An antimicrobial peptide-based colorimetric bioassay for rapid and sensitive detection of *E. coli* O157:H7

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1. Detection of *E. coli* O157:H7 by the antibody-based biosensor

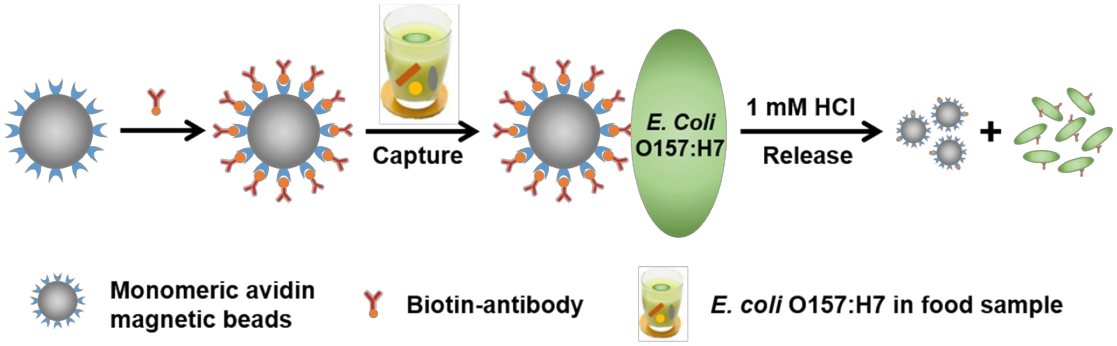
The preparation of antibody-HRP probes was the same as that of the AMP-based bioassay: biotinylated antibody for *E. coli* (50 μg/mL) were mixed with HRP-conjugated streptavidin (25 μg/mL) and then the mixture was placed in a programmable rotating-mixer at 15 rpm for 30 min at RT. The molar ratio of antibody and HRP was selected as 1.5:1 according to the optimization tests. Finally, the antibody-HRP probe was obtained with a final antibody concentration of 50 μg/mL. Under optimized conditions (the procedure is the same as the optimization of these of AMP-HRP), 800 μL of serially diluted *E. coli* O157:H7 and negative control were mixed with 5 μL of antibody-HRP probes and incubated for 30 min in the filter tubes at RT. Finally, the unbound probes were removed and the signals were measured as described in the detection by the AMP-based assay.

2. Capture efficiency and release efficiency of IMBs against *E. coli* O157:H7

Serial dilutions of the pure cultures of *E. coli* O157:H7, were prepared in PBS as above. 20 μL of the prepared IMBs was mixed with 200 μL of culture (10^3, 10^4 and 10^5 cfu/mL) and rotated at 15 rpm for 30 min at RT. The IMB-bacteria complexes were washed two times and re-suspended in 200 μL of PBS. The uncaptured cells in supernatant were also collected and appropriately diluted if needed. A 100 μL of the captured samples and uncaptured samples was plated on selective agars and incubated at 37°C for 24 h for bacterial enumeration. The same level for all the original cultures was used as a positive control. All enumeration experiments were performed in triplicate. The capture efficiency (CE, %) was calculated with the following equation (Xiong et al., 2014): $CE\ (\%) = (1 - N_U/N_O) \times 100\%$. where N_O is the number of original cells, N_U is the number of uncaptured cells (in supernatant and washed solution). and N_C is the number of captured cells. The calculation was considered valid only when N_C was in the same magnitude as N_O.

After captured by IMBs, the bacteria were released as mentioned in section 2.7. It's impossible to do enumeration experiments after the bacteria were released because

most of them were killed by elution buffer. Therefore, the biotin labeled horseradish peroxidase (bio-HRP) was used to substitute for biotin labeled antibody because the bio-HRP can simulate the bio-antibody in the process of elution and it's simple to signal output. The HRP labeled magnetic beads were prepared as the same as preparation of immuno-magnetic beads. Subsequently, an aliquot of 5 μL of HRP labeled magnetic beads were separated and re-suspended in 100 μL of elution buffer (1 mmol/L HCl) for 1 min to separate the magnetic beads and bio-HRP. These washed magnetic beads re-bound with 0.1 mg/mL bio-HRP. Finally, the signal output reaction was started by adding 100 μL of TMB and measured by the microplate reader. The release efficiency (RE, %) was calculated with the following equation: RE (%)=OD_R/OD_O × 100%. where OD_R is the optical intensity of rebound HRP labeled magnetic beads, OD_O is the optical intensity of original HRP labeled magnetic beads.



Scheme S1. Schematic representation of capture-release procedure.

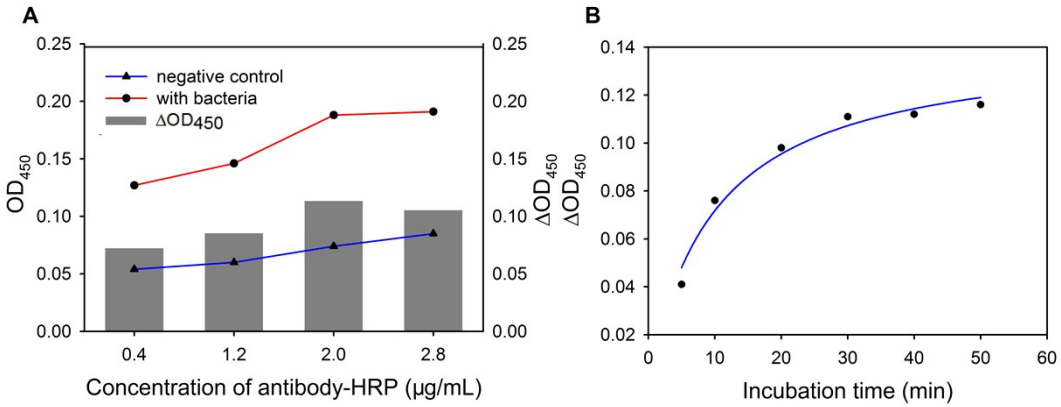


Fig. S1 (A) The influence of antibody-HRP at different concentrations on the optical signal. (B) The influence of different incubation times on the optical signal. The concentration of antibody-HRP is 2 μg/mL, and the concentration of *E. coli* O157:H7 is 10^5 cfu/mL.

Table. S1. CE of IMBs against *E. coli* O157:H7.

N_O (cfu)	N_U (cfu)	CE (%)	Average CE (%)
$(2.64 \pm 0.068) \times 10^2$	3	99.2	98.9 ± 0.4
	3	99.2	
	4	98.5	
$(2.72 \pm 0.0929) \times 10^3$	51	98.1	97.8 ± 2.2
	3	99.9	
	121	95.6	
$(2.48 \pm 0.0854) \times 10^4$	200	99.2	97.8 ± 2.8
	1344	94.6	
	52	99.8	

Table. S2. RE of IMBs based on bio-HRP colorimetric reaction.

OD_O (ΔOD_{450})	OD_R (ΔOD_{450})	RE (%)	Average RE (%)
0.306	0.270	88.1	86.9 ± 1.7
0.312	0.265	84.9	
0.298	0.261	87.7	

References

1. Q. Xiong, X. Cui, J. K. Saini, D. Liu, S. Shan, Y. Jin and W. Lai, Food Control, 2014, **37**, 41-45.