

## Supporting information

### **Rapid and Sensitive detection of *Staphylococcus aureus* and *Klebsiella pneumonia* based on Bacitracin-modified Fe<sub>3</sub>O<sub>4</sub>@PDA magnetic beads combined with Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry**

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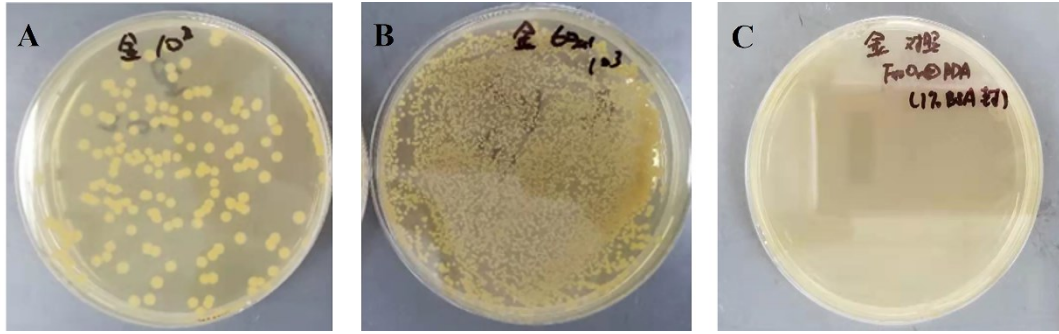
