Electronic Supplementary Material (ESI) for Analytical Methods.

Supplementary Information

Simultaneous quantitative analysis of *Listeria monocytogenes* and *Staphylococcus aureus* based on antibiotic-introduced lateral flow immunoassay

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Table S1 Optimization of the ratio of Van, AuNPs and BSA in the probe

group	Van	BSA	AuNPs	Colorimetric effect	Background interference
1	0.5 mg	1 mg	5 mL	+	+
2	1 mg	1 mg	5 mL	+ +	+
3	2 mg	1 mg	5 mL	+ + +	+
4	**5 mg**	**1 mg**	**10 mL**	**+ + + +**	**+**
5	10 mg	1 mg	10 mL	+ + + +	+ +
6	20 mg	1 mg	10 mL	+ + + +	+ +

Principle and results of the method work for a gram-negative bacteria

Salmonella enteritidis is one of gram-negative bacteria, which was selected as target model to evaluated the LFA. According to section 2.4, the antibody of *S.enteriti* was coated on the NC film as a T line to establish a test strip sensor; according to section 2.5, a standard sample of *S.enteriti* (10^8 CFU/mL) was prepared; according to section 2.6, 10^8 CFU/mL of *S.enteriti* was tested. As shown in Fig. S1A, a negative test result was obtained, which showed that the proposed LFA based on Van-probe can only identify gram-positive bacteria, so it cannot detect gram-negative bacteria. In addition, according to the method of construction of Van-BSA-Au probe in section 2.3, ampicillin (Amp) was used instead of Van to prepare the Amp-BSA-Au probe, and the LFA was developed to test *S.enteriti*. The principle was illustrated in Fig. S2 and a positive result was obtained in Fig. S1B. Thus, the test strips based on antibiotic probes can promote the detection of more types of bacteria, whether it is gram-positive or negative bacteria, which only need to replace antibiotics (broad-spectrum antibacterial properties and low production cost) and antibodies on the T line (guarantee specificity) to complete the detection of multiple bacteria.

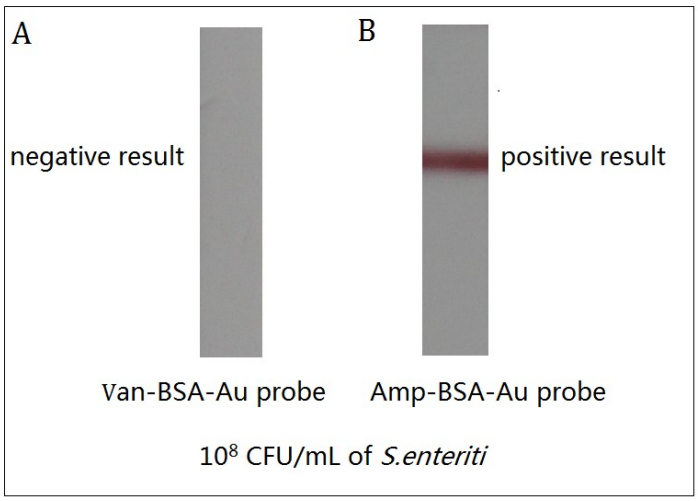

Fig. S1 Detection results of gram-negative bacteria (S.enteriti) by LFA based on different antibiotic probes

