Supporting Information

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

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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 cephallexin, 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.

<table>
<thead>
<tr>
<th></th>
<th>Amp-BSA</th>
<th>Amo-BSA</th>
<th>Cep-BSA</th>
<th>Cefa-BSA</th>
</tr>
</thead>
<tbody>
<tr>
<td>A&lt;sub&gt;max&lt;/sub&gt;&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.84</td>
<td>1.40</td>
<td>0.19</td>
<td>1.23</td>
</tr>
<tr>
<td>A&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.86</td>
<td>0.81</td>
<td>0.12</td>
<td>0.69</td>
</tr>
<tr>
<td>1-A/A&lt;sub&gt;max&lt;/sub&gt;*100%</td>
<td>53.3%</td>
<td>42.1%</td>
<td>-</td>
<td>43.9%</td>
</tr>
</tbody>
</table>

<sup>a</sup> A<sub>max</sub> represents the absorbance of negative sample (PBS).

<sup>b</sup>A represents the absorbance of positive sample (2 ng/mL of benzylpenicillin).