Supporting Information

A Portable Fluorescent Microsphere-Based Lateral Flow Immunosensor for the Simultaneous Detection of Colistin and Bacitracin in Milk

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MATERIALS AND METHODS

Chemicals and Instruments. The 3,3',5,5'-tetramethylbenzidine (TMB) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Horseradish peroxidase (HRP)-labeled goat anti-mouse IgG was purchased from Jackson ImmunoResearch Laboratories (PA, USA). All other reagents were analytical grade or higher and were provided by the National Pharmaceutical Group Chemical Reagent Co., Ltd. (Shanghai, China). The ELISA results were collected by a Multiskan Spectrum (Thermo Fisher Scientific, Boston, MA, USA).

Buffers and solutions. All buffer solutions were prepared using ultrapure water produced by the Milli-Q ultrapure system (Bedford, MA, USA). The phosphate-buffered solution (PBS, 10 mM, pH 7.4) was used as dialysis buffer. The coating buffer was carbonate buffer (CB, 5 mM, pH 9.6), the washing solution was 10 mM PBS containing 0.05% (v/v) Tween-20 (PBST), the blocking buffer was 5 mM CB

containing 0.2% (m/v) gelatin, the antibody dilution buffer was 1 mM PBST containing 0.1% (m/v) gelatin, the substrate solution was a mixture of citrate buffer (0.1 M citrate phosphate buffer containing 0.018% (v/v) of 30% H_2O_2, pH 5.0) and TMB solution (0.06% m/v in ethylene glycol) at a ratio of 5:1 (v/v), and the stop reagent was 2 M sulfuric acid.

Ic-ELISA procedure. The 96-well plates were coated with COL-BSA or Baci-BSA (100 μL/well) in CB and incubated at 37°C for 2 h. The plates were washed three times with PBST followed by blocking with blocking buffer (200 μL/well) at 37°C for 2 h. After blocking, the plates were washed twice and dried at 37°C for 30–60 min. COL or Baci diluted in PBS was then added to the plate (50 μL/well) followed by antibodies (50 μL/well) diluted in antibody dilution buffer and then incubated at 37°C for 30 min. After washing four times, HRP-labeled goat anti-mouse IgG diluted by 4000-times with antibody dilution buffer was added to plates (100 μL/well). Following another 30 min incubation at 37°C and four washes, the substrate solution (100 μL/well) was then added and reacted at 37°C for 15 min in the dark. The enzyme-catalyzed reaction was stopped by addition of stop reagent (50 μL/well). The absorbance was recorded on a Multiskan Spectrum at 450 nm.
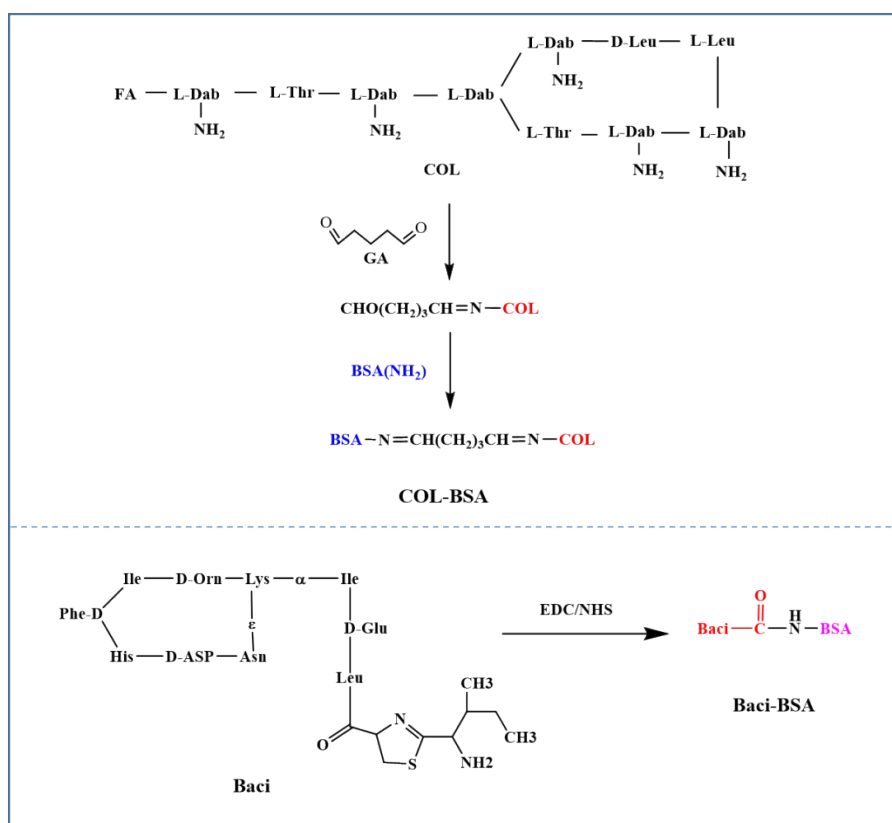
RESULTS

