

Supporting information for

**A Robust Homogeneous Fluorescence Polarization Immunoassay for
Rapid Determination of Erythromycin in Milk**

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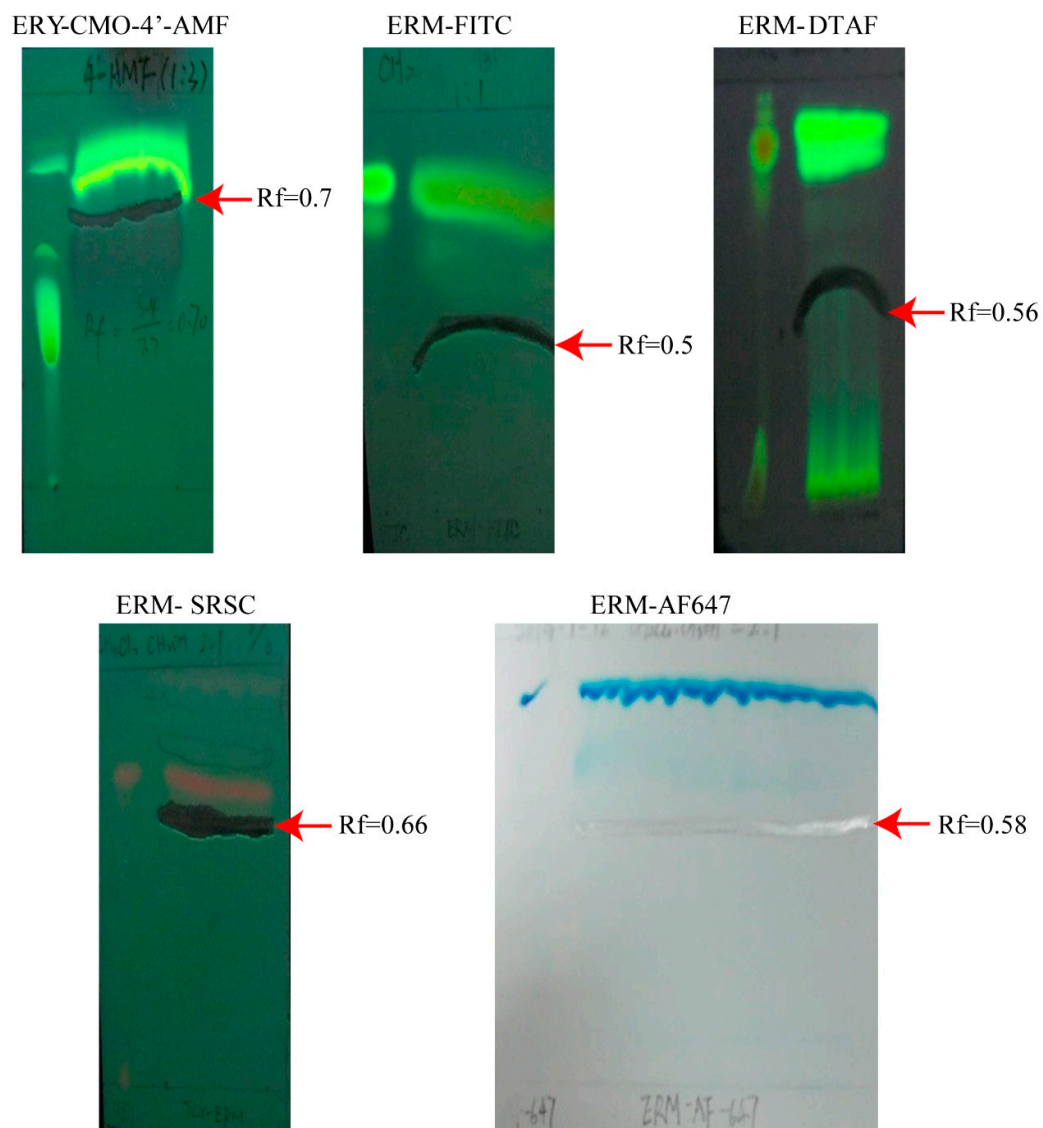


Figure S1 The TLC purification of tracers.

