Electronic Supplementary Material (ESI) for RSC Advances.

Supplemental information for "A sensitivity-enhanced heterologous immunochromatographic assay based on monoclonal antibody for the rapid detection of histamine in saury samples"

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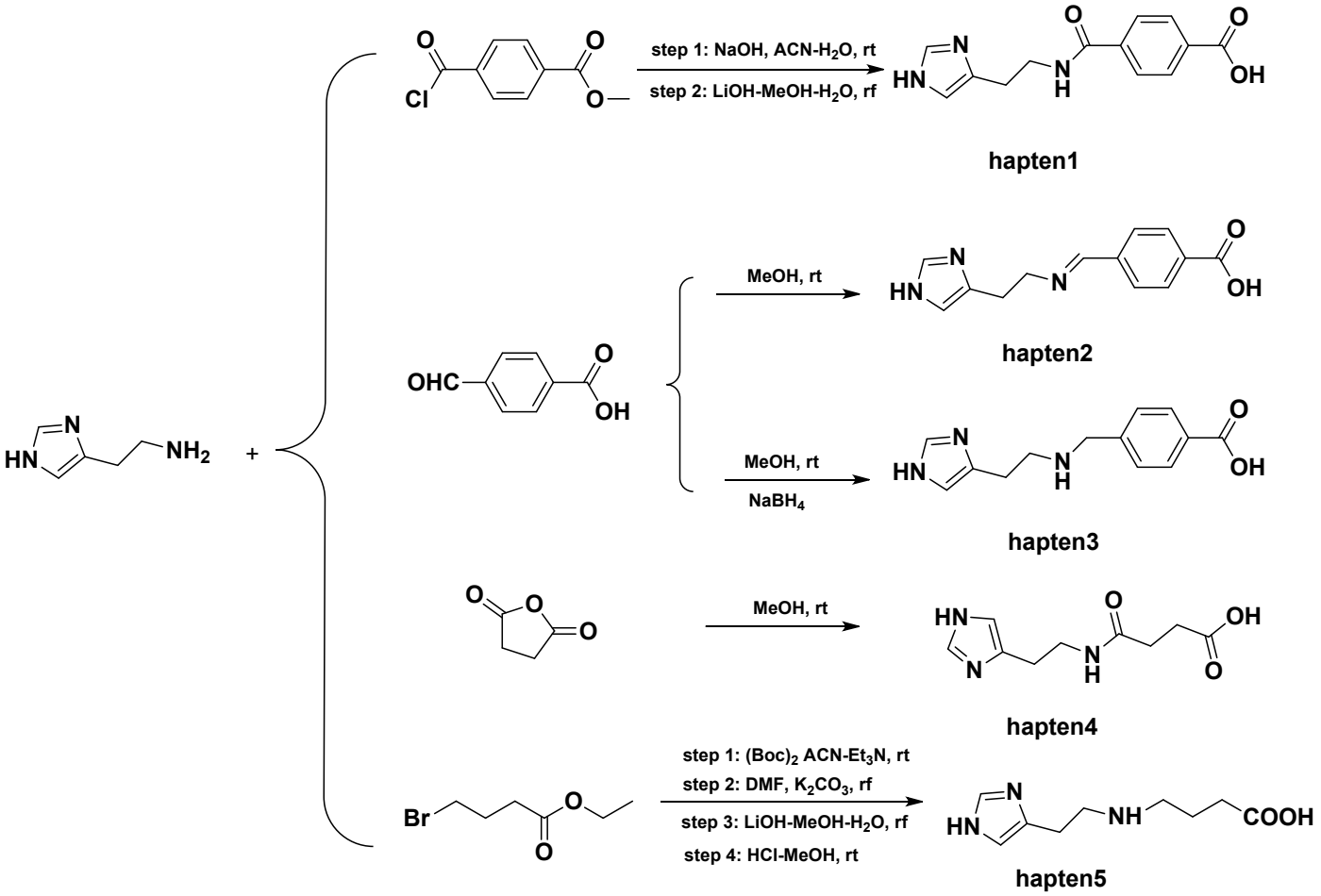

Fig. S1. Synthetic route for hapten1~5

Synthetic procedures:

hapten1: *4-((2-(1H-imidazol-4-yl)ethyl)carbamoyl)benzoic acid*. Hapten1 was synthesized according to previous work [18]. ESI analysis (negative ion) m/z 258 $[M–H]^–$; 1H NMR (400 MHz, Methanol-d4) δ 8.54 (d, J = 1.4 Hz, 1H), 8.12–8.05 (m, 2H), 7.90–7.82 (m, 2H), 7.27 (d, J = 1.3 Hz, 1H), 3.72 (t, J = 6.8 Hz, 2H), 3.04 (t, J = 6.8 Hz, 2H).

