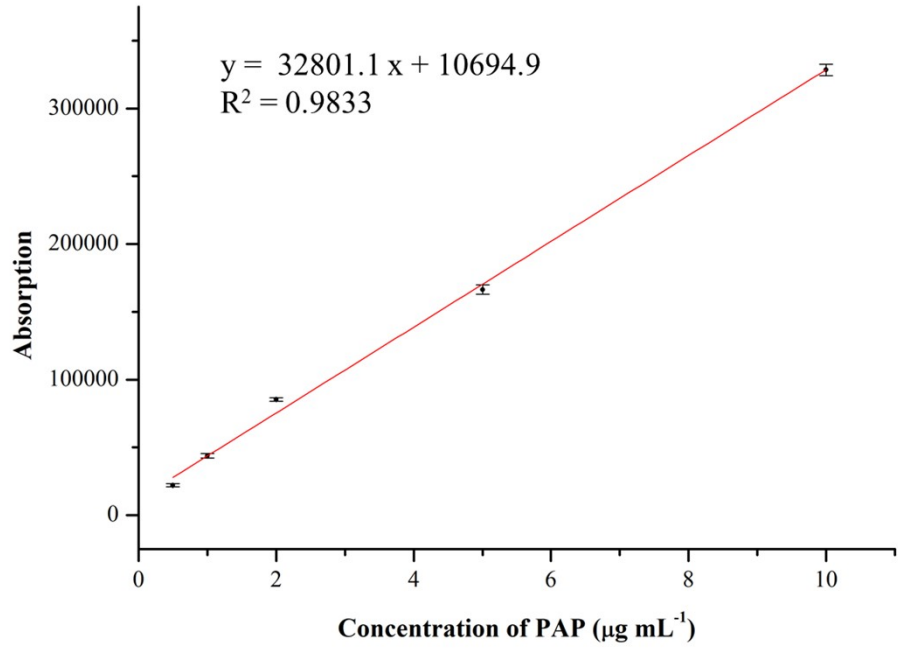
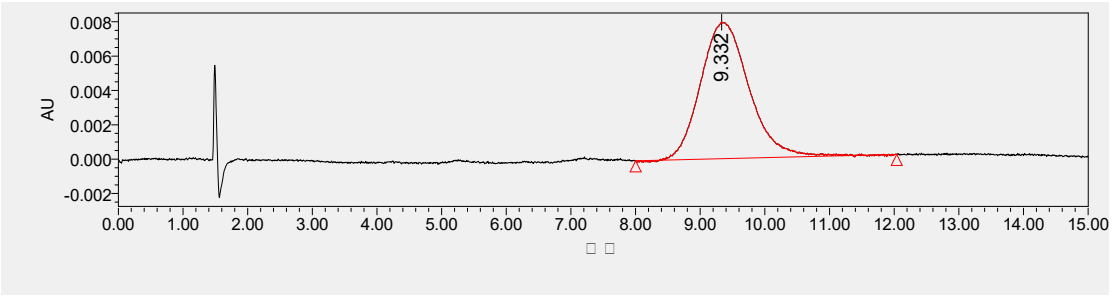


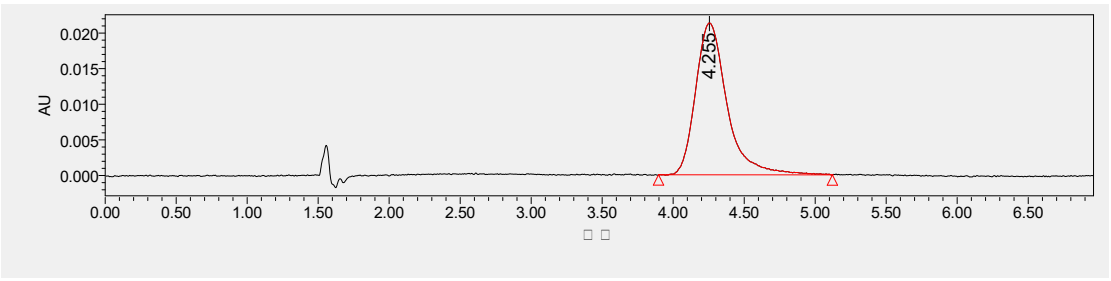
Figure S7. The regression equation of PAP in acetonitrile by HPLC.

1. PNCT standard



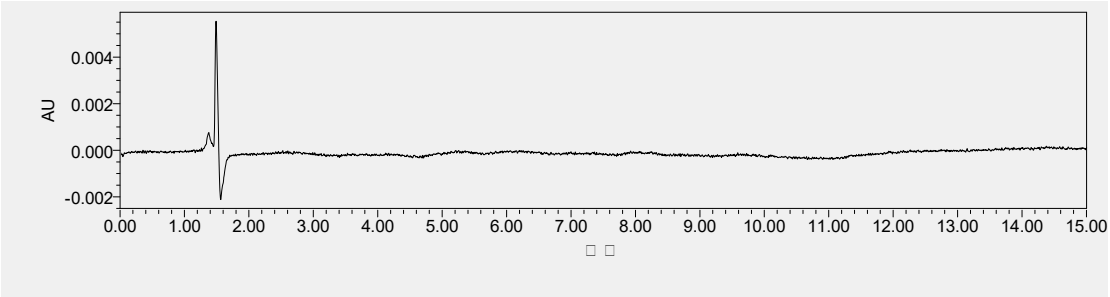
Nane	Retention time (min)	Area	% Area
PNCT standard	9.332	414761	100.00

