Supporting Information

Universal and ultrasensitive detection of foodborne bacteria on a lateral flow assay strip by using wheat germ agglutinin-modified magnetic SERS nanotag

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S1. Experimental section

S1.1. Instruments and SERS measurement conditions

TEM images of Au@MNP-WGA-bacteria and HRTEM images of Au@MNP were taken on Hitachi H-7650 TEM and Tecnai G2 F20 microscope, respectively. The scanning electron microscope (SEM) images were obtained by JEOL JSM-7001F instrument. Elemental mapping images were recorded by energy-dispersive X-ray spectroscopy (EDS) using a Philips Tecnai G2 F20 microscope equipped with a STEM unit. Zeta potential was determined using a Mastersizer 2000 (Malvern, UK). UV-vis spectra were obtained with a Shimadzu 2600 spectrometer. Raman spectra was recorded by a B&W Tek portable Raman spectrometer (i-Raman Plus BWS465-785H).

The SERS detection was standardized by setting uniform detection conditions and data recording was performed using a portable Raman spectrometer with 785 nm laser excitation (B&W Tek, i-Raman Plus BWS465-785H). The laser power, integration time, and spot size of the Raman instrument were set at 5 mW, 10 s, and 100 μm respectively to unify the signals of the material and the test lines of LFA. To make the SERS results more accurate and reduce experimental errors, the front part of test line of each LFA was randomly measured 20 times to calculate the average value for data analysis. Finally, all data were smoothed and baseline corrected using the bundled software.

The SERS mapping images of the test line were obtained using a Renishaw invia plus Raman system excited by a 785 nm excitation source, with laser power and acquisition time set at 1% and 1 s respectively. The positions of each test line were observed by the Olympus BX51 optical microscope and a computer-controlled x–y translational stage was used to scan the selected area in 800 × 600 μm (containing 300 pixels).

S1.2. Preparation of bacteria sample and evaluation the capture efficiency of Au@MNP-WGA to bacteria

The concentration of bacteria was obtained by routine plate counting method. The steps are briefly described as follows: *L. mono*, *S. aureus*, and *C. jejuni* were grown on 5% sheep blood agar plates. *L. mono* and *S. aureus* were incubated at 37°C, 5% CO_2 for 12 hours, and *C. jejuni* at 37°C, 10% CO_2, 2.5% O_2 for 36 hours. Then 20 colonies were obtained from the plate and transferred into 1 mL PBS solution (10 mM, pH 7.4) as the initial bacterial solution. After diluting the stock solution $1\times10^5 \sim 1\times10^7$ times, 0.1 mL of the solution was placed on a blood agar plate and incubated under the above conditions. Then the number of colony forming units (CFU) was measured. According to the CFU counting results, the initial bacterial solution was adjusted to the detected concentration.

The ability of the WGA-modified Fe_3O_4@Au MNPs to capture different bacteria from a complex solution was investigated. First, 1 mL of bacteria solution containing 10^3 cells mL^{-1} of bacteria was prepared. Then, 0.1 mL of bacteria solution was placed

on a blood agar plate as the blank control. Subsequently, 5 μL of Au@MNP-WGA or Au@MNP (10 mg mL^{-1}) was incubated with the rest of bacterial samples (10^3 cells mL^{-1}) for 20 min, and the complexes were enriched by magnet. 0.1 mL of the supernatant was then coated onto the blood agar plate as positive sample. Finally, the bacteria colonies in blank and positive samples were counted after plate culture for 12 h, and the capture rate (%) was then calculated.

S1.3. Preparation of immuno-Au@MNP-based SERS-LFA

The conjugation of Au@MNP and anti-*S. aureus* antibodies was conducted via the carbodiimide chemistry. First, 1mL of Au@MNP was washed and resuspended in 500 μL MES buffer (100 mM, pH 5.5), and then mixed with 5 μL of EDC (0.1 M) and 10 μL of sulfo-NHS (0.1 M). After activation for 15 min, the mixture was washed to remove the excess activators (EDC/sulfo-NHS) and dispersed in 200 μL of PBS buffer (10 mM, pH 7.4). Then, the activated Au@MNP solution was incubated with 30 μg of *S. aureus* antibodies for 2 h, followed by surface blocking with 100 μL BSA solution (100 cells mL^{-1}) for 30 min. The antibody-conjugated Au@MNPs were magnetically collected, and resuspended with 200 μL of storage solution (10 mM PBS containing 1 mg BSA (w/v), 0.5% sucrose (w/v), 0.02% NaN_3 (w/v)).