hapten2: *(E)-4-(((2-(1H-imidazol-4-yl)ethyl)imino)methyl)benzoicacid.* Histamine dihydrochloride (1.84 g, 10 mmol) and 4-formylbenzoic acid (1.5 g, 10 mmol) were dissolved in methanol, the resulting mixture was stirred for 1 h at room temperature. Then, the white precipitate was filtered off and washed with cold methanol, dried to yield hapten2 (19.6 g, 80%). ESI analysis (negative ion) m/z 244 $[M-H]^–$; ^{1}H NMR (600 MHz, DMSO-*d6*) 8.37 (s, 1H), 7.98 (d, J=8.1, 2H), 7.80 (d, J=8.2, 2H), 7.51 (s, 1H), 6.76 (s, 1H), 3.83 (t, J=7.1, 2H), 2.85 (t, J=7.3, 2H).

hapten3: *4-(((2-(1H-imidazol-4-yl)ethyl)amino)methyl)benzoic acid*. Histamine dihydrochloride (1.84 g, 10 mmol) and sodium methoxide (1.08 g, 20 mmol) were dissolved in methanol, then 4-formylbenzoic acid (1.5 g, 10 mmol) was added to the resulting mixture under stirring. After 1 h, $NaBH_4$ (0.37 g, 10 mmol) was added to the mixture, and the reaction proceeded another 0.5 h. Then the solvent was removed under rotoevaporation, the residue was purified on a silica gel column, using $CHCl_3$/MeOH/NH_4OH (10:5:1) as the eluent, and then crystallized from cold ethanol (10 mL) to afford hapten3 (1.68 g, 69%). ESI analysis (positive ion) m/z 246 $[M+H]^+$; ^{1}H NMR (600 MHz, D_2O) δ = 8.48 (d, J=1.2, 1H), 7.72 (d, J=8.1, 2H), 7.36 (d, J=8.2, 2H), 7.22 (s, 1H), 4.20 (s, 2H), 3.32 (t, J=7.6, 2H), 3.08 (t, J=7.6, 2H).

hapten4: *4-((2-(1H-imidazol-4-yl)ethyl)amino)-4-oxobutanoic acid*. Hapten4 was synthesized according to previous work [18]. ESI analysis (negative ion) m/z 210 $[M–H]^–$; 1H NMR (600

MHz, D2O) δ 8.54 (s, 1H), 7.26 (s, 1H), 3.45 (t, J = 6.3 Hz, 2H), 2.89 (t, J = 6.4 Hz, 2H), 2.44–2.35 (m, 4H).

hapten5: *4-((2-(1H-imidazol-4-yl)ethyl)amino)butanoic acid*. Hapten5 was synthesized according to previous work [18]. ESI analysis (negative ion) m/z 196 $[M–H]^-$; 1H NMR (600 MHz, Methanol-d4) δ 8.97 (s, 1H), 7.61 (s, 1H), 4.30–4.26 (m, 2H), 3.30–3.28 (m, 1H), 3.14 (t, J = 7.5 Hz, 2H), 2.45 (t, J = 7.2 Hz, 2H), 2.21–2.18 (m, 2H).

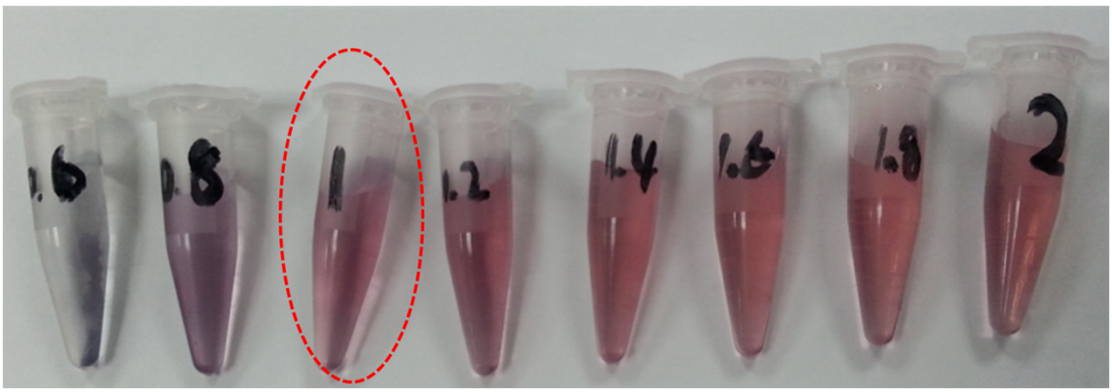

Fig.S2. Results of screening the minimum mAb amount needed for conjugating with GNPs. From left to right, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8, 2.0 μL anti-NPHA mAb were added to 500 μL of GNPs. The minimum amount mAb which can make GNPs solution free from color change to blue was considered as the minimum mAb amount needed for labeling.

