Electronic Supplementary Material (ESI) for Analyst.

Supporting Information

Rapid Detection of 21 β-Lactams using Immunochromatographic Assay Based on Mutant BlaR-CTD Protein from *Bacillus Licheniformis*

Yue Li[a,b], Xinxin Xu[a,b], Liqiang Liu[a,b], Hua Kuang[a,b], Liguang Xu[a,b*], Chuanlai Xu[a,b*]

[a]*State Key Laboratory of Food Science and Technology, Jiangnan University, Wuxi, People's Republic of China;*
[b]*International Joint Research Laboratory for Biointerface and Biodetection, and School of Food Science and Technology, Jiangnan University, Wuxi, People's Republic of China;*

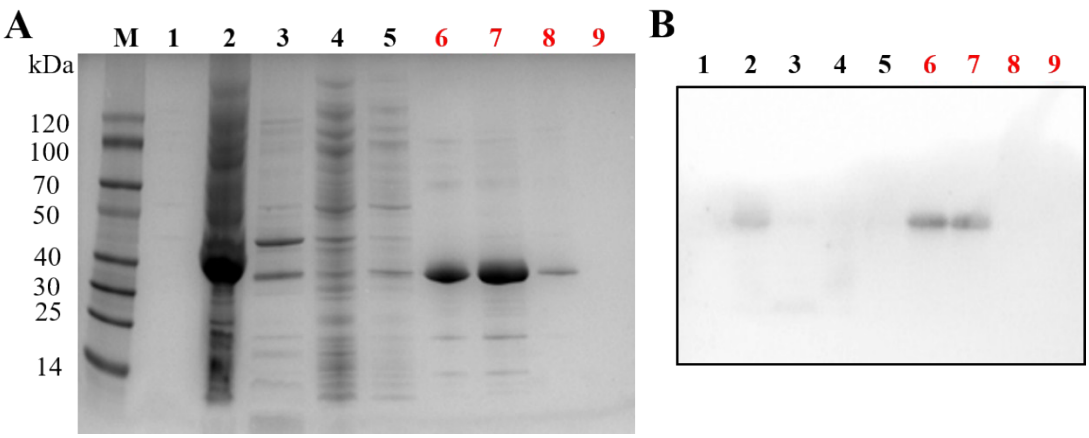

Fig. S1 SDS-PAGE (A) and Western-Blot (B) analysis of BlaR-CTD-M expressed in recombinant *E. coli* BL21(DE3)/pET-28a(+)-BlaR-CTD-M and purified using nickel affinity chromatography. Proteins were separated on a 4-20% separation gel and stained with Coomassie Brilliant Blue. Lane 1, total cellular protein of recombinant *E. coli* without IPTG induction; Lane 2, soluble fractions of total cellular protein of recombinant *E. coli* with IPTG induction; Lane 3, insoluble fractions of total cellular protein of recombinant *E. coli* with IPTG induction; Lane 4, flow-through from the nickel affinity chromatography; Lane 5, washing fraction from the nickel affinity chromatography; Lanes 6–9, elution fractions from the nickel affinity chromatography with 50, 100, 250, and 500 mM imidazole.

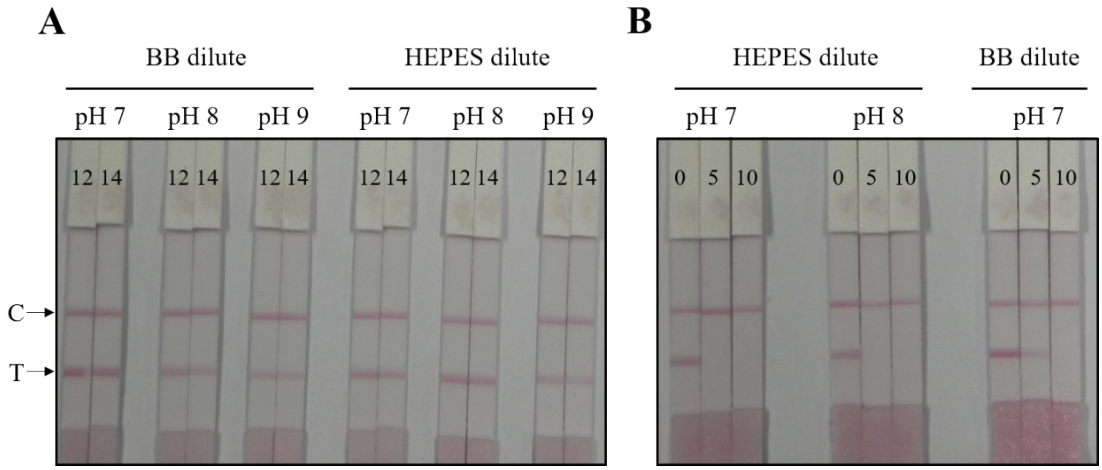

Fig. S2 Optimization of pH for CG-labelled BlaR-CTD-M preparation. Evaluation of the resultant CG-labelled BlaR-CTD-M was performed by testing negative milk sample **(A)** and benzylpenicillin standard **(B)** using GICA strips (12 and 14 represent the sample pad was treated with basic suspension buffer containing 5% Brij35 and 5% Rhodasurf® On-870 (an ethoxylated oleyl alcohol), respectively. 0, 5, and 10 represent the benzylpenicillin standard concentrations were 0, 5, and 10 ng/mL, respectively, prepared in milk). Each test was repeated thrice.