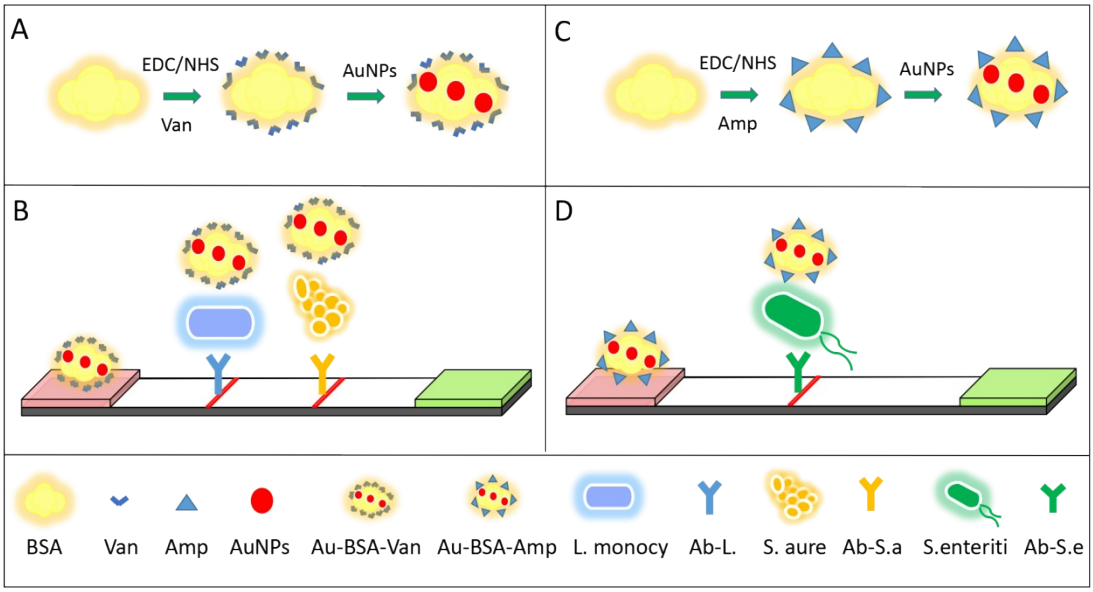

Fig. S2 Probe design and test strip principle for the detection of gram-positive bacteria (*L.monocy* and *S.aure*) (1A, 1B) and gram-negative bacteria (*S.enteriti*) (1C, 1D).

The overall view of the developed LFA

From bottom to top, there are conjugate pad (also called sample pad in this work), NC membrane and absorbent pad. This figure shows the detection results of 10^7 and 10^8 CFU/mL of *S.aure* and *L.monocy*. In order to display and compare the results more clearly and neatly, we only intercepted the image of the detection area in test results.

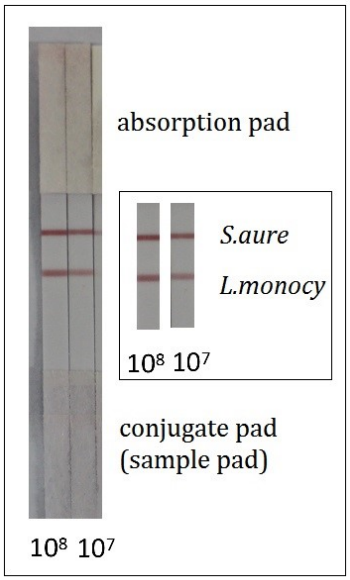

Fig. S3 The overall view of the test strip and the cropped image of the detection area.

Specific reaction evaluation in real samples

Potable water, milk and broth identified as aseptic foods by plate culture counting method were used as real samples to evaluate specific interactions. All these samples were artificially contaminated with 10^7 CFU/mL of these kinds of bacteria, including *L.monocy*, *S.aure*, *S.enteri*, *L.acidophilus*, *E.coli*, *S.cerevisiae*, *Acetobacter and S.lactis*and, and tested with the developed bio-sensors in accordance with the analysis process. The test results was shown in Fig. S4, which indicated that the test strips were free from interference of other bacteria and still maintained good specificity.

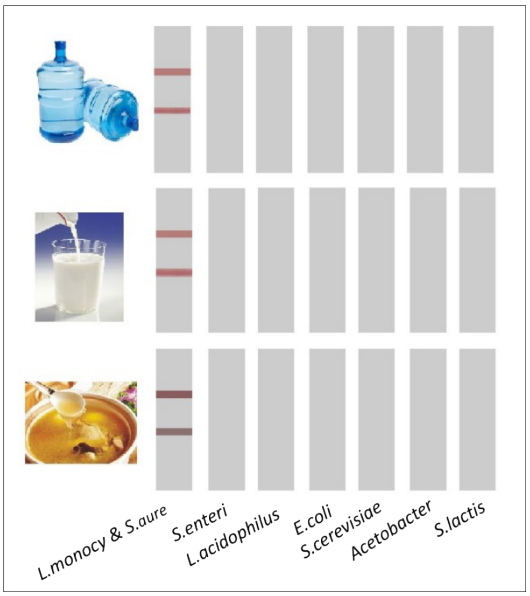

Fig. S4 Specific evaluation results in real samples.

