Supplementary material

Quadruple-labeling luminescence strategy for multiplexed immunoassay of 51 drugs in milk with automated pretreatment system

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The preparation of immunomagenetic beads

The preparation of immunomagnetic beads was according to the manufacture's product specifications. Briefly, streptavidin magnetic beads were added to centrifuge tubes, 0.02 M PB was added to and made the concentration of streptavidin magnetic beads to be 1 mg·mL^{-1} (1 mL). The biotinylated mAb (30.0 μg) was putted into centrifuge tubes and incubated for 30 min at room temperature, the immunomagnetic beads were formed and separated from the solution by magnetic grate, and washed for 4 times with 0.01 M PBS (containing 0.1% BSA). The immunomagnetic beads were resuspended with 0.1 mL of 0.02 M PB and placed at 4 °C before use.

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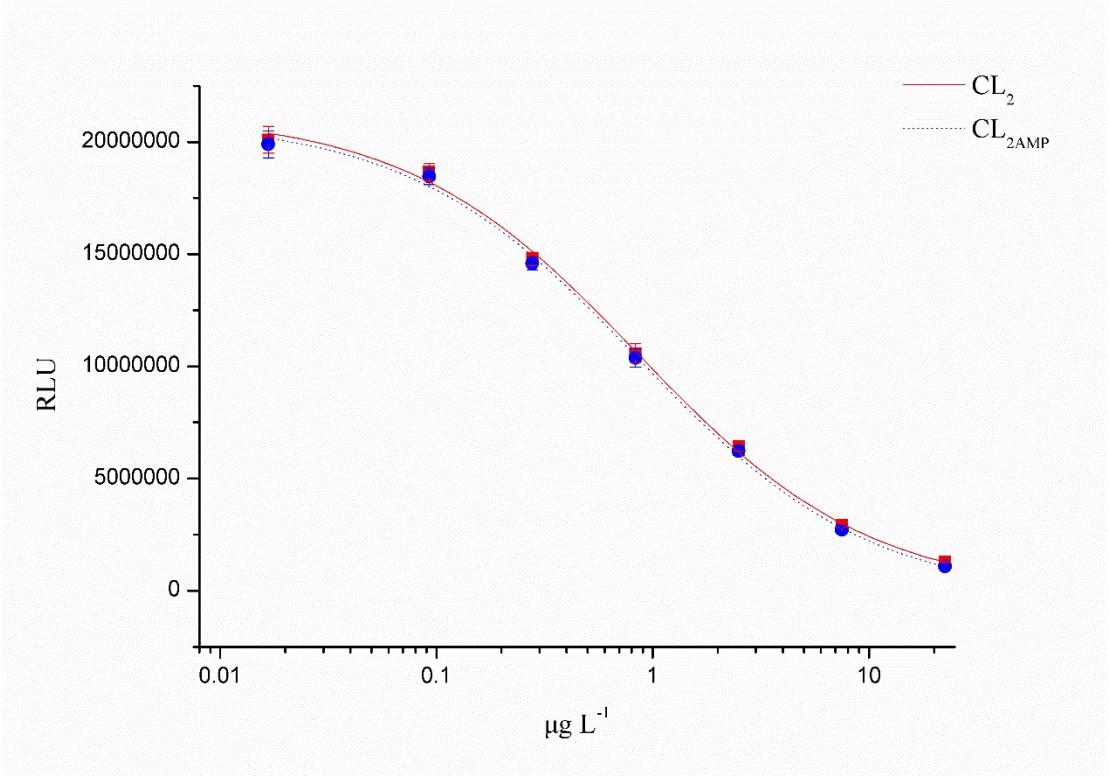
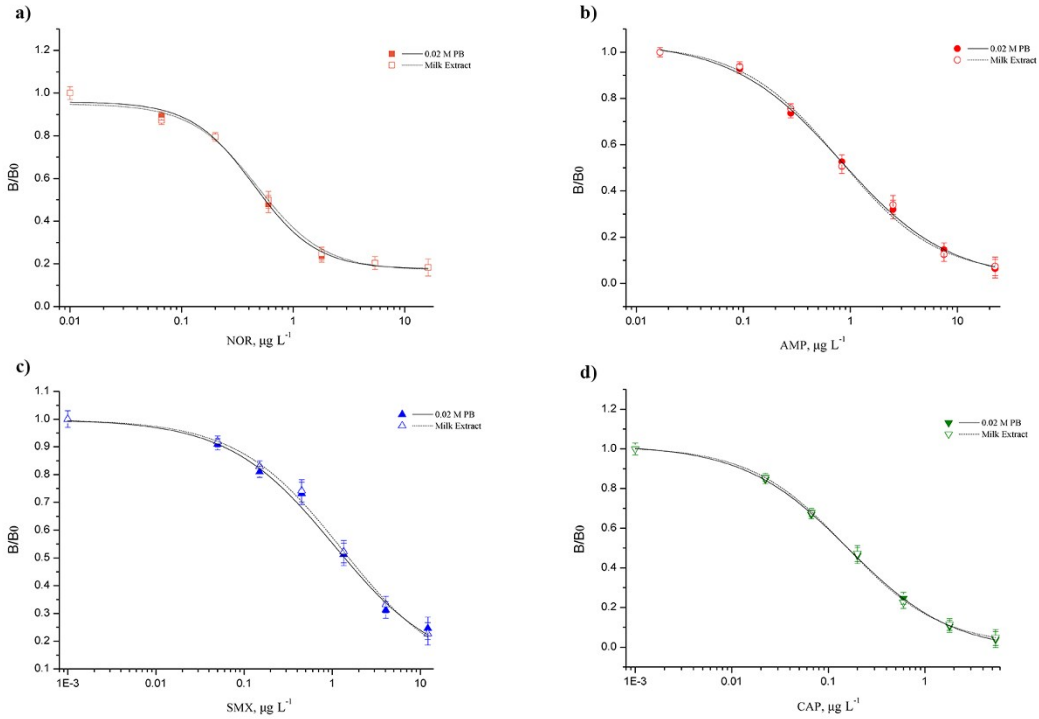