S1.4. Detection of bacteria by using immuno-Au@MNP-based SERS-LFA

The detection assay for *S. aureus* was performed in a 1.5 mL EP tube and on a test strip. First, 5 μL of Au@MNPs were added into 1 mL of sample solution, and the mixture was shaken for 15 min on an oscillator at room temperature. Then, the formed Au@MNP-antibody-bacteria complexes were rapidly separated by using an external magnet, resuspended in 100 μL of loading solution (10 mM PBS, pH 7.4, 10% FBS, 1% Tween 20), and then loaded onto the sample pad of tested strip to start the chromatographic reaction. After 15 min, the SERS signal on the test line was detected using a portable Raman instrument.

S2. Optimization of the Au@MNP-WGA-based SERS-LFA

Our previous studies verified that PBS solution (10 mM, pH 7.4) containing 1% Tween

can ensure the good transport of big magnetic SERS tags (~200 nm) on the LFA strip [1]. On this basis, different sealing agents were added into the PBST (1% Tween) buffer to increase the sensitivity and specificity of the LFA method. As displayed in Fig. S1, using PBS solution containing 0.5% milk, 10% FBS, and 1% Tween as the running buffer can effectively reduce non-specific signals and maximize the signal-to-noise ratio (SNR) value for bacterial detection. The concentration of the detection antibody on the test line greatly affects the sensitivity of the LFA method and was therefore studied as well. As displayed in Fig. S2, 1 mg mL^{-1} anti-*L. mono*, 1 mg mL^{-1} anti-*C. jejuni*, and 1.4 mg mL^{-1} anti-*S. aureus* antibodies achieved the highest sensitivity for the corresponding pathogens. Next, we assessed the optimal immunoreaction time of LFA method for bacteria detection as it is the key index for rapid testing. The results in Fig. S3 demonstrate that 15 min of chromatographic separation time was suitable for SERS–LFA to generate the highest SNR on the test line.

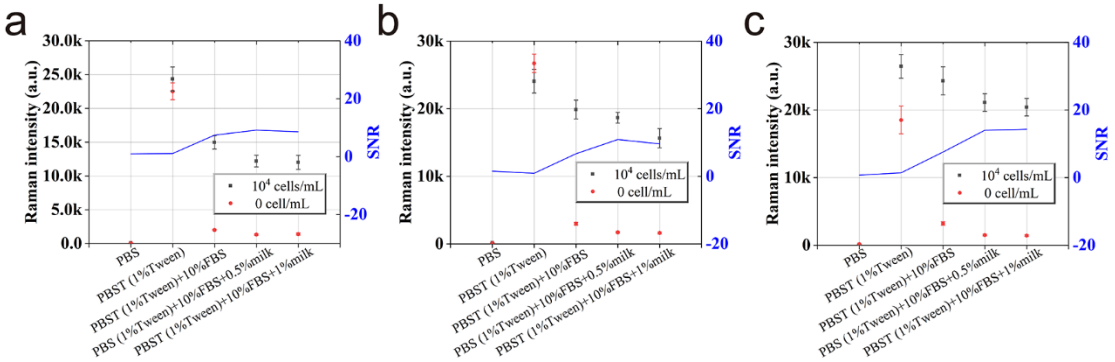

Fig. S1 Optimization of running buffer for (a) *L. mono* strip, (b) *C. jejuni* strip, and (c) *S. aureus* strip. The error bars indicate standard deviations calculated from three measurements.

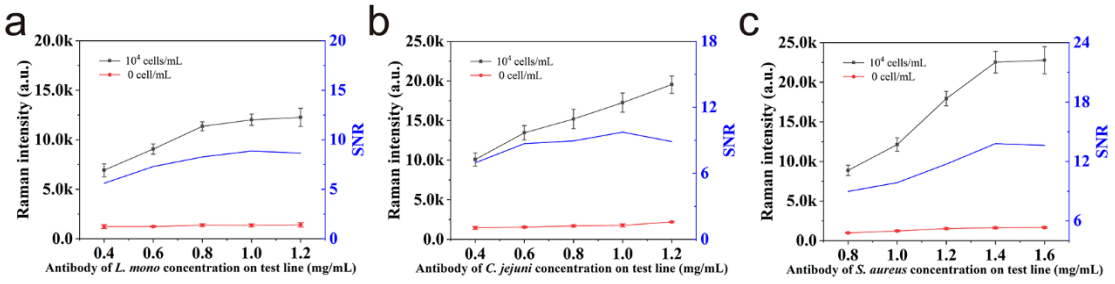

Fig. S2 Optimization of capture antibody concentration on test line for (a) *L. mono*, (b) *C. jejuni*, and (c) *S. aureus*. The error bars indicate standard deviations calculated from

three measurements.