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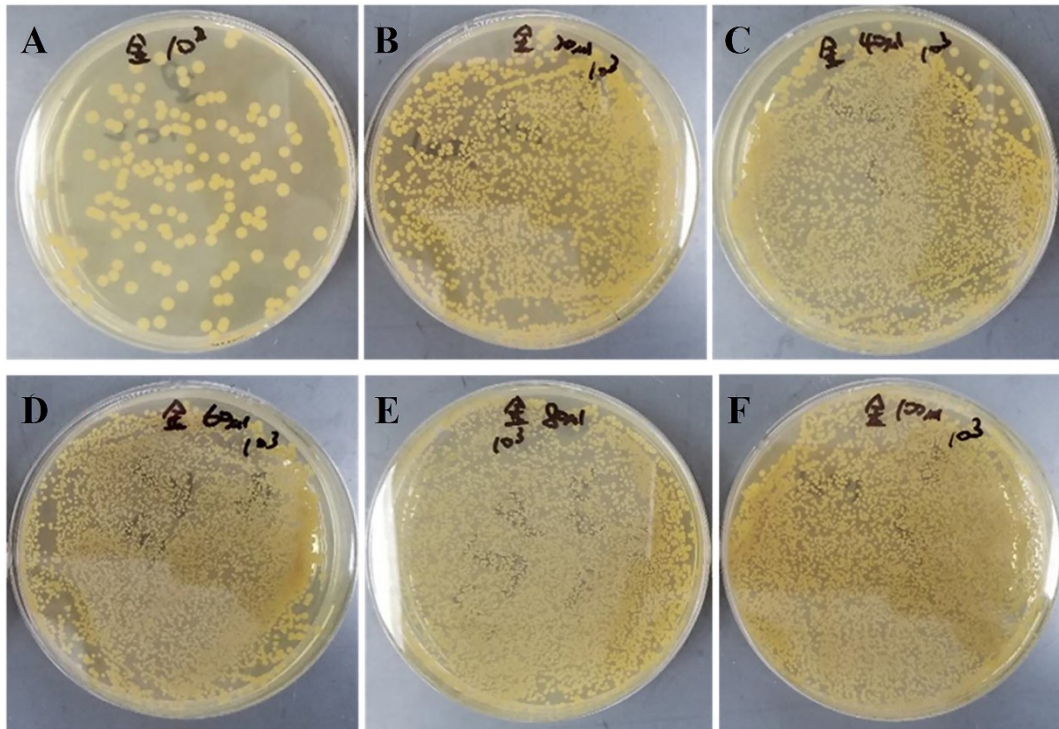
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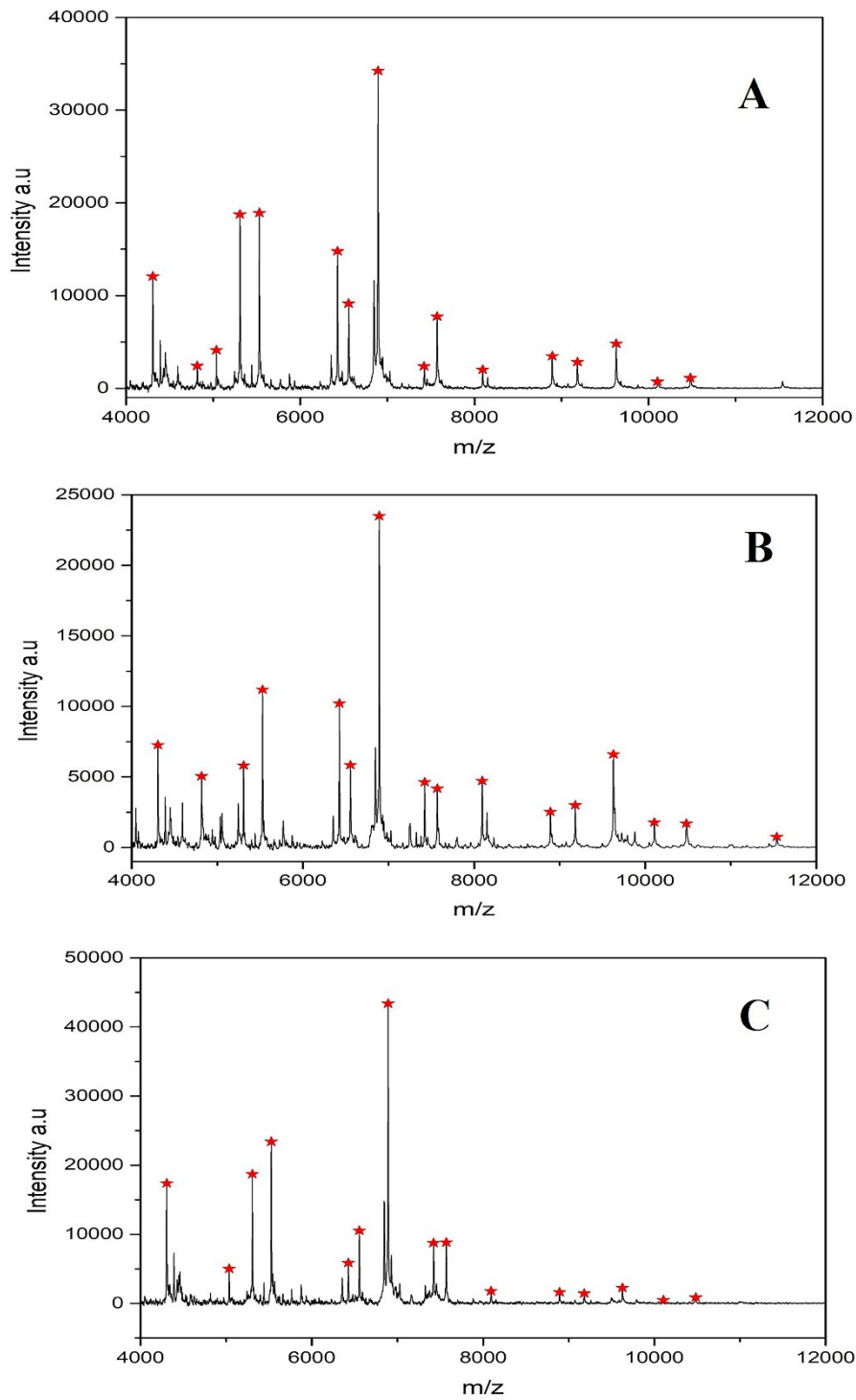
**Fig.S1** The effect of adding  $\text{Fe}_3\text{O}_4@\text{PDA}@\text{Bacitracin}$  and  $\text{Fe}_3\text{O}_4@\text{PDA}$  (negative control) on the enrichment *S. aureus*

