Supporting information for:

Multiplex Lateral Flow Immunoassay for Mycotoxin Determination

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EXPERIMENTAL SECTION

Preparation of immunogens

For preparation of AFB1-COM-BSA, AFB1 (1 mg) was dissolved in 500 μL of pyridine, and then 0.34 mg carboxymethyloxime (CMO) was added. The mixture was incubated for 5 h at 70 °C before evaporated to dryness by vacuum drying. Then the pellet was redissolved in 300 μL of DMSO/H_2O (3:1, v/v) and 6.7 mg EDC was added into the mixture. The solution was stirred at room temperature for 2 h. The prepared AFB1-CMO was added to BSA solution (5 mg BSA dissolved in 1 mL of PBS buffer, pH=7.4), and the mixture was stirred overnight at room temperature. After centrifugation (4500g, 10 min), the supernatant was dialyzed by PBS (0.5 M，pH=7.4) at 4 °C.

For preparation of ZEA-COM-BSA, 1.5918 mg ZEA was dissolved in 300 μL of pyridine, then 1.1 mg CMO was added, and the mixture was incubated for 5 h at 70 °C. The solution was then evaporated to dryness and the pellet was redissolved in 300 μL of DMF. Then the mixture was stirred overnight at 4 °C after added 1.2 mg NHS and 4.2 mg DCC. Finally, 150 μL of the prepared CMO-ZEN was added to BSA solution (33.5 mg BSA dissolved in 0.9 mL of CBS buffer, pH=9.6), and the mixture was stirred 2 h at room temperature and overnight at 4 °C for further reaction. After centrifugation (4500g, 10min), the supernatant was dialyzed by PBS (0.5 M，pH=7.4) at 4 °C.

For preparation of DON-HS-BSA, l-butaneboronic acid (30 mg) and 10 mg of DON were dissolved in 0.5 mL of pyridine. The mixture was reacted overnight at room temperature. Then 0.5 mL of 1.7 M succinic anhydride was added into the solution and the mixture was stirred for 3 h in a boiling water bath to yielding 3-O-hemisuccinyl-7, 15-O-(butylborony1)-DON. The solution was evaporated to dryness. The residue was then dissolved in 2 mL DMF, into which 10 mg NHS and 20 mg DCC were added. The mixture was slowly added into BSA solution (90 mg BSA in 6 mL PBS) and then stirred overnight at 4 °C for further reaction. After centrifugation (4500g, 10min), the supernatant was dialyzed by PBS (0.5 M，pH=7.4) at 4 °C.

Preparation of Colloidal Gold Nanoparticles

Colloidal gold nanoparticles (CG) with a mean diameter of 25 nm were prepared as following procedures. 2 mL of chlorauric acid solution (1%, w/v) was added to 200

mL of distilled water and brought to a boiling point with vigorous stirring. Then, 5 mL of freshly prepared 1% sodium citrate was added with constant stirring. After the color changed to wine-red, the solution continued to be boiled for another 7~10 min; the solution was cooled to room temperature, and 200 mL deionized water was added. The solution was stored at 4 °C until use.

Preparation of Ab-CG Conjugates

The CG solution was first adjusted to pH 8.0 with 1% potassium carbonate. To 10 mL of CG, 0.1 mg of anti-AFB1 was added, with stirring for 40 min at room temperature. The free colloidal gold was blocked by adding 1 mL of BSA solution (5%, w/v) for 30 min at room temperature. After centrifuged (10,000 rpm, 4 °C) for 30 min, the supernatant was discarded and the pellet was washed twice with PBS buffer (pH 7.4, containing 0.1 % BSA and 0.05 % sodium azide) and finally resuspended by 1 mL of the same buffer for use. Furthermore the anti-DON-CG and anti-ZEA-CG conjugates were prepared with the same procedure of anti-AFB1-CG conjugates.

Selection of NC membrane

For the selection of NC membrane of LFA, several factors, such as the CI of test line, spreading speed of samples and the interference of the background, should be taken into account. In our study, different from Whatman-AE99 and Sartorius CN140, PALL vivid 170 showed better performances for its best CI developed in the test line and lowest CI on the background. In addition, LFA with PALL vivid 170 gave a better spreading speed for samples, which would influence the stability of antigen-antibody compounds.