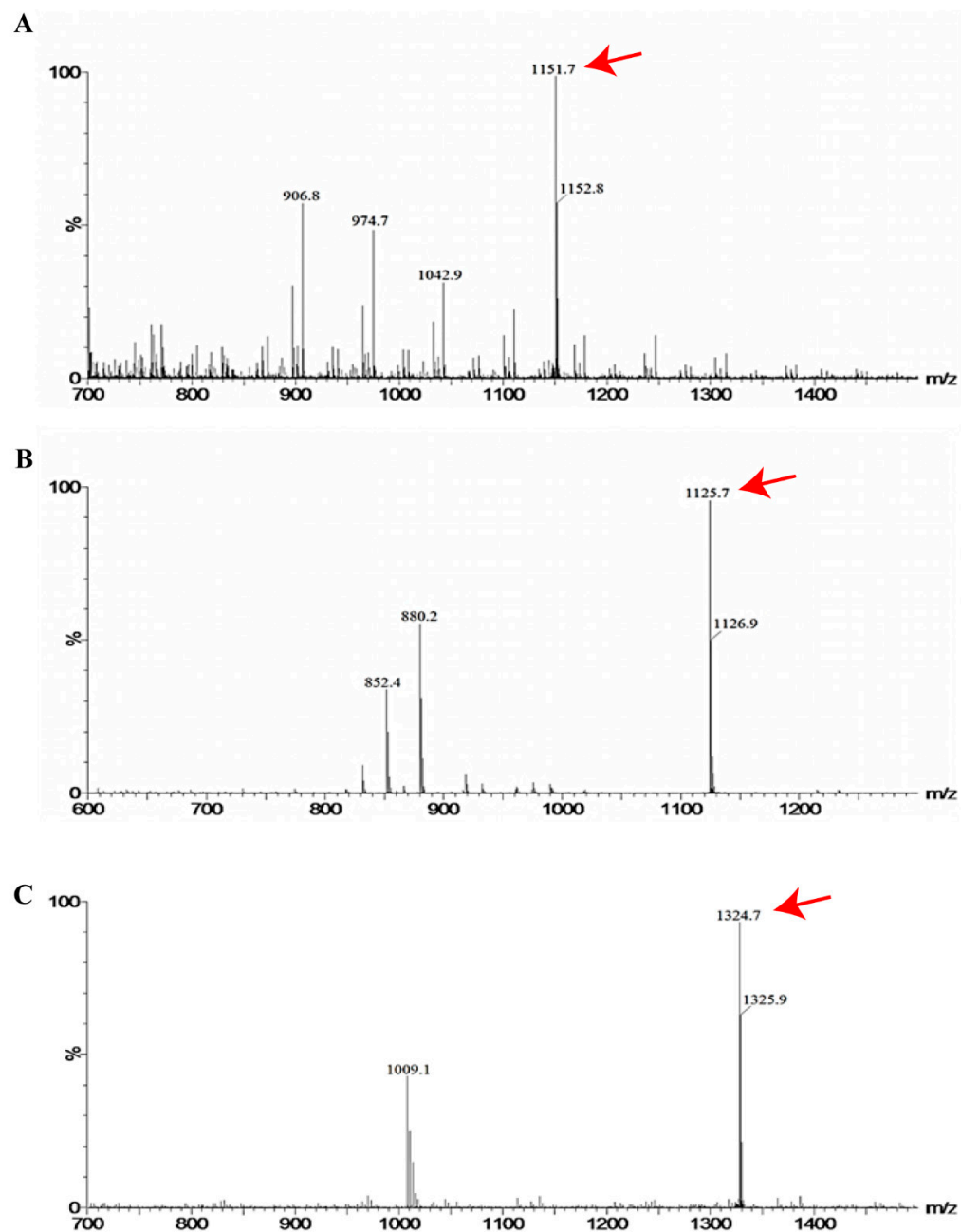


Figure S2 Mass spectra of the ERY-CMO-4'-AMF(A), ERM-FITC (B) and ERM-SRSC (C).