Figure S1. Synthetic routs of antigen conjugates.
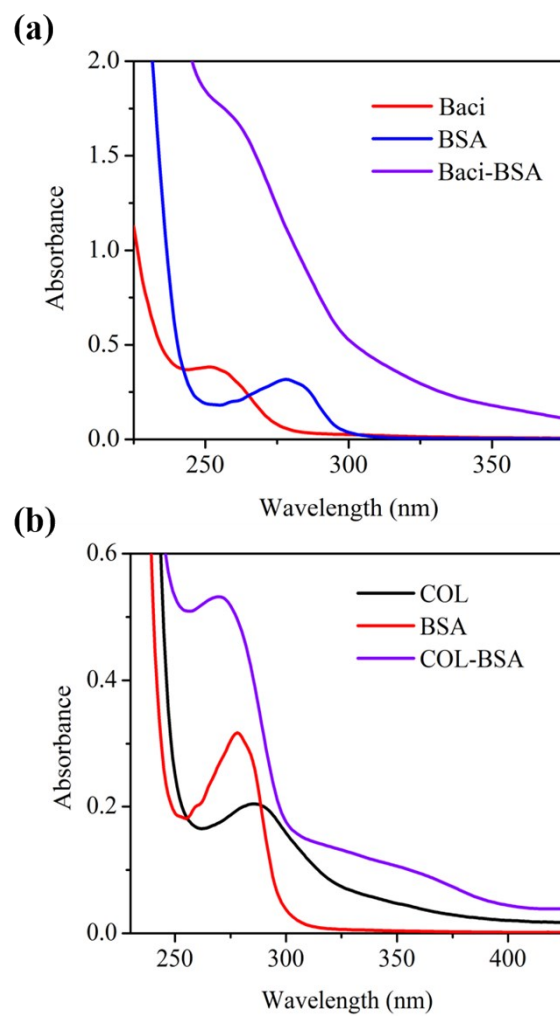
(a)

(b)

Figure S2. Verification of the Baci-BSA conjugate (a) and COL-BSA conjugate (b) using UV–Vis spectrometry.

Figure S3. Optimization of surfactants in probe resuspend buffer by testing 0 and 100 ng/mL of COL and Baci in PBS using strip sensor. The concentrations of goat anti-mouse IgG (C line), Baci-BSA (T1 line) and COL-BSA (T2 line) were 0.1, 0.2, and 0.1 mg/mL.
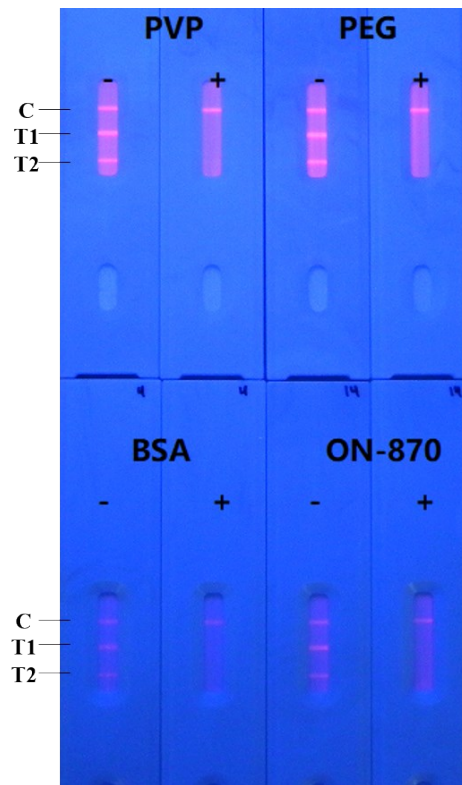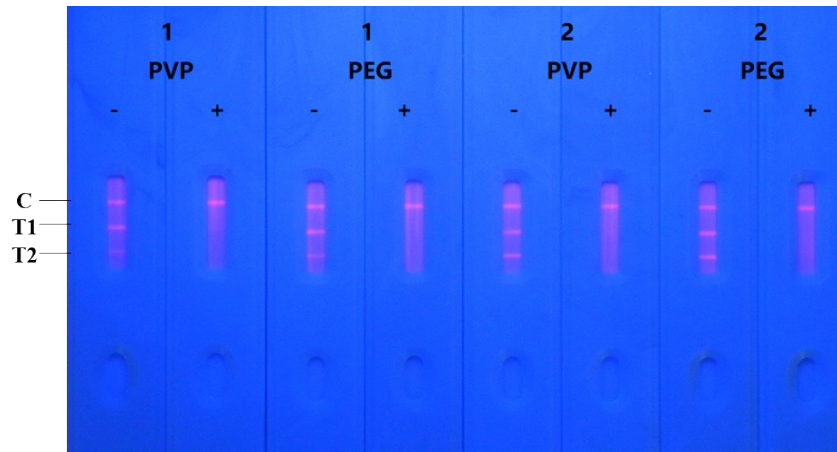
Figure S4. Optimization of surfactants in probe resuspend buffer by testing 0 and 100 ng/mL of COL and Baci in milk using strip sensor. The concentrations of goat anti-mouse IgG (C line), Baci-BSA (T1 line) and COL-BSA (T2 line) were 0.01, 0.2, and 0.2 (represent by 1) or 0.4 (represent by 2) mg/mL.