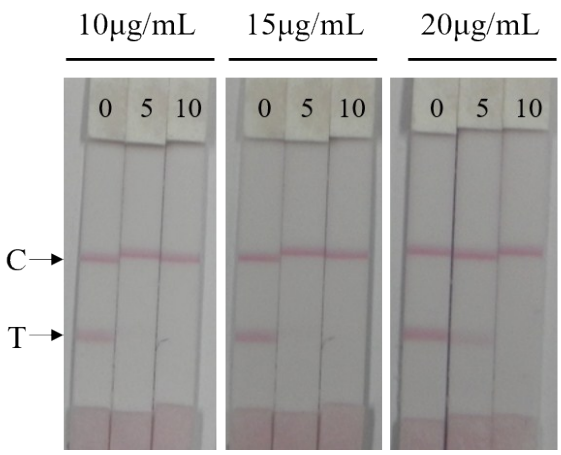

Fig. S3 Optimization of the amount of labelled BlaR-CTD-M. Evaluation of the resultant CG-labelled BlaR-CTD-M was performed by testing benzylpenicillin standard prepared in milk (0, 5, and 10 represent the benzylpenicillin standard concentrations were 0, 5, and 10 ng/mL, respectively). Each test was repeated thrice.

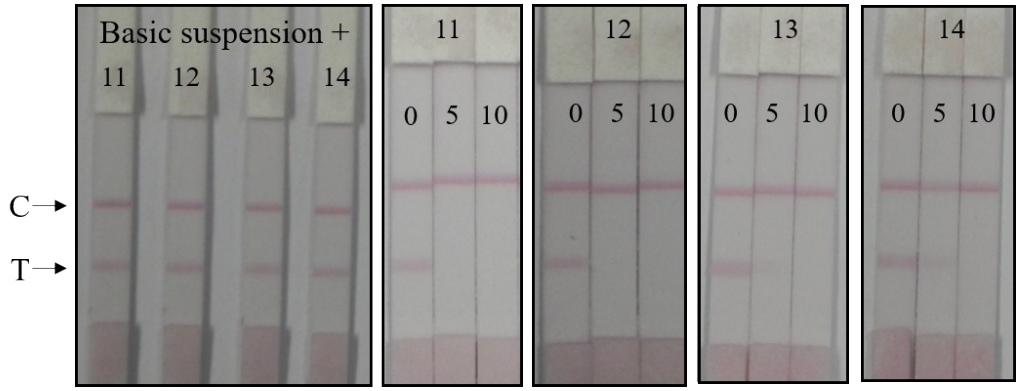

Fig. S4 Result of using different surfactants (11, 12, 13, and 14 represent tween-20, Brij 35, triton X-100, and On-870, respectively. 0, 5, and 10 represent the benzylpenicillin standard concentrations were 0, 5, and 10 ng mL^{-1}, respectively, prepared in milk.

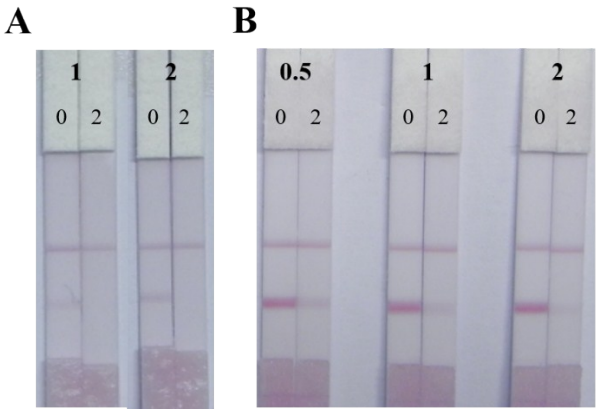

Fig. S5 Optimization of the concentration of antigen on the T line by testing benzylpenicillin standard prepared in milk **(A)** and chicken sample **(B)** (0 and 2 represent the benzylpenicillin standard concentrations were 0 and 2 ng/mL, respectively).

