

## Supplementary Materials for

# Development of Nanobody-Based Flow-through Dot ELISA and Lateral-flow Immunoassay for Rapid Detection of 3- Phenoxybenzoic Acid

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## **Acquisition of ROC curve**

IBM SPSS statistic 23 software was used to design the ROC curve.

The negative and positive results (recorded as '0' and '1') and corresponding concentrations were counted by ROC analysis in the SPSS. The output results ('1-Specificity' and 'Sensitivity') were plotted on X- axis and Y- axis. Youden's index is a method to evaluate the authenticity of test. Youden index is the sum of sensitivity and 1- specificity. The larger the Youden's index, the better the screening effect and the greater the authenticity. We found out the maximum Youden's index of flow-through dot ELISA and lateral-flow immunoassay, the corresponding value was recorded as the cut-off point. The details are as follows:

For flow-through dot ELISA:

Specificity: 0.857

Sensitivity: 0.833

Cut-off point: 0.011

Area under Curve (AUC): 0.897

For lateral-flow immunoassay:

Specificity: 0.824

Sensitivity: 0.875

Cut-off point: 0.107

Area under Curve (AUC): 0.926

## Figure and Table Captions

**Figure S1.** The characterization of GNPs. A: TEM analysis; B: Particle size analysis.

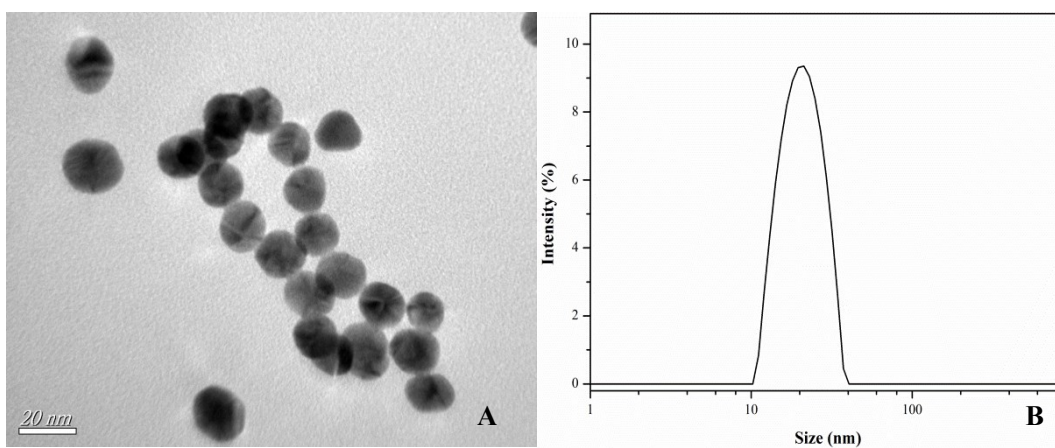
**Figure S2.** Matrix effect evaluated by plate ELISA, Nbs-based flow-through dot ELISA and GNPs-Nbs lateral-flow immunoassay (n=3). (a) inhibition curves by plate ELISA; (b) matrix effect conducted by Nbs-based flow-through dot ELISA and GNPs-Nbs lateral-flow immunoassay.

**Figure S3.** (a) The variation of gray scale of flow-through dot ELISA and GNPs-Nbs lateral-flow immunoassay; (b) Standard curves of flow-through dot ELISA and GNPs-Nbs lateral-flow immunoassay quantitatively analyzed by image software.

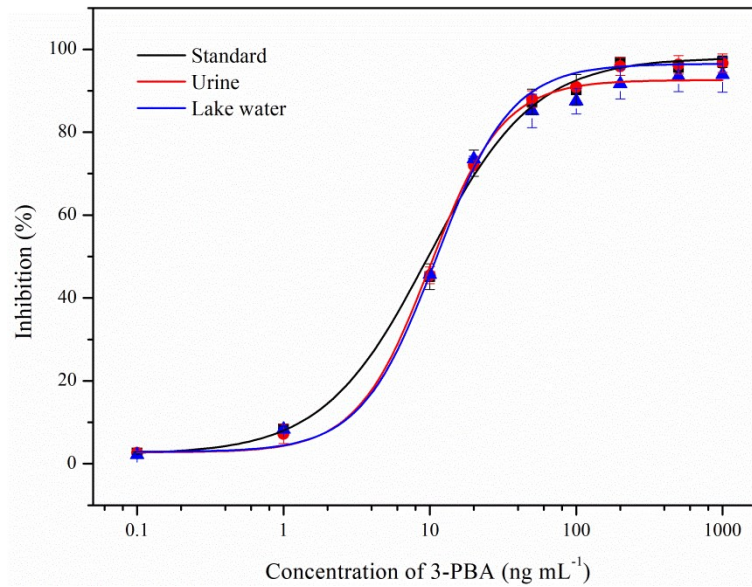
**Figure S4.** Correlation curves of flow-through dot ELISA ( $R^2=0.982$ ) and GNPs-Nbs lateral-flow immunoassay ( $R^2=0.973$ ) between LC-MS.