Table. S1 Cross-reactivity (CR) of anti-COL mAb and anti-Baci mAb with common antibacterial drugs.

Analyte	Anti-COL mAb 1C10		Anti-Baci mAb 4B11	
	IC_{50} (ng/mL)	CR (%)[a]	IC_{50} (ng/mL)	CR (%)
Colistin	2.7	100	>2000	<1
Polymyxin B	24.8	10.8	>2000	<1
Bacitracin	>2000	<2	18.3	100
Penicillin G	>2000	<2	>2000	<1
Ampicillin	>2000	<2	>2000	<1
Vancomycin	>2000	<2	>2000	<1
Chloramphenicol	>2000	<2	>2000	<1
Tetracycline	>2000	<2	>2000	<1
Sulfadimidine	>2000	<2	>2000	<1
Enrofloxacin	>2000	<2	>2000	<1

[a] Cross-reactivity of antibodies was calculated as the ratio of IC_{50} COL or Baci/ IC_{50} analogue expressed as a percentage.

Table. S2 Optimization of surfactants in probe resuspend buffer by testing 0 and 500 ng/mL of COL and Baci in PBS using strip sensor.

Surfactants	Targets concentration (ng/mL)	Color intensity			T/C values	
		T1	T2	C	T1/C	T2/C
PVP	0	19943	11062	14060	1.418	0.786
	500	0	353	13442	0	0.026
PEG	0	28775	18757	19982	1.440	0.93
	500	224	208	19875	0.011	0.010
BSA	0	10879	5213	7838	1.39	0.666
	500	49	22	6625	0.007	0.003
ON-870	0	20144	11845	13314	1.513	0.890
	500	323	670	13239	0.024	0.051

Table. S3 Optimization of surfactants in probe resuspend buffer by testing 0 and 500 ng/mL of COL and Baci in milk using strip sensor.

Concentration of COL-BSA (T2 line) (mg/mL)	Surfactants	Targets concentration (ng/mL)	Color intensity			T/C values	
			T1	T2	C	T1/C	T2/C
0.2	PVP	0	11136	4208	10291	1.082	0.409
		500	0	228	11718	0	0.019
	PEG	0	10628	3638	9991	1.064	0.364
		500	102	0	10097	0.010	0
0.4	PVP	0	7567	8339	11787	0.642	0.707
		500	340	452	12012	0.028	0.038
	PEG	0	14566	11702	11614	1.254	1.007
		500	218	377	15398	0.014	0.024

Table. S4 Individual analysis of Baci and COL using LFIA immunosensor.

Targets concentration (ng/mL)		Color intensity			T/C values	
Baci	COL	T1	T2	C	T1/C	T2/C
0	0	18229	13666	14398	1.266	0.949
5	0	8110	9560	10483	0.774	0.912
10	0	4411	10428	10569	0.417	0.987
25	0	2829	12361	14375	0.197	0.860
50	0	1660	12856	13874	0.120	0.927
100	0	558	12122	14213	0.039	0.853
250	0	372	12779	13271	0.028	0.963
500	0	0	13266	14118	0	0.940
0	0	21710	14066	12289	1.767	1.145
0	2	15983	5350	9980	1.602	0.536
0	5	16845	3166	11290	1.490	0.276
0	10	18719	1810	12108	1.546	0.149
0	25	17160	973	12424	1.381	0.078
0	50	19114	853	11943	1.600	0.071
0	100	20097	850	14581	1.378	0.058
0	250	19077	0	12432	1.535	0

Table. S5 Sensitivity assessment of LFIA immunosensor.

Matrix	Targets concentration (ng/mL)		Color intensity			T/C values	
	Baci	COL	T1	T2	C	T1/C	T2/C
PBS	0	0	28551	16757	18170	1.571	0.922
	5	2	7591	6960	14528	0.523	0.479
	10	5	4401	3481	19800	0.222	0.176
	25	10	2015	2069	23092	0.087	0.090
	50	25	1619	1777	20655	0.078	0.086
	100	50	825	985	19011	0.043	0.052
	250	100	544	1094	23373	0.023	0.047
	500	250	0	0	22755	0	0
Milk	0	0	9874	9053	10900	0.906	0.831
	2	1	9306	6013	9794	0.950	0.614
	5	2	4126	2346	4277	0.965	0.549
	10	5	2692	1070	4090	0.658	0.262
	25	10	1925	1251	5914	0.325	0.212
	50	25	902	583	6937	0.130	0.084
	100	50	634	284	7188	0.088	0.040
	250	100	185	120	4680	0.040	0.026
	500	250	0	0	8480	0	0