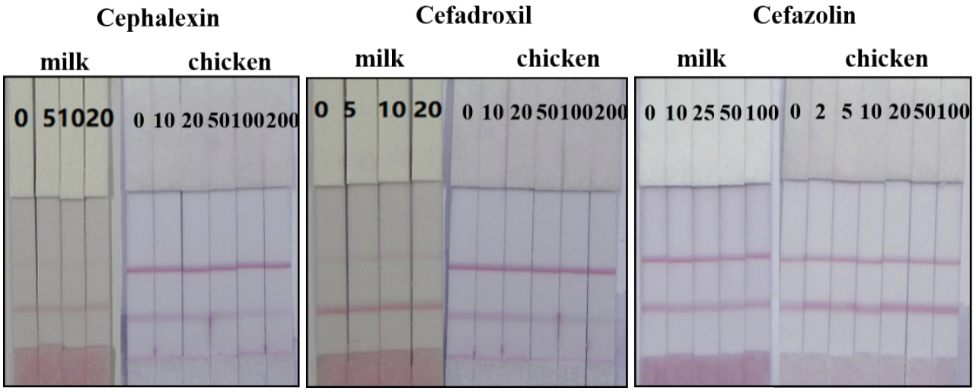

Fig. S6 Results of detecting cephalexin, cefadroxil, cefazolin in milk and chicken samples.