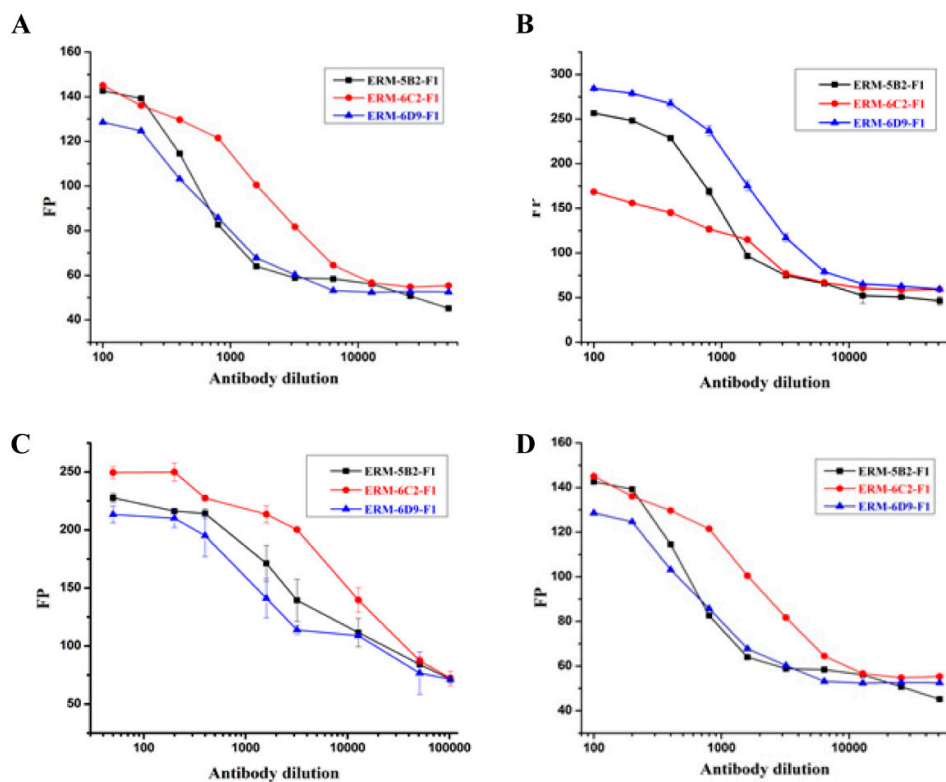


Figure S3 Binding curves for three antibodies with the ERY-CMO-4'-AMF (A), ERM-FITC (B), ERM-AF647 (C) and ERM-SRSC (D), respectively.