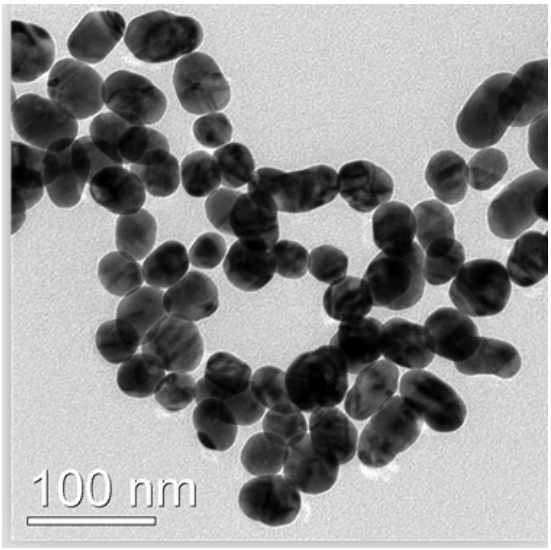

Fig. S3. TEM image of the prepared colloidal gold nanoparticles in this study

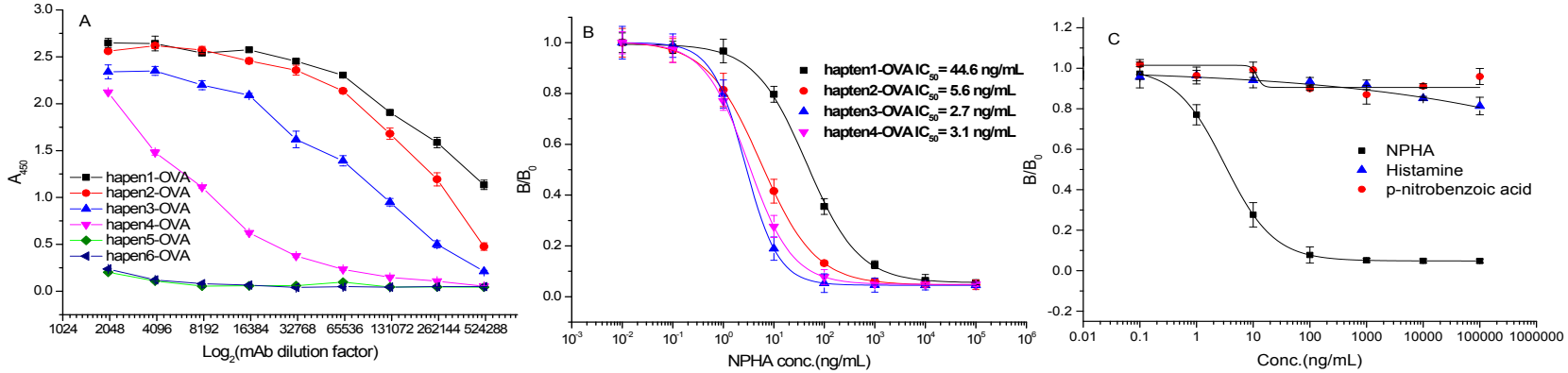

Fig. S4. (A).anti-NPHA mAb titer curve against each of the four coating antigens (n=3). Each coating antigens was coated at 0.5 μg mL^{-1}. Ordinate represents the absorbance value at 450 nm of wells containing serial diluted mAb (1:2000, 1:4000, 1:8000, 1:16000, 1:32000, 1:64000, 1:128000, 1:256000). (B). Dose-dependent competitive indirect ELISA curves for NPHA against each of the four effective coating antigens. B_0 is the A_{450} of the well containing no NPHA; B is the A_{450} of the well containing certain amounts of NPHA. (C) The specificity of anti-NPHA mAb was tested by ic-ELISA under hapten4-OVA coating using NPHA, histamine and p-nitrobenzoic acid as competitor.