The plate results of *S.aureus* (A) before enrichment, (B) enrichment by 60 $\mu\text{L}$   $\text{Fe}_3\text{O}_4@\text{PDA}@\text{Bacitracin}$ , (C) enrichment by 60 $\mu\text{L}$   $\text{Fe}_3\text{O}_4@\text{PDA}$ (negative control)

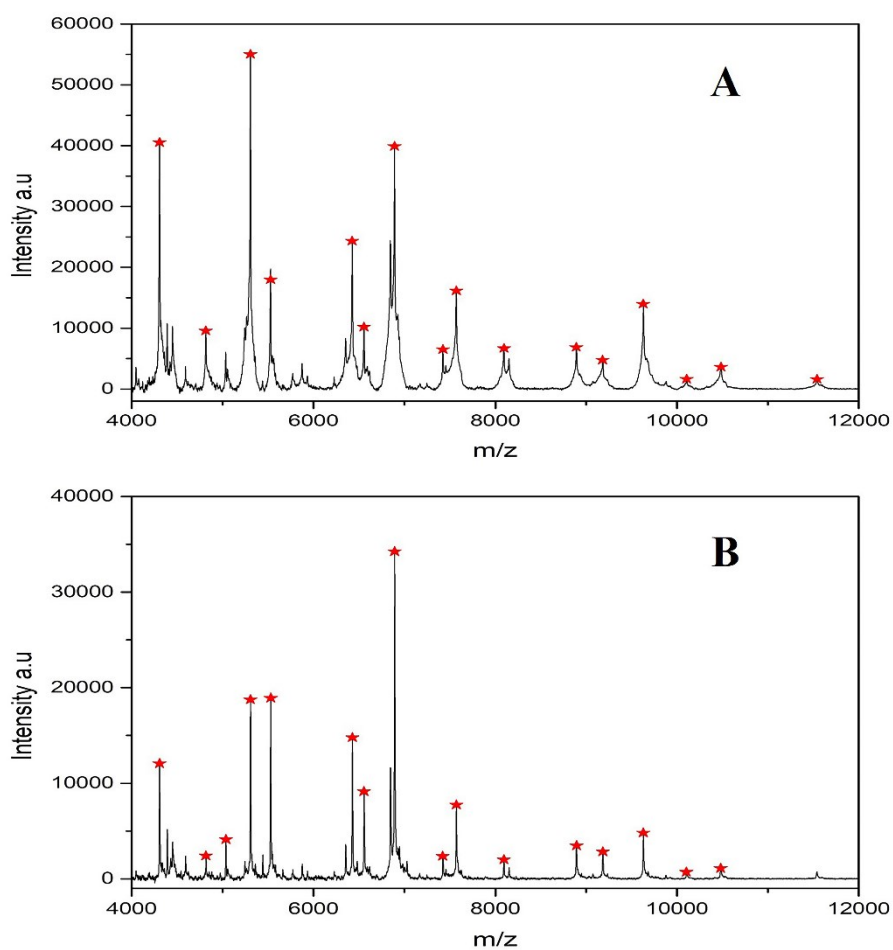


**Fig.S2** Effect of the different volume of  $\text{Fe}_3\text{O}_4@\text{PDA}@\text{Bacitracin}$  on the enrichment of *Staphylococcus aureus*.

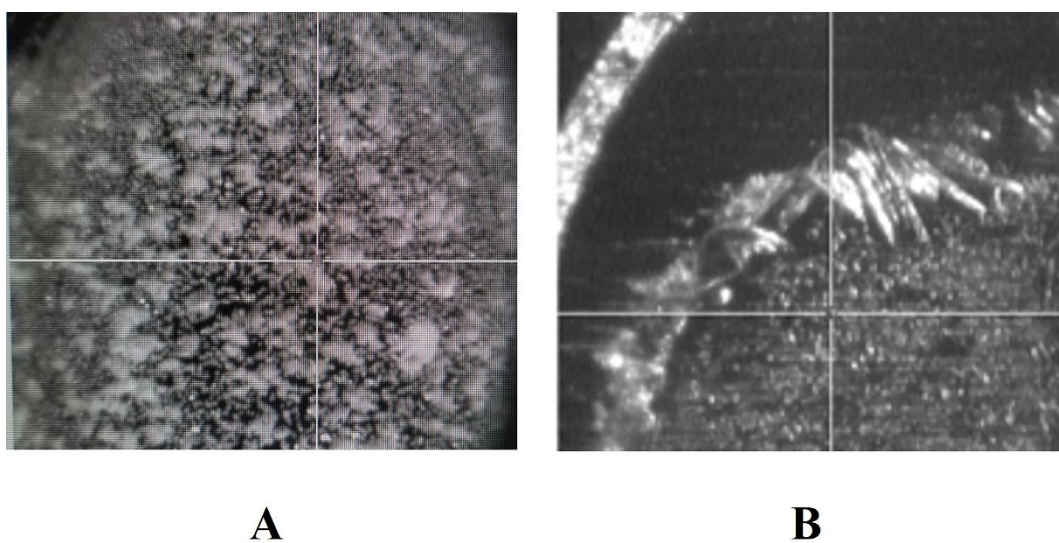
(A)The plate results of the count of  $10^2 \text{CFU}\cdot\text{mL}^{-1}$  *S. aureus* (before enrichment); (B)-(F). The plate results of the count of  $10^3 \text{CFU}\cdot\text{mL}^{-1}$  *S.aureus* after enriched by 20,40,60,80,100  $\mu\text{L}$   $\text{Fe}_3\text{O}_4@\text{PDA}@\text{Bacitracin}$  in turn