Preparation of 10^8 CFU/mL of bacteria standard solution

The absorbance of bacteria at OD_{600} is proportional to the number of cells per unit volume (CFU/mL), so the bacterial cells per unit volume of solution can be determined based on the absorbance. The absorbance of a solution containing a certain number of cells was measured, and then it was determined by colony counting method. The specific operation steps are as follows: The original solution was diluted into a series of gradient solutions (10×, 100×, 1000×, 10000×,10000× and so on). Then, 200 μL of the diluted solution was spread on the plate. After incubating for a period of time, a plate with clear and countable colonies was selected to count the colonies, which represented the number of colonies in 200 μL. The number of colonies can be further converted into the number contained in 1 mL, and then multiplied by the dilution factor to obtain the number of cells contained in 1 mL of the original solution. Based on this method, at least five bacterial solutions were measured for absorbance and the number of cells (CFU/mL), and these five sets of data were used as a standard curve. According to our rich experience, when the absorbance (OD_{600}) is in the range of 0.4-0.5, the number of cells is 10^8 CFU/mL.

Sensitivity comparison of the developed LFA with traditional LFA

According to different design principles, as shown in the Fig. S5, we have prepared traditional double-antibody-based LFA. The sensitivity of the two kinds of LFA, including visual detection limit (VDL) and limit of detection (LOD) obtained by T lines intensity analysis, were evaluated and compared. The results are shown in the Fig. S6, the VDL were 10^3 CFU/mL (*S.aure*) and 10^5 CFU/mL (*L.monocy*) in antibiotic-based LFA, and 10^4 CFU/mL (*S.aure*) and 10*5* CFU/mL (*L.monocy*) in double-antibody-based LFA, respectively, showing that the VDL in this work was 10 times lower than that of traditional LFA in the assay of *S.aure*. Similarly, the LOD obtained by T lines intensity analysis were 5×10^2 CFU/mL (*S.aure*) and 5×10^4 CFU/mL (*L.monocy*) in antibiotic-based LFA, and 10^4 CFU/mL (*S.aure*) and 10^5 CFU/mL (*L.monocy*) in double-antibody-based LFA, respectively, indicating that the LODs were 20 times lower than that of traditional LFA in the detection of *S.aure*, and 2 times lower than that of traditional LFA in the detection of *L.monocy*, respectively. The results showed that the antibiotic-based LFA we developed have improved sensitivity than that of traditional double-antibody-based LFA.

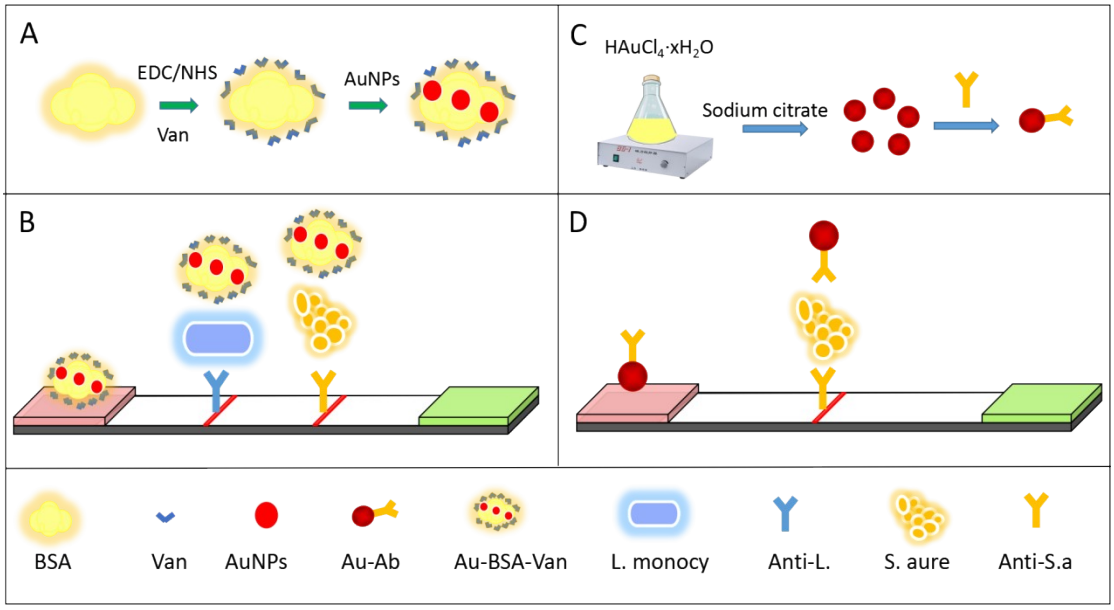

Fig. S5 Comparison (probe design and principle) of the developed LFA with traditional antibody-based LFA for the detection of *L.monocy* and *S.aure*.

