Supporting information

Colloidal gold immunochromatographic assay for mycophenolic acid in human plasma

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Contents

The specific process of subtype determination

The procedure was performed according to the previously reported method[1, 2], as follows: First, the coating antigen MPA-OVA required was serially diluted in a gradient manner starting from an initial concentration of 1 μg/mL, using 0.05 M/L CB (pH 9.6) as the diluent to ensure uniform and accurate dilution. Subsequently, 100 μL of the diluted coating antigen solution was evenly added to each well of a 96-well ELISA plate. After sample addition, the plate was incubated at 37 °C in a constant-temperature oven for 2 h to allow the coating antigen to be fully adsorbed and immobilized on the inner walls of the wells.

The liquid in the ELISA plate was then completely discarded by flicking the plate. Next, 300 μL of plate washing solution (0.01 M PBS containing 0.05% (v/v) Tween-20) was added to each well. The plate was shaken on a horizontal shaker to thoroughly wash away the unbound coating antigen residues on the well walls. After shaking, the washing solution was discarded by flicking, and this washing-flicking step was repeated three times. Finally, the ELISA plate was inverted and gently patted dry on clean absorbent paper to ensure no residual liquid remained in the wells.

Subsequently, 200 μL of blocking solution (0.05% CB, pH 9.6, containing 2% gelatin) was added to each well. The plate was again incubated at 37 °C in a constant-temperature oven for 2 h. The gelatin acted as a blocking agent to occupy the non-specific binding sites on the well walls that were not bound by the coating antigen, thereby avoiding non-specific adsorption of irrelevant substances in subsequent reactions and improving the specificity of the assay.

Antiserum detection was then carried out. First, the mouse antiserum was serially diluted in a gradient manner using a dedicated antibody diluent. Meanwhile, the target analyte standard was diluted correspondingly with 0.01 M PBS (K^+). For the ELISA plate that had completed the blocking step, the aforementioned plate washing and patting-drying procedure was repeated to remove residual blocking solution from the wells. Then, 50 μL of target-free blank buffer (negative control) or target analyte standard solution, along with 50 μL of diluted antiserum solution, were sequentially added to each well. After sample addition, the plate was incubated at 37 °C for 0.5 h.

Next, the goat anti-mouse IgG antibody was diluted at a ratio of 1:3000 (v/v) using the antibody diluent. For the ELISA plate that had finished antiserum incubation, the previous plate washing process was repeated. After patting dry, 100 μL of the diluted goat anti-mouse IgG antibody solution was added to each well, and the plate was then incubated at 37 °C for 0.5 h to form the coating antigen-primary antibody-secondary antibody immune complex.

Finally, after repeating the plate washing step three times, 100 μL of chromogenic mixture was added to each well. This mixture was freshly prepared at a volume ratio of 5:1 (chromogenic solution A: chromogenic solution B) and used immediately; the core component of the chromogenic solution was an ethylene glycol solution containing 0.06% (m/v) tetramethylbenzidine (TMB). After sample addition, the plate was incubated at 37 °C for 15 min to allow the enzyme-catalyzed chromogenic reaction of TMB, which produced a blue-colored product.

The chromogenic reaction was then terminated: after 15 min of reaction, 50 μL of

2 M sulfuric acid solution was directly added to each well, immediately stopping the chromogenic reaction and ensuring the stability of the detection results.