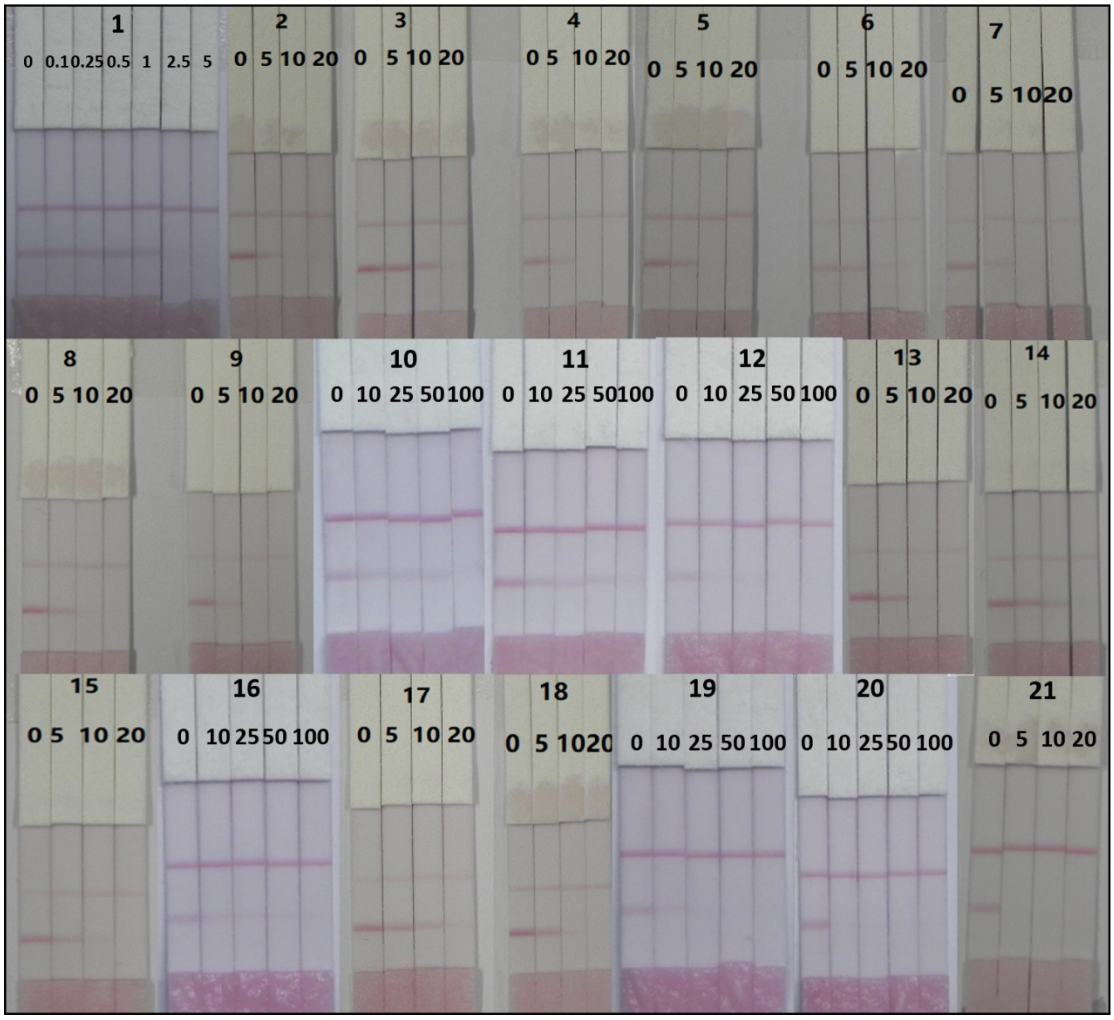

Fig. S7 Detection of β-lactam antibiotics in milk samples using CG-BlaR-CTD-M-based GICA strips. 1 = benzylpenicillin, 2 = ampicillin, 3 = amoxicillin, 4 = cloxacillin, 5 = dicloxacillin, 6 = nafcillin, 7 = cephalothin，8 = cefapirin，9 = cefoperazone, 10 = cefotaxime, 11 = cefuroxime, 12 = ceftiofur, 13 = cefamandole, 14 = oxacillin, 15 = moxalactam, 16 = cefaclor, 17 = meropenem, 18 = cefalotin, 19 = ceftriaxone, 20 =cefquinome, 21 = penicillin V.

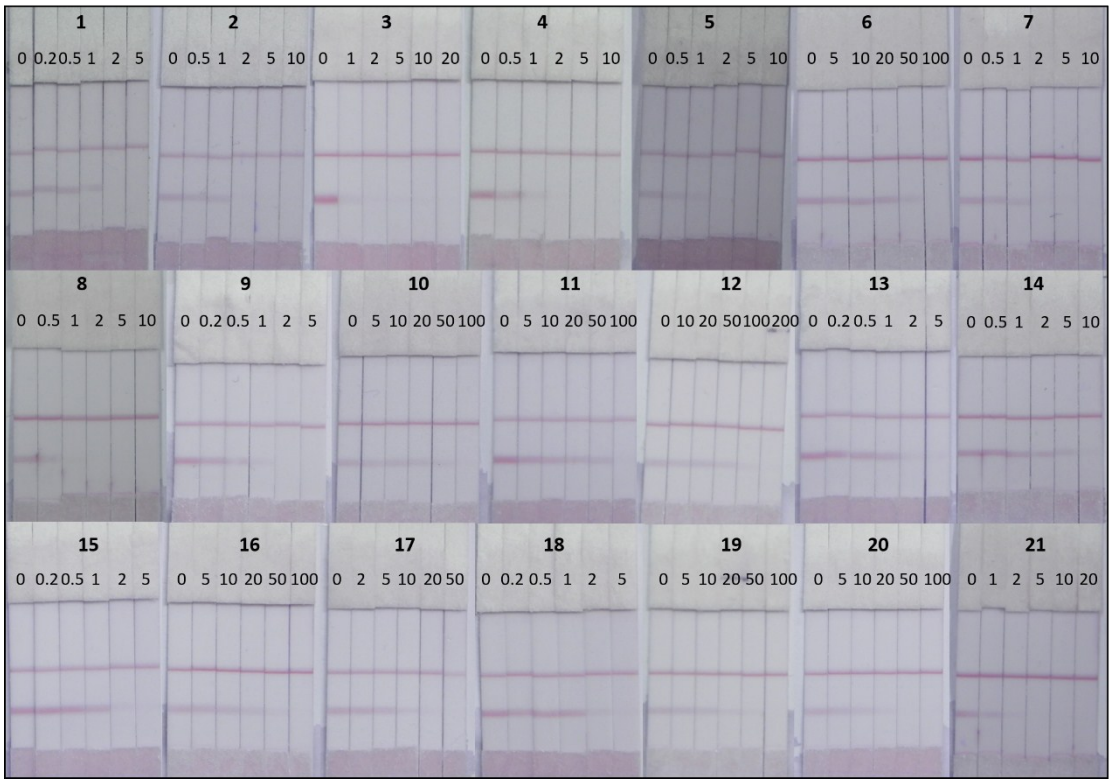

Fig. S8 Detection of β-lactam antibiotics in chicken samples using CG-BlaR-CTD-M-based GICA strips.1 = benzylpenicillin, 2 = ampicillin, 3 = amoxicillin, 4 = cloxacillin, 5 = dicloxacillin, 6 = nafcillin, 7 = cephalothin, 8 = cefapirin, 9 = cefoperazone, 10 = cefotaxime, 11 = cefuroxime, 12 = ceftiofur, 13 = cefamandole, 14 = oxacillin, 15 = moxalactam, 16 = cefaclor, 17 = meropenem, 18 = cefalotin, 19 = ceftriaxone, 20 =cefquinome, 21 = penicillin V.

Table S1. Benzylpenicillin detection based on different antigens.

	Amp-BSA	**Amo-BSA**	**Cep-BSA**	**Cefa-BSA**
A_{max}[a]	1.84	1.40	0.19	1.23
A[b]	0.86	0.81	0.12	0.69
$1-A/A_{max}*100\%$	53.3%	42.1%	-	43.9%

[a] A_{max} represents the absorbance of negative sample (PBS).

[b]A represents the absorbance of positive sample (2 ng/mL of benzylpenicillin).