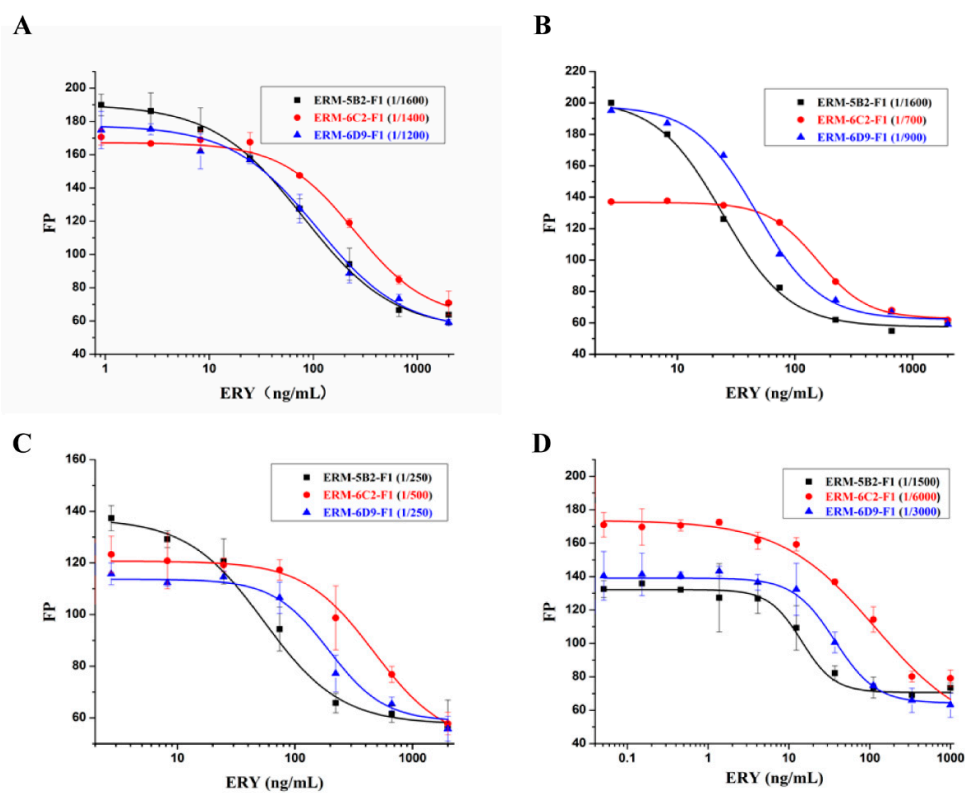
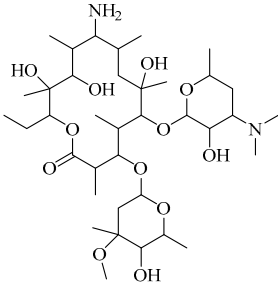
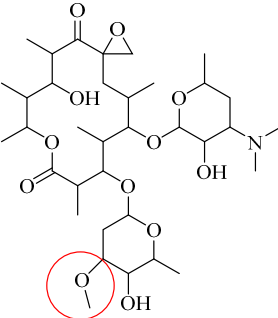
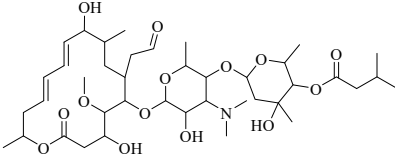
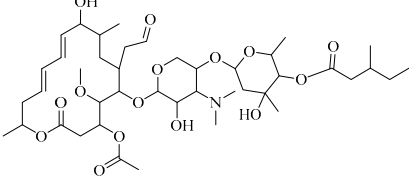
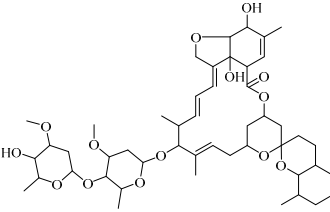
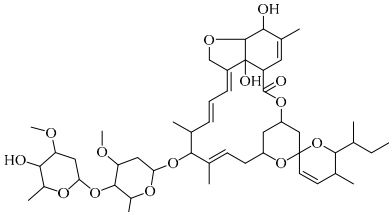
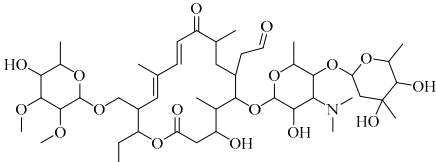


Figure S4 Standard curves for three antibodies with ERY-CMO-4'-AMF (A), ERM-FITC (B), ERM-SRSC (C) and ERM-AF647 (D).

Table S1. The IC₅₀ values and CRs of FPIA.

