Supplementary information

A gold-based immunochromatographic strip for the specific detection of tacrolimus in whole blood

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Experimental section

LC-MS/MS analysis

LC-MS/MS analysis of tacrolimus was performed based on a past report with some modifications[1]. The chromatographic conditions were shown in Table S2. Positive electrospray ionization was chosen. The source temperature was 500 ℃, and the ion spray voltage was 5500 V. The curtain gas, ion source gas 1, and ion source gas 2 were at 40, 50, and 60 psi, respectively. Multiple reaction monitoring (MRM) was applied and the parameters were shown in Table S3. Subsequently, a calibration curve of LC-MS/MS was established by diluting a series of tacrolimus standards in acetonitrile, according to the above conditions.

Sample preparation for LC-MS/MS

Human whole blood samples were prepared as previously described with slight modifications[1]. Briefly, tacrolimus standard (10 μL) was fully mixed with a blank human whole blood sample (290 μL). Then, a mixed solution of 0.1 M zinc sulfate and

acetonitrile (600 μL, 1:4, v/v) was added to the whole blood sample to lyse the red cells and precipitate proteins. The solution was mixed thoroughly for 30 s, and then centrifuged (10,000 rpm, 10 min). The supernatant was collected for LC-MS/MS analysis.

Table S1. The antisera and coating antigens screening by ic-ELISA.

Coating antigen	Immunized by Tacrolimus-1-KLH		Immunized by Tacrolimus-2-KLH	
	Titer	IC_{50} (ng/mL)	Titer	IC_{50} (ng/mL)
Tacrolimus-1-BSA	1:8100	2.44	1:200	-
Tacrolimus-2-BSA	1:100	-	1:2700	20.31

Table S2. The Chromatographic conditions of LC-MS/MS analysis for tacrolimus detection.

Instrument conditions			
Chromatographic column	C18 ethylene-bridged column (2.1 × 50.0 mm, 2.5 μm XBridge, Waters Corporation, Milford, MA, USA)		
Column temperature	50℃		
Injection volume	2 μL		
Flow rate	0.3 mL/min		
Mobile phase A	20 mM ammonium formate in water		
Mobile phase B	acetonitrile		
Gradient elution	time	A (%)	B (%)
	0	95	5
	0.5	95	5
	4	5	95
	6	5	95
	6.1	95	5
	9	95	5

Table S3. The MS/MS parameters for tacrolimus detection.

	Parents (m/z)	Daughter (m/z)	Declustering Potential (V)	Entrance Potential (V)	Collision Energy (V)
Tacrolimus	821.5	768.5	80	10	29
	821.5	576.5	80	10	32

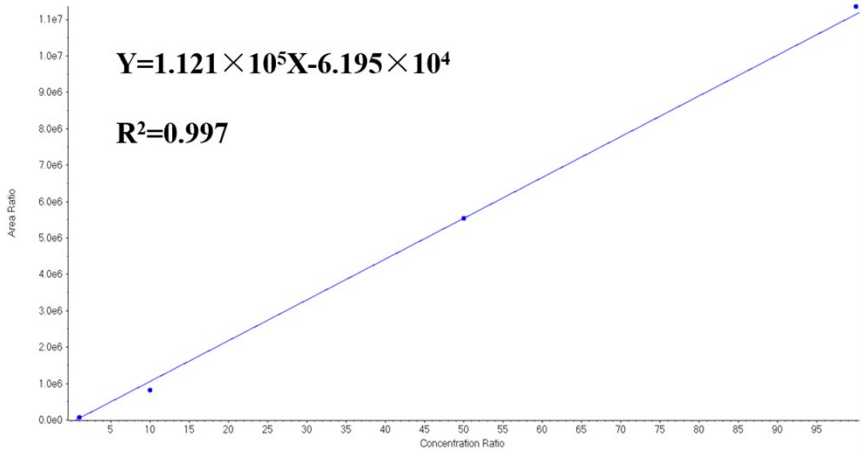

Fig.S1 The calibration curve of tacrolimus in acetonitrile by LC-MS/MS.

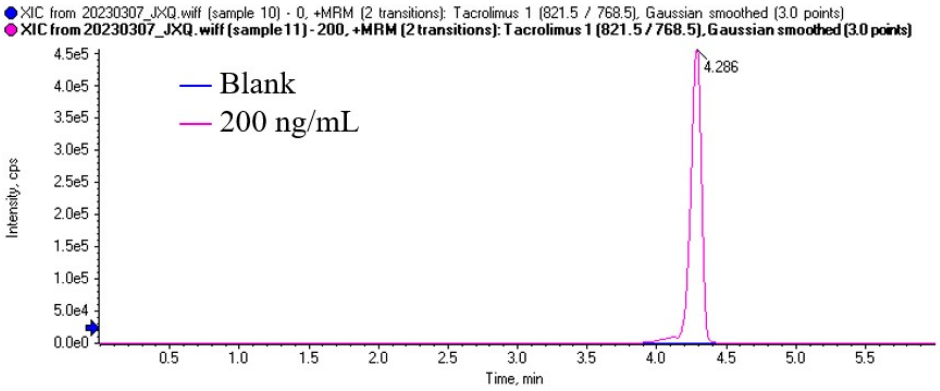

Fig.S2 LC-MS/MS chromatograms of 0 and 200.0 ng/mL tacrolimus spiked in human whole blood.