**Figure S5.** ROC curve of flow-through dot ELISA and lateral-flow immunoassay.

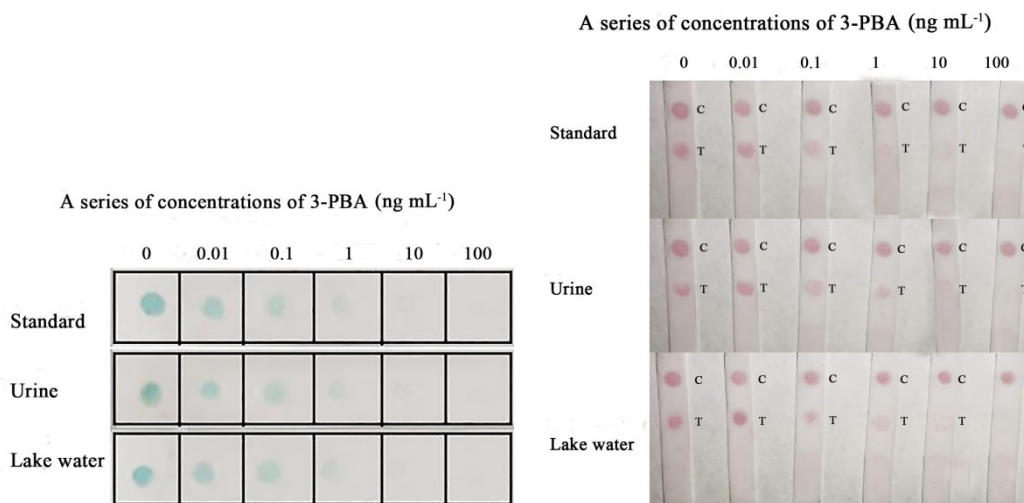
**Table S1.** Quantitatively analysis of the visual results



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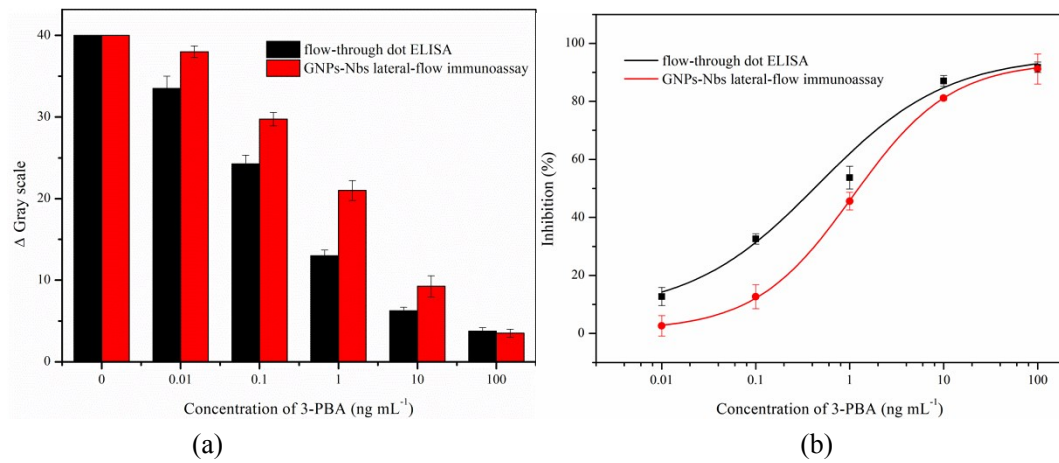


(a)