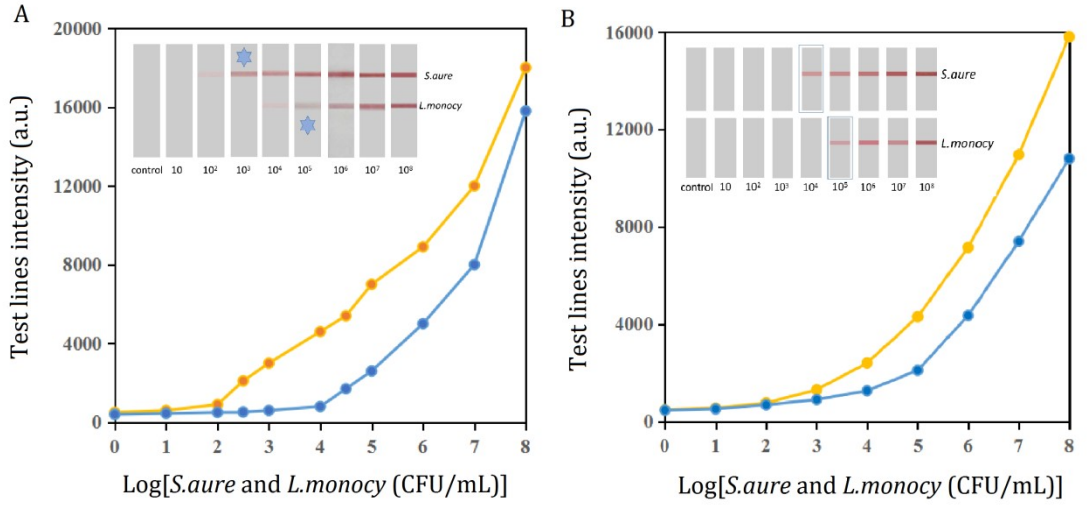

Fig. S6 Sensitivity comparison of the developed LFA with traditional antibody-based LFA for the detection of *L.monocy* and *S.aure*. VDL and LOD of *S.aure* and *L.monocy* (yellow line: *S.aure*; blue line: *L.monocy*).

The application of the developed LFA in contaminated food samples

To evaluate the application of LFA in contaminated food samples, three kinds of actual food samples (potable water, milk and broth) identified as aseptic foods by plate culture counting method were inoculated with *S.aure* and *L.monocy* to an initial bacteria cells of 10^2 CFU/mL. They were cultured on a shaker at 37°C for 24, 48 and 72 hours, respectively. The samples were quantitatively analyzed by ELISA and tested with the designed LFA. The results were shown in the Table. S2. The bacteria cells grew slowly in potable water and milk, and our test strips cannot detect less than 5×10^4 CFU/mL of *L.monocy* and 5×10^2 CFU/mL of *S.aure*, leading negative results. When the number of bacteria cells in the food sample was higher than the LOD of the test strip, the results were positive and quantitative analysis was almost the same as the gold standard ELISA, indicating that the LFA has good analytical performance in actual analysis.

Table S2 Application of the designed LFA in contaminated food samples

Bacteria target	Sample	Culture time	ELISA (log CFU/mL)	LFA (log CFU/mL)
L.monocy	potable water	24	2.04	negative
		48	2.10	negative
		72	2.14	negative
	milk	24	2.20	negative
		48	3.52	negative
		72	6.24	6.39
	broth	24	3.42	negative
		48	5.76	5.82
		72	7.68	7.93
S.aure	potable water	24	2.11	negative
		48	2.18	negative
		72	2.22	negative
	milk	24	2.36	negative
		48	3.89	negative
		72	5.86	5.62
	broth	24	4.16	negative
		48	6.35	6.66
		72	8.26	8.15