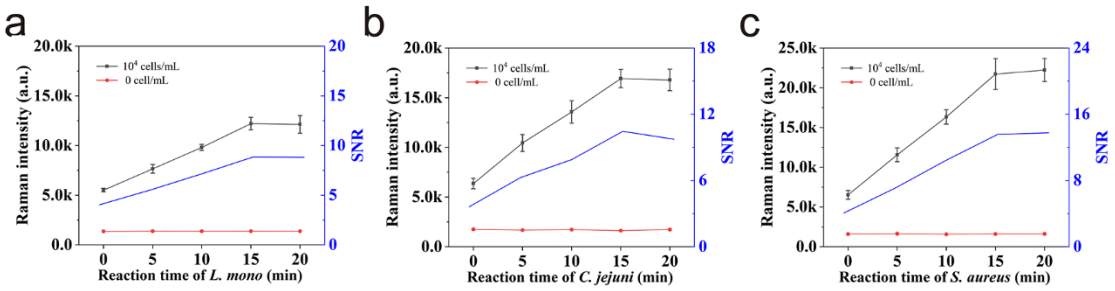

Fig. S3 Optimization of (a) *L. mono*, (b) *C. jejuni*, and (c) *S. aureus* reaction time on the test line for the LFA detection system. The error bars indicate standard deviations calculated from three measurements.

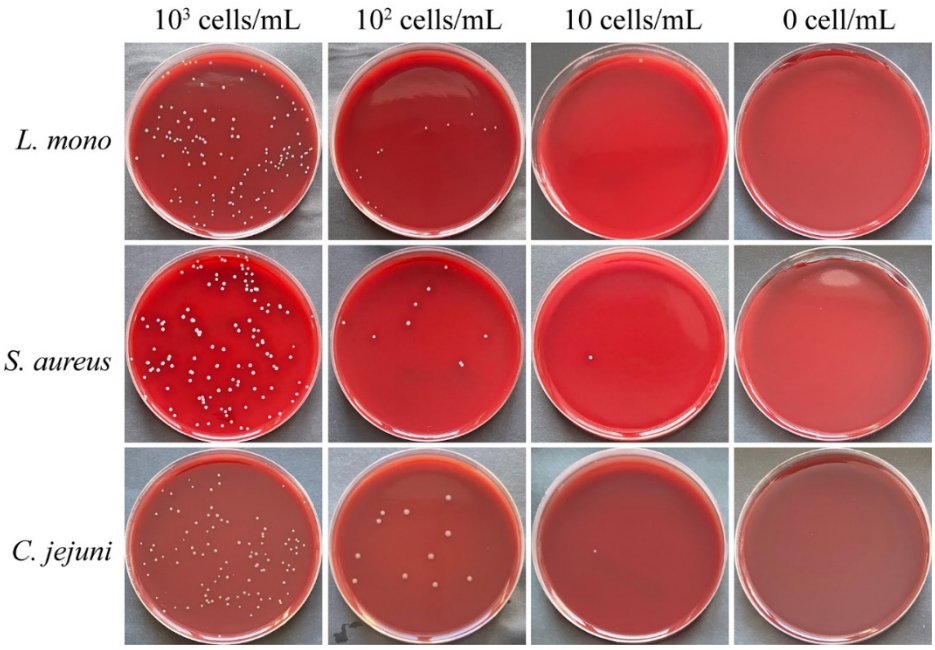

Fig. S4 Standard plate counting method for quantitative determination of *L. mono*, *S. aureus*, and *C. jejuni*.