2. PAP standard

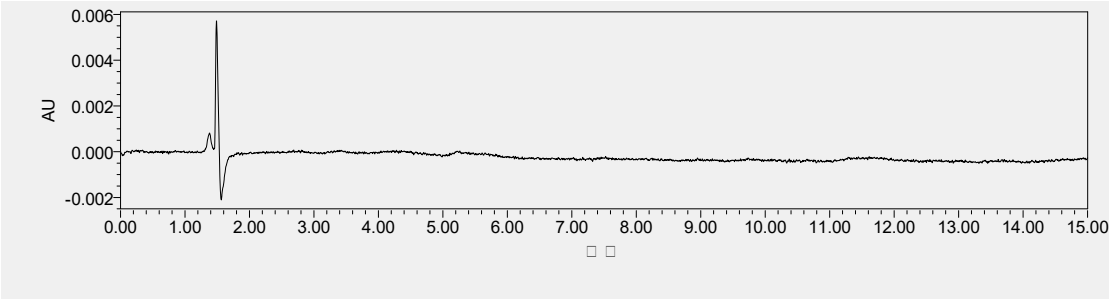


	Nane	Retention time (min)	Area	% Area
1	PAP standard	4.255	3280408	100.00

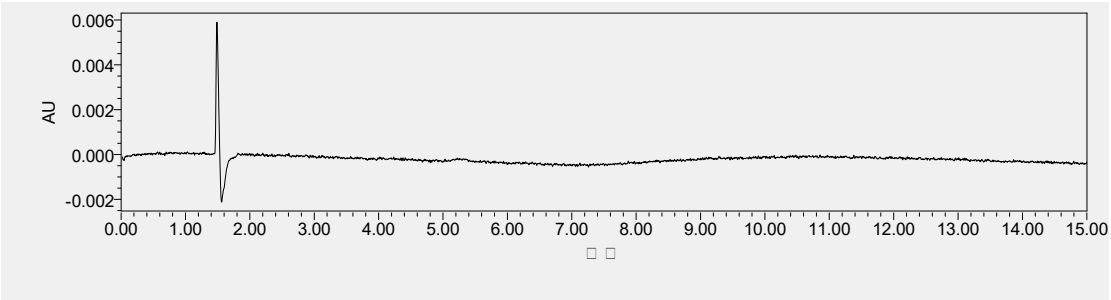
3. Soda water



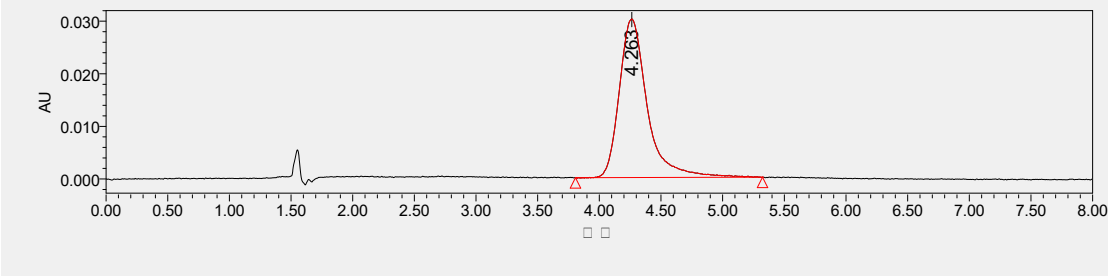
4. Carbonate beverage



5. Tea beverage

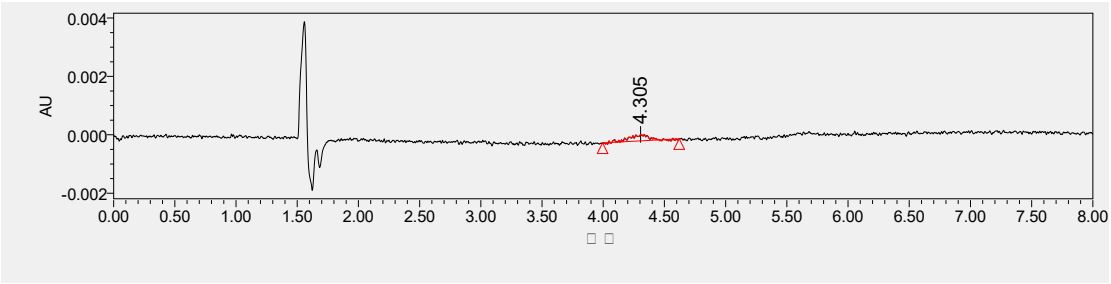


6. PAP-drug-10 ppm



	Nane	Retention time (min)	Area	% Area
1	PAP-drug	4.263	323287	100.00

7. PAP-drug-2



	Nane	Retention time (min)	Area	% Area
1	PAP-drug-2	4.305	3035	100.00

Figure S8. HPLC chromatograms of beverages samples and PAP-containing drug sample. 1-5 stand for the HPLC chromatograms of PNCT standard at 10 μg mL^{-1}, PNCT standard at 10 μg mL^{-1}, soda water, carbonate beverage, and tea beverage, respectively; 6, 7 stand for the HPLC chromatograms of PAP-containing drug sample extract at 10 μg mL^{-1} and 100 ng mL^{-1} in MeOH, respectively.