Supplementary Figure 1 Gross standard inhibition curves and revised standard inhibition curves in two detection steps



Supplementary Figure 2 Inhibition curves of NOR, AMP, SMX and CAP in buffer and milk extract

Supplementary Table 1 Limits of detection for FQs, β-lactams, SAs and CAP, and MRL (MRPL) set by EU, USA and China in milk

Compoud		LOD	MRL/MPL ($ng \cdot L^{-1}$)		
		$\mu g \cdot L^{-1}$	EU [20, 21]	USA[22]	China[3]
FQs	CIP, ENR, NOR, PEF	≤0.04	10.0-100.0	10.0-100.0	30.0-100.0
	DAN, LEV, OFL, AMI, ENO, ORB, FLER, SPA, MAR, LOM	<0.08			
	FLU, PAZ	<0.20			
	PRU, SAR, DIF	<1.60			
	TRO	<4.00			
β-lactams	PEN, AMP, AMO, NAF, CEF, CEQ	≤0.06	4.0-125.0	5.0-10.0	4.0-100.0
	DIC, OXA, CEM, CEZ, CEP, CET	<0.24			
	CLO, CER	<1.20			
	CEL	<6.00			100.0
SAs	SMX, SDM, SDT, SIM, SMD, SMM, SMP, SPY, SNT	≤0.06	25.0-100.0	10.0-100.0	25.0-100.0
	SCP, SMR, SMZ, acetyl-SMZ, SQX	<0.30			
	SDZ	<1.20			
CAP	CAP	0.006	—[a] (MRPL 0.3 $\mu g \cdot L^{-1}$)	—	—

[a] No level of chloramphenicol is acceptable