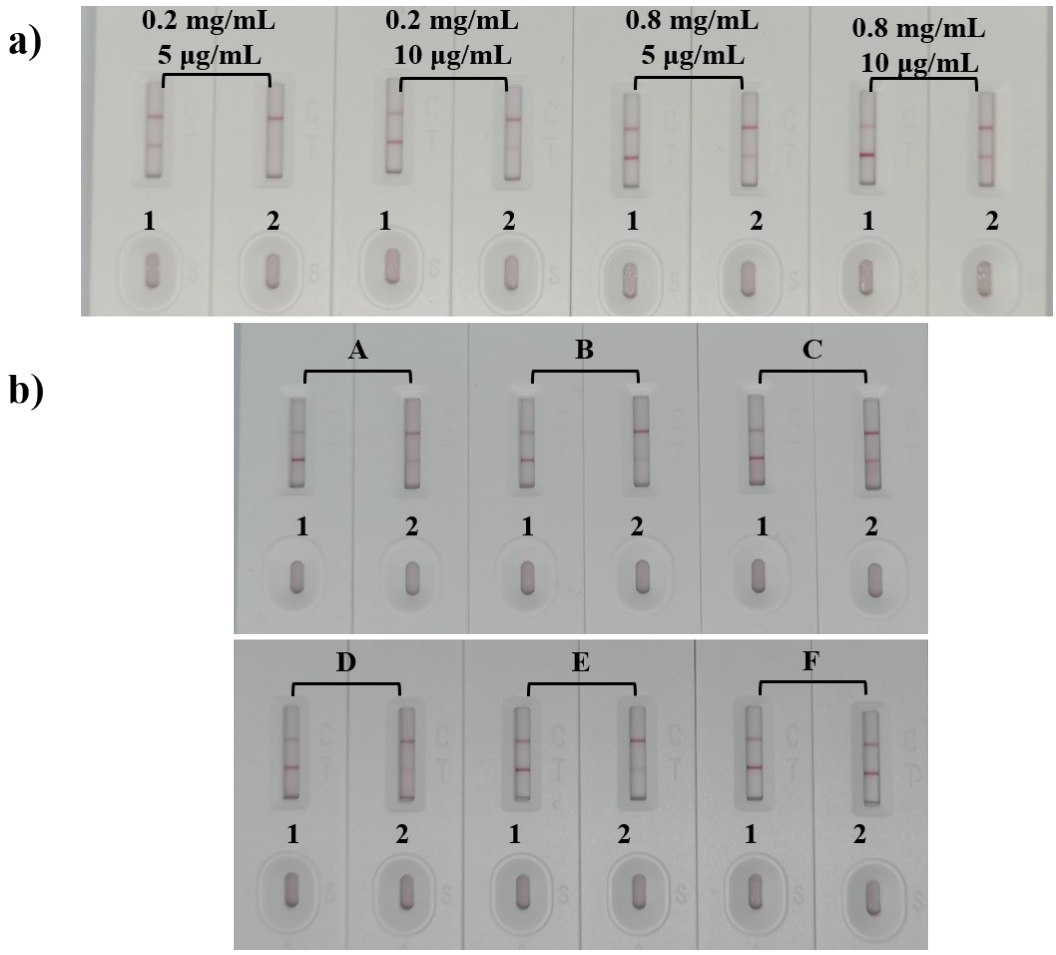

Fig. S3 Optimization of the CG-ICS in human whole blood samples: (a) the concentrations of tacrolimus-1-BSA (0.2 and 0.8 mg/mL) and GNP-labeled mAbs (5 and 10 μg/mL); (b) the resuspension buffer types. A = the basic buffer. B-F = the basic buffer with 5% PEG, 5% BSA, 5% PVP, 5% glucan and 5% On-870, respectively. 1 = 0 ng/mL, and 2 = 5.0 ng/mL.

References

(1) Krnac, D.; Reiffova, K.; Rolinski, B. A new HPLC-MS/MS method for simultaneous determination of Cyclosporine A, Tacrolimus, Sirolimus and Everolimus for routine therapeutic drug monitoring. *J. Chromatogr. B* **2019**, *1128*, 8, Article. DOI: 10.1016/j.jchromb.2019.121772.