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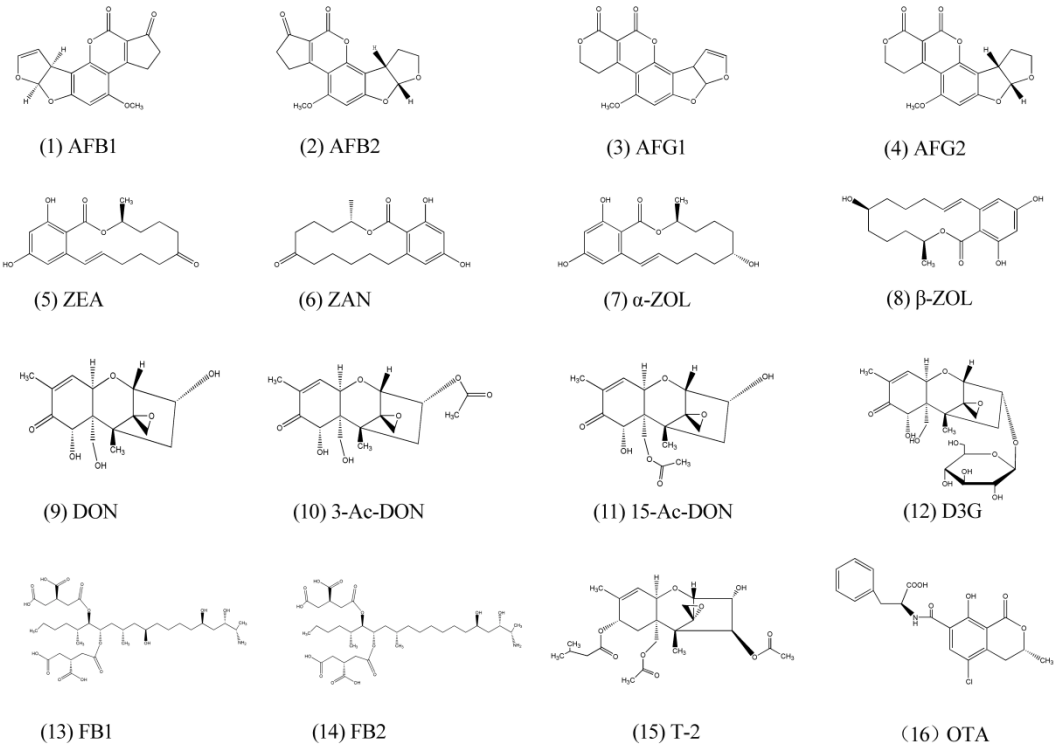

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Table S-1

Optimized MS/MS parameters for AFB1, DON and ZEA analysis in negative and positive modes.

Compound	Mode	Precursor ion	Product ion (m/z)	Q1 Pre Bias(V)	Collision energy (eV)	Q2 Pre Bias(V)
AFB1	ESI^+	313 $[M+H]^+$	285.05*	-25	-25	-20
			241.05	-25	-40	-16
ZEA	ESI^-	317 $[M-H]^-$	175.00*	22	25	8
			273.00	22	25	10
DON	ESI^-	295 $[M-H]^-$	265.25*	20	10	10
			138.15	20	15	10

Table S-2
The influence of different methods for sample preparation. (n=4)

		Method A (μg/kg)	Method B (μg/kg)	MLs for maize (μg/kg)
AFB1	IC_{20}	2	0.3	2
	IC_{50}	9	2	-
	IC_{80}	40	6	-
ZEA	IC_{20}	49	9	350
	IC_{50}	125	38	-
	IC_{80}	363	186	-
DON	IC_{20}	154	27	1750
	IC_{50}	500	80	-
	IC_{80}	>1750	596	-

Method A, the sample was diluted 3.5 times with PBS and loaded onto the LFA strips.
Method B, the sample was first dried and then dissolved with equal volume of PBS.

Table S-3

Calibration curve parameters of three mycotoxins in maize and wheat. (n=4)

Matrix	Analytes	Equation	R^2	cLOD (μg/kg)	IC_{50}	work range	Cutoff	vLOD (μg/kg)
Maize	AFB1	y = -18.66Ln(x) + 67.10	0.98	0.3	2	0.3-15	20	0.5
	ZEA	y = -19.34Ln(x) + 120.98	0.99	9	38	9-186	250	10
	DON	y = -24.74Ln(x) + 159.73	0.98	19	80	27-597	800	40
Wheat	AFB1	y = -18.83Ln(x) + 71.59	0.98	0.4	3	0.4-16	20	0.5
	ZEA	y = -18.80Ln(x) + 112.58	0.98	7	26	7-131	200	10
	DON	y = -19.60Ln(x) + 140.37	0.97	21	110	21-439	600	40