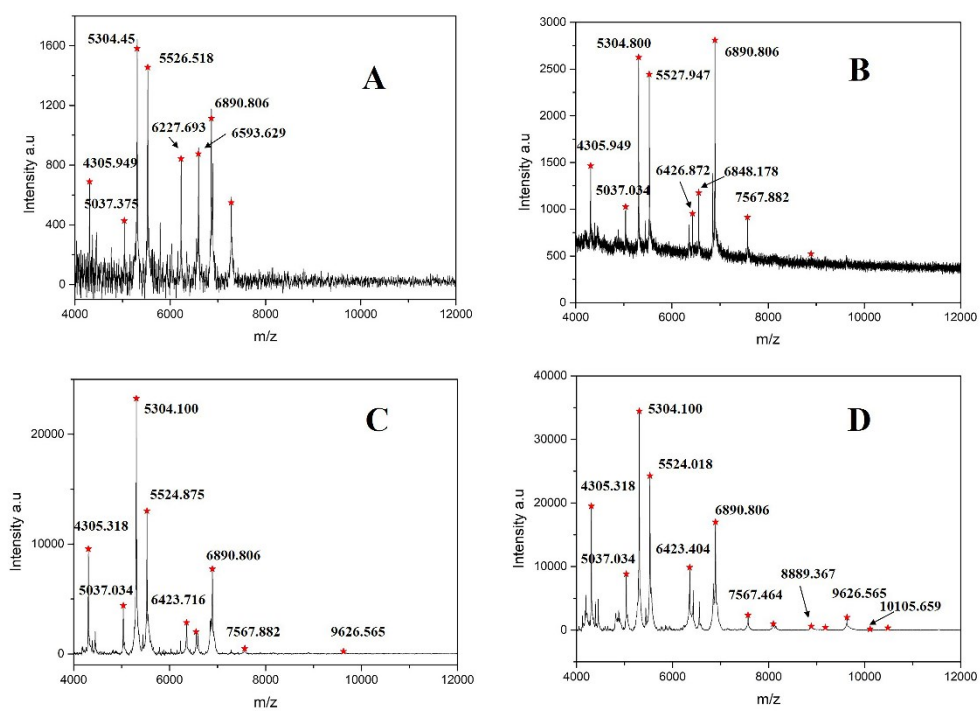
**Fig. S3** Effect of different bacterial protein extraction pre-treatment methods on MALDI-TOF MS assay. (A) 70% formic acid treatment before targeting, (B) 70% formic acid / acetonitrile (1:1) treatment before targeting, (C) target first and then treated with 70% formic acid