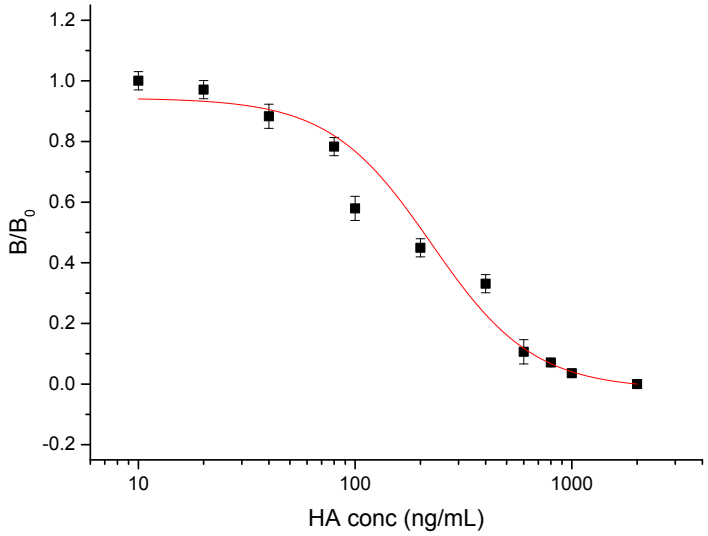

Fig. S5. Calibration curve for HA using ICA strip. A_T and A_C were color intensity of T line and C line recorded by strip reader, respectively. The A_T/A_C ratio of negative and positive samples were designated B_0 and B_x. The calibration curve constructed by plotting the B_x/B_0 ratio against the logarithm of HA concentration.

Table S1 Effects of Tween-20 contained in working solution on the colloidal gold immunochromatographic assay of HA

Tween-20 content (%) in 0.01M PBS, pH 7.4		NPHA standard concentration (ng mL^{-1})					
		0	20	50	100	200	400
0	T line	+++	++	+±	+	±	–
	C line	+++	+++	+++	+++	+++	+++
0.01	T line	+++	++±	++	+±	+	±
	C line	+++	+++	+++	+++	+++	+++
0.05	T line	+++	+++	++±	++	+±	+
	C line	+++	+++	+++	+++	+++	+++
0.1	T line	+++	+++	+++	++±	++	+±
	C line	+++	+++	+++	+++	+++	+++

+++: Red line appeared.

++±: Red line appeared but was weaker than +++.

++: Red line appeared but was weaker than ++±.

+±: Red line appeared but was weaker than++.

+: Red line appeared but was weaker than +±.

±: Red line appeared but was weaker than +.

–: Red line did not appear.

Table S2 Optimization of PO_4^{3-} concentrations of working solution for the colloidal gold immunochromatographic assay of HA

PO_4^{3-} concentrations (M) of PBS pH 7.4		NPHA standard concentration (ng mL^{-1})					
		0	20	50	100	200	400
0.01	T line	+++	++	+±	+	±	–
	C line	+++	+++	+++	+++	+++	+++
0.02	T line	+++	++	+±	±	–	–
	C line	+++	+++	+++	+++	+++	+++
0.05	T line	+++	++	±	–	–	–
	C line	+++	+++	+++	+++	+++	+++
0.1	T line	++±	++	+	±	–	–
	C line	+++	+++	+++	+++	+++	+++

+++: Red line appeared.

++±: Red line appeared but was weaker than +++.

++: Red line appeared but was weaker than ++±.

+±: Red line appeared but was weaker than++.

+: Red line appeared but was weaker than +±.

±: Red line appeared but was weaker than +.

–: Red line did not appear.

Table S3 Optimizaiton of pH values of working solution for the colloidal gold immunochromatographic assay of HA

pH values of 0.05 MPBS pH 7.4		NPHA standard concentration (ng mL^{-1})					
		0	20	50	100	200	400
6.2	T line	+++	++	+±	+	±	–
	C line	+++	+++	+++	+++	+++	+++
7.4	T line	+++	++	±	–	–	–
	C line	+++	+++	+++	+++	+++	+++
8.2	T line	++±	++	±	–	–	–
	C line	+++	+++	+++	+++	+++	+++
9.0	T line	++	+±	±	–	–	–
	C line	+++	+++	+++	+++	+++	+++
10.0	T line	+±	+	–	–	–	–
	C line	+++	+++	+++	+++	+++	+++

+++: Red line appeared.

++±: Red line appeared but was weaker than +++.

++: Red line appeared but was weaker than ++±.

+±: Red line appeared but was weaker than++.

+: Red line appeared but was weaker than +±.

±: Red line appeared but was weaker than +.

–: Red line did not appear.

Table S4. Effcets of acetonitrile concentration in working solution on the colloidal gold immunochromatographic assay of HA

Acetonitrile concentration (%) in working solution		NPHA standard concentration (ng mL^{-1})					
		0	20	50	100	200	400
0	T line	+++	++	±	–	–	–
	C line	+++	+++	+++	+++	+++	+++
2	T line	+++	++	+±	±	–	–
	C line	+++	+++	+++	+++	+++	+++
5	T line	+++	++±	++	+	±	–
	C line	+++	+++	+++	+++	+++	+++
10	T line	++±	++±	++±	++	+±	+
	C line	+++	+++	+++	+++	+++	+++
20	T line	++	++	++	++	+±	+
	C line	+++	+++	+++	+++	+++	+++

+++: Red line appeared.

++±: Red line appeared but was weaker than +++.

++: Red line appeared but was weaker than ++±.

+±: Red line appeared but was weaker than++.

+: Red line appeared but was weaker than +±.

±: Red line appeared but was weaker than +.

–: Red line did not appear.