Type	Name	Structures	IC ₅₀ (ng/mL)	CRs (%)
14- membered macrolides	ERY	The chemical structure of Erythromycin (ERY) is a 14-membered macrolide. It features a 14-membered ring with a ketone at C2, a hydroxyl group at C3, and a dimethylamino group at C14. It is substituted with a 2,6-dideoxy-3,4-dihydroxy-5-methyl-erythro-pentose at C3 and a 2,6-dideoxy-3,4-dihydroxy-5-methyl-erythro-pentose at C14.	7.39	100.0
	DIR	The chemical structure of Dirithromycin (DIR) is a 14-membered macrolide. It features a 14-membered ring with a ketone at C2, a hydroxyl group at C3, and a dimethylamino group at C14. It is substituted with a 2,6-dideoxy-3,4-dihydroxy-5-methyl-erythro-pentose at C3 and a 2,6-dideoxy-3,4-dihydroxy-5-methyl-erythro-pentose at C14. It also has a 2,6-dideoxy-3,4-dihydroxy-5-methyl-erythro-pentose at C14.	4.71	156.9
	ROX	The chemical structure of Roxithromycin (ROX) is a 14-membered macrolide. It features a 14-membered ring with a ketone at C2, a hydroxyl group at C3, and a dimethylamino group at C14. It is substituted with a 2,6-dideoxy-3,4-dihydroxy-5-methyl-erythro-pentose at C3 and a 2,6-dideoxy-3,4-dihydroxy-5-methyl-erythro-pentose at C14. It also has a 2,6-dideoxy-3,4-dihydroxy-5-methyl-erythro-pentose at C14.	8.05	91.8
	ERE	The chemical structure of Erythromycin Ethylsuccinate (ERE) is a 14-membered macrolide. It features a 14-membered ring with a ketone at C2, a hydroxyl group at C3, and a dimethylamino group at C14. It is substituted with a 2,6-dideoxy-3,4-dihydroxy-5-methyl-erythro-pentose at C3 and a 2,6-dideoxy-3,4-dihydroxy-5-methyl-erythro-pentose at C14. It also has a 2,6-dideoxy-3,4-dihydroxy-5-methyl-erythro-pentose at C14.	16.91	43.7
	CLA	The chemical structure of Clarithromycin (CLA) is a 14-membered macrolide. It features a 14-membered ring with a ketone at C2, a hydroxyl group at C3, and a dimethylamino group at C14. It is substituted with a 2,6-dideoxy-3,4-dihydroxy-5-methyl-erythro-pentose at C3 and a 2,6-dideoxy-3,4-dihydroxy-5-methyl-erythro-pentose at C14. It also has a 2,6-dideoxy-3,4-dihydroxy-5-methyl-erythro-pentose at C14.	27.67	26.7

	ERM		16.79	44.0
	OLE		—	< 0.1
16-membered macrolides	KIT		—	< 0.1
	JOS		—	< 0.1
	IVM		—	< 0.1
	AVE		—	< 0.1
	TYL		—	< 0.1

	SPI		—	< 0.1
pleuromutilin	VAL		—	< 0.1
	TAM tiamulin		—	< 0.1

Table S2. The parameters of standard curves in BB, BB with skimmed milk powder, and milk matrix, respectively.

Standard curve	δmP (mP)	IC_{50} (ng/mL)	IC_{10} (ng/mL)	$\text{IC}_{20}\text{-IC}_{80}$ (ng/mL)	R^2
Milk matrix	47.0	4.95	1.2	2.02-12.14	0.9971
BB with skimmed milk powder	55.8	5.66	1.05	1.95-16.39	0.9931
BB	74.2	7.39	0.83	1.85-29.47	0.9969

Table S3. Summary of typically reported immunoassays for the determination of ERY.

Methods	Samples	LOD/LOQ ($\mu\text{g/L}$ or $\mu\text{g/kg}$)	Assay Time	
			(from added samples)	Reference
ciELISA	milk	0.3	2h 30 min	[28]
ciELISA	milk	0.03	2h 40 min	[36]
IGCA	milk	5	10 min	[37]
MISPE-HPLC-ELSD	milk	10	15 min	[13]
HPLC	milk	25	15 min	[1]
MISPE-LC-MS/MS	muscle	1.0	15 min	[12]
LC-MS/MS	muscle	5	20 min	[38]
FPIA	milk	14.08	5 min	This study

ciELISA: competitive indirect enzyme-linked immunosorbent assay

IGCA: immunogold chromatographic assay

MISPE-HPLC-ELSD: molecularly imprinted polymers solid phase extraction combining with High Performance Liquid Chromatography-Evaporation Light Scattering Detection

HPLC: High Performance Liquid Chromatography

MISPE-LC-MS/MS: Molecularly imprinted polymer solid phase extraction combining with LC-MS/MS

LC-MS/MS: liquid chromatography-tandem mass spectrometry