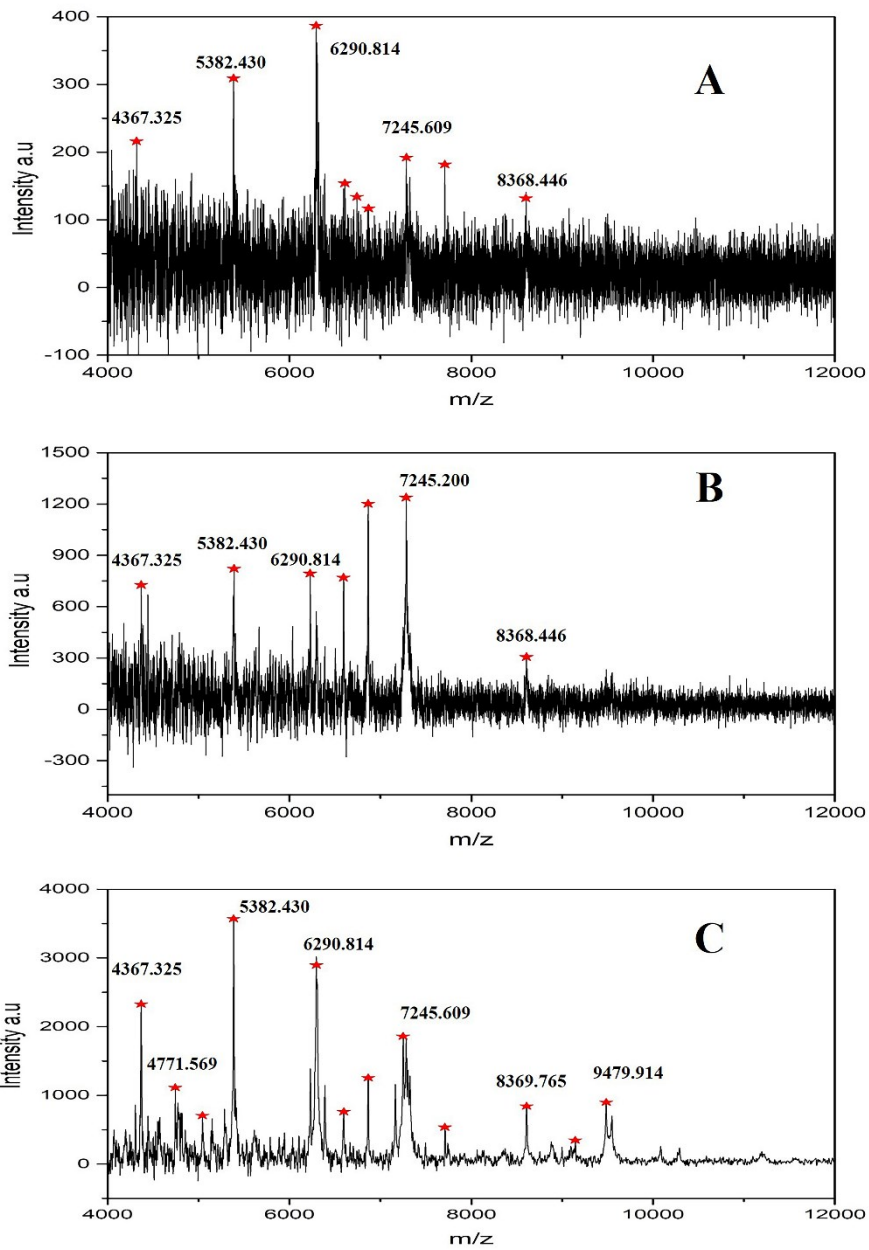
**Fig.S4** Effects of different matrixes on the MALDI-TOF MS analysis  
(A)  $\alpha$ -CHCA (B) DHB