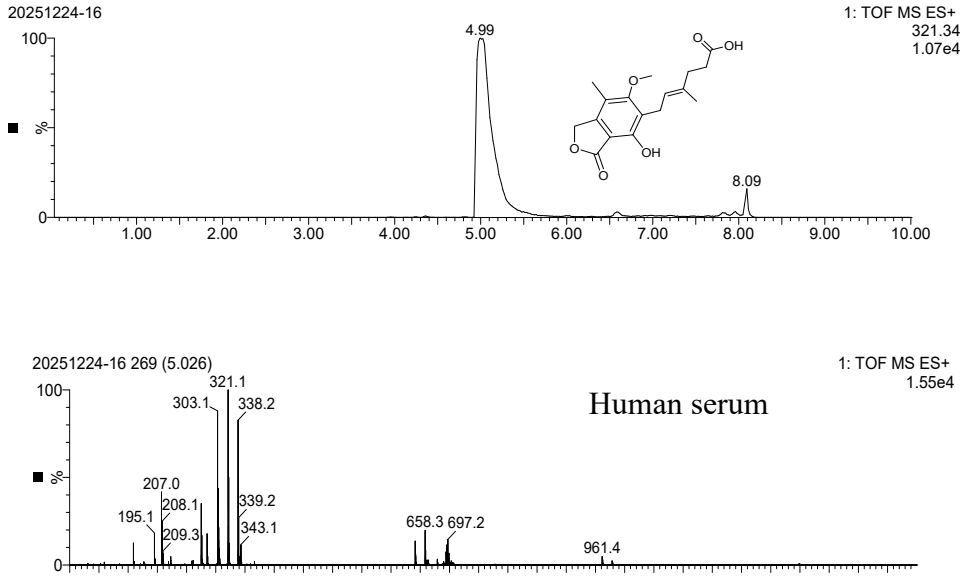


Figure S1. Identification of positive samples by LC-MS/MS.

Table S1. Evaluation of antibody titer and affinity of antisera from mice.

Number	Antibody titers[a]	Inhibition ratios[b](%)		IC_{50}[c](ng/mL)
		Second immunization	Third immunization	
1	27000	60.40	71.20	0.21
2	24000	53.10	59.50	0.38
3	18000	25.40	38.20	3.56
4	9000	8.90	12.40	8.19
5	6000	7.50	12.50	9.11
6	3000	6.10	7.80	-[d]
7	1000	2.20	5.10	-[d]
8	-[d]	-[d]	-[d]	-[d]
9	-[d]	-[d]	-[d]	-[d]
10	-[d]	-[d]	-[d]	-[d]

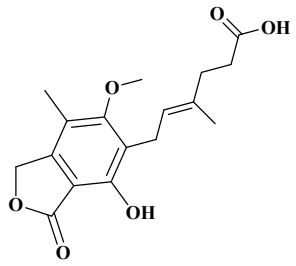
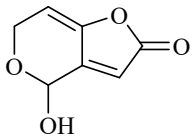
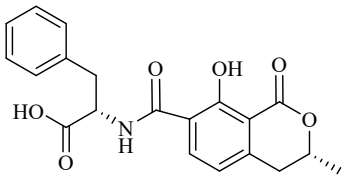
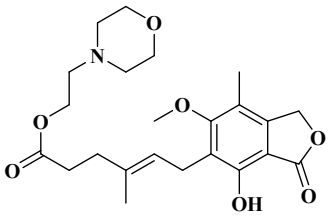
[a] The antibody titer was an average of eight mice and each mouse was obtained in triplicate.

[b] The concentration of MPA used in the ic-ELISA for the inhibition ratios determination was 1 ng/mL.

[c] The mouse with the lowest inhibition ratio was determined IC_{50}.

[d] No antibody titers or inhibitions were detected.

Table S2. Cross-reaction results of the anti-MPA mAb with structural analogues.

Chemicals	Structure	IC_{50} (ng/mL)	CR[a] (%)
Mycophenolic acid		0.22	100
Patulin		>50	<0.5
Ochratoxin B		>50	<0.5
Mycophenolate mofetil		>50	<0.5

[a] CR, cross-reactivity (%) of antibody was calculated as the ratio of IC_{50} of MPA/ IC_{50} of the tested compound expressed as a percentage.

Reference

1. X. L. Lei, L. G. Xu, S. S. Song, L. Q. Liu and H. Kuang, Development of an ultrasensitive ic-ELISA and immunochromatographic strip assay for the simultaneous detection of florfenicol and thiamphenicol in eggs, *Food and Agricultural Immunology*, 2018, 29, 254-266.
2. H. Li, S. Q. He, G. L. Liu, C. Li, Z. Q. Ma and X. Zhang, Residue and dissipation kinetics of toosendanin in cabbage, tobacco and soil using IC-ELISA detection, *Food Chemistry*, 2021, 335, 127600.