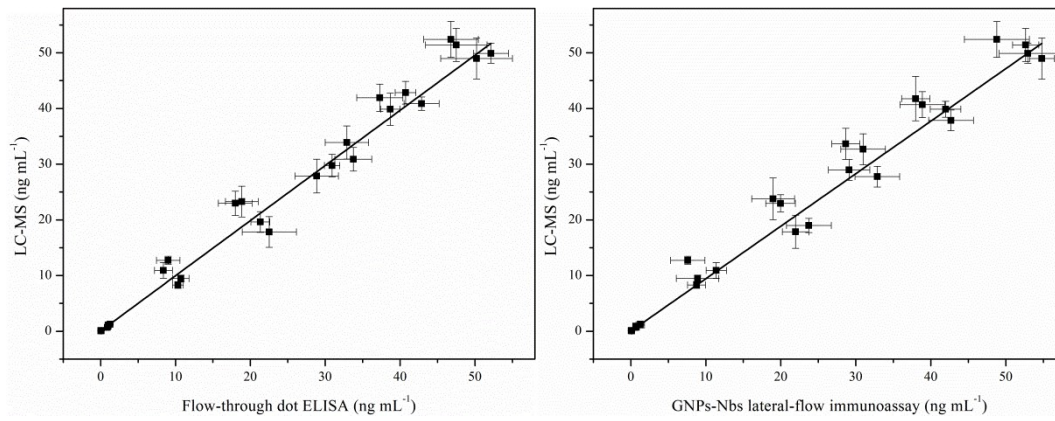


(b)

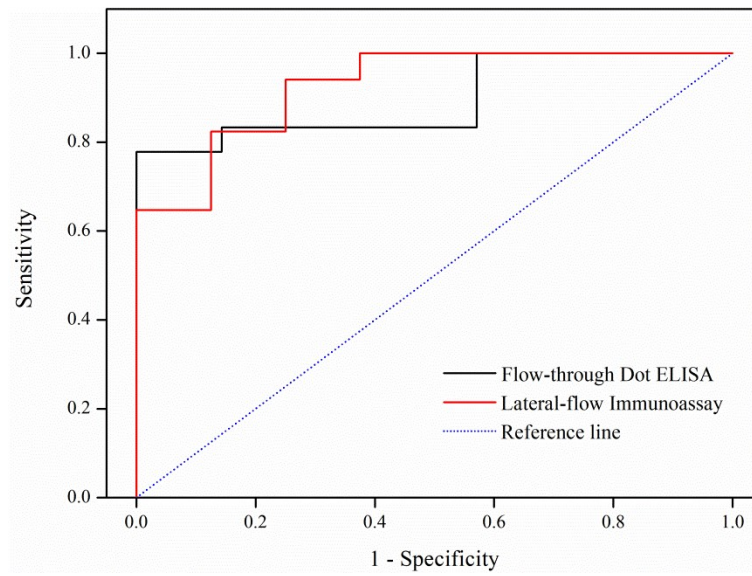
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**Figure S3.** (a) The variation of gray scale of flow-through dot ELISA and GNPs-Nbs lateral-flow immunoassay; (b) Standard curves of flow-through dot ELISA and GNPs-Nbs lateral-flow immunoassay quantitatively analyzed by image software.



**Figure S4.** Correlation curves of flow-through dot ELISA ( $R^2=0.982$ ) and GNPs-Nbs lateral-flow immunoassay ( $R^2=0.973$ ) between LC-MS ( $n=4$ ).



**Figure S5.** ROC curve of flow-through dot ELISA and lateral-flow immunoassay.



**Table S1.** Quantitatively analysis of the visual results (n=4).

Spiked (ng mL <sup>-1</sup> )	Quantitatively analysis by software		LC-MS (ng mL <sup>-1</sup> )
	Flow-through dot ELISA (ng mL <sup>-1</sup> )	GNPs-Nbs lateral-flow immunoassay (ng mL <sup>-1</sup> )	
0.1	0.084 ± 0.047	0.073 ± 0.031	0.097 ± 0.070
1	1.04 ± 0.13	0.99 ± 0.18	0.98 ± 0.045
10	9.6 ± 0.9	9.2 ± 1.4	10.3 ± 0.8
50	49.1 ± 2.1	52.3 ± 2.2	50.7 ± 2.6