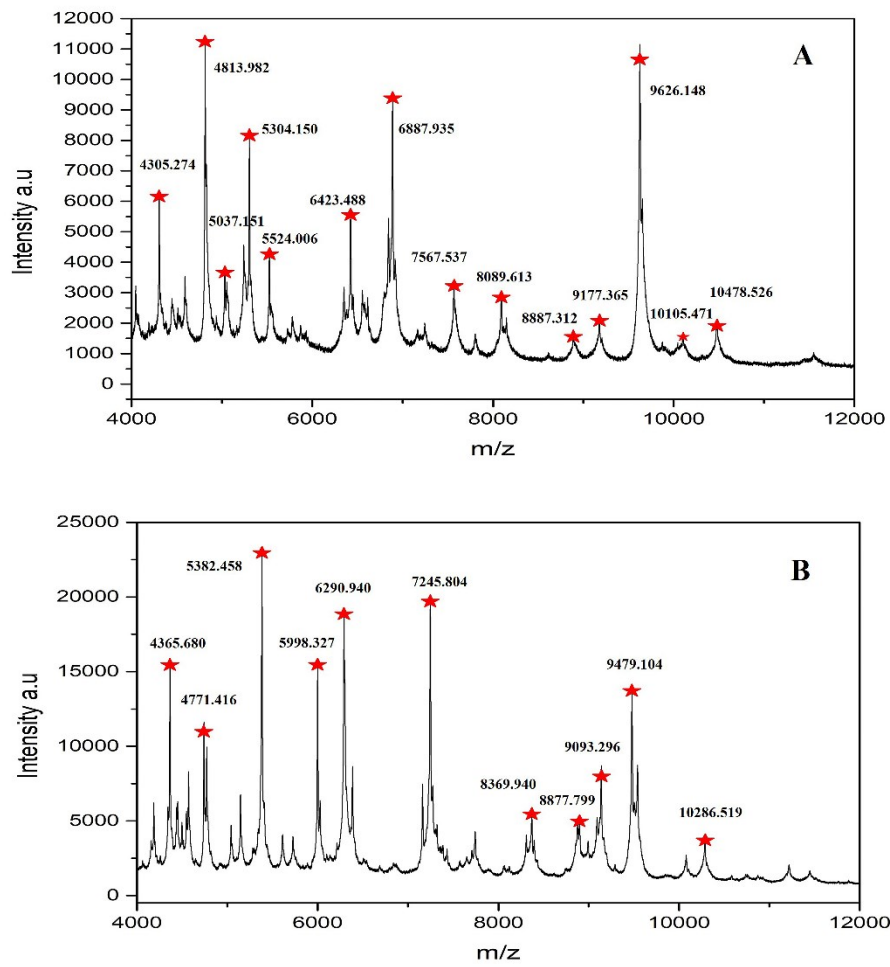
**Fig.S5** Imaging of *S. aureus* protein treated by different matrixes in visual field  
(A)  $\alpha$ -CHCA (B) DHB



**Fig.S6** MALDI-TOF-MS spectra of different concentrations of the *S. aureus* in aqueous solution after enrichment by Fe<sub>3</sub>O<sub>4</sub>@PDA@Bacitracin: (A) 10<sup>4</sup>CFU·mL<sup>-1</sup> (B) 10<sup>5</sup>CFU·mL<sup>-1</sup> (C) 10<sup>6</sup>CFU·mL<sup>-1</sup> (D) 10<sup>7</sup>CFU·mL<sup>-1</sup>



**Fig.S7** MALDI-TOF-MS spectra of different concentrations of the *K. pneumoniae* in aqueous solution after enrichment by Fe<sub>3</sub>O<sub>4</sub>@PDA@Bacitracin: (A) 10<sup>5</sup>CFU·mL<sup>-1</sup> (B) 10<sup>6</sup>CFU·mL<sup>-1</sup> (C) 10<sup>7</sup>CFU·mL<sup>-1</sup>



**Fig.S8** Reference MS spectra of (A) *Staphylococcus aureus* and (B) *Klebsiella pneumonia*

**Table S1 Enrichment of Fe<sub>3</sub>O<sub>4</sub>@PDA@Bacitracin to different bacteria**

Name of bacteria	Plate counting results (CFU·mL <sup>-1</sup> )	Enrichment ratio
<i>S.aureus</i>	1.45×10 <sup>3</sup>	45.2%
<i>K.pneumoniae</i>	4.3×10 <sup>3</sup>	37.0%
<i>P.aeruginosa</i>	2.57×10 <sup>3</sup>	20.9%
<i>Listeria monocytogenes</i>	1.47×10 <sup>3</sup>	8.61%
<i>Pyogeniccoccus</i>	2×10 <sup>3</sup>	6.62%
<i>E. Coli</i>	1.26×10 <sup>3</sup>	6.42%
<i>shigella flexneri</i>	2.75×10 <sup>3</sup>	1.92%
<i>beta hemolytic streptococcus</i>	1.42×10 <sup>3</sup>	1.21%
<i>E. coli</i> O157:H7	8.85×10 <sup>3</sup>	0%
<i>Enterobacter sakazakii</i>	4.95×10 <sup>3</sup>	0%