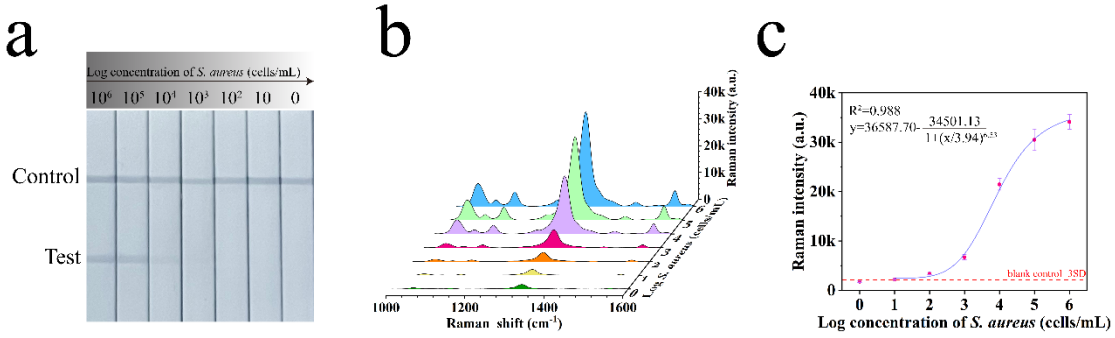

Fig. S5 (a) Photograph of LFA strip with C line for *S. aureus*. (b) Corresponding Raman

spectra of test lines for different concentrations of *S. aureus*. (c) Corresponding calibration line of *S. aureus*. The error bars indicate the standard deviations calculated from five independent measurements.

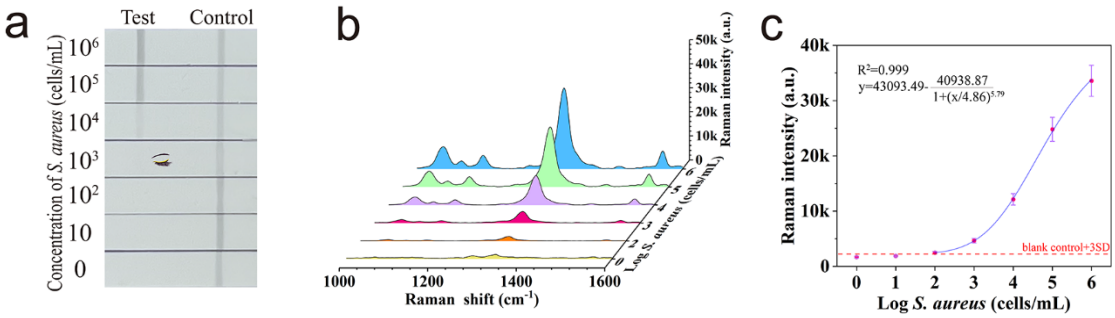

Fig. S6 (a) Photograph of LFA strip for *S. aureus*. (b) Corresponding Raman spectra of test lines for different concentrations of *S. aureus*. (c) Corresponding calibration line of *S. aureus*. The error bars indicate the standard deviations calculated from five independent measurements.

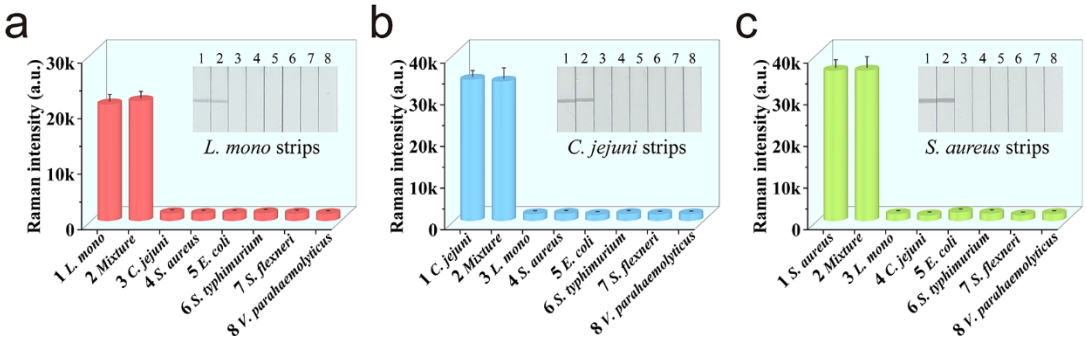

Fig. S7 Specificity of the Au@MNP-WGA-based SERS–LFA. Photographic images and Raman intensities at 1331 cm^{-1} of the (a) *L. mono* strips, (b) *C. jejuni* strips, and (c) *S. aureus* strips for different bacterial samples at 1.0×10^6 cells mL^{-1}. The mixture sample contains *L. mono* (10^6 cells mL^{-1}), *C. jejuni* (10^6 cells mL^{-1}), and *S. aureus* (10^6 cells mL^{-1}). Error bars indicate the standard deviations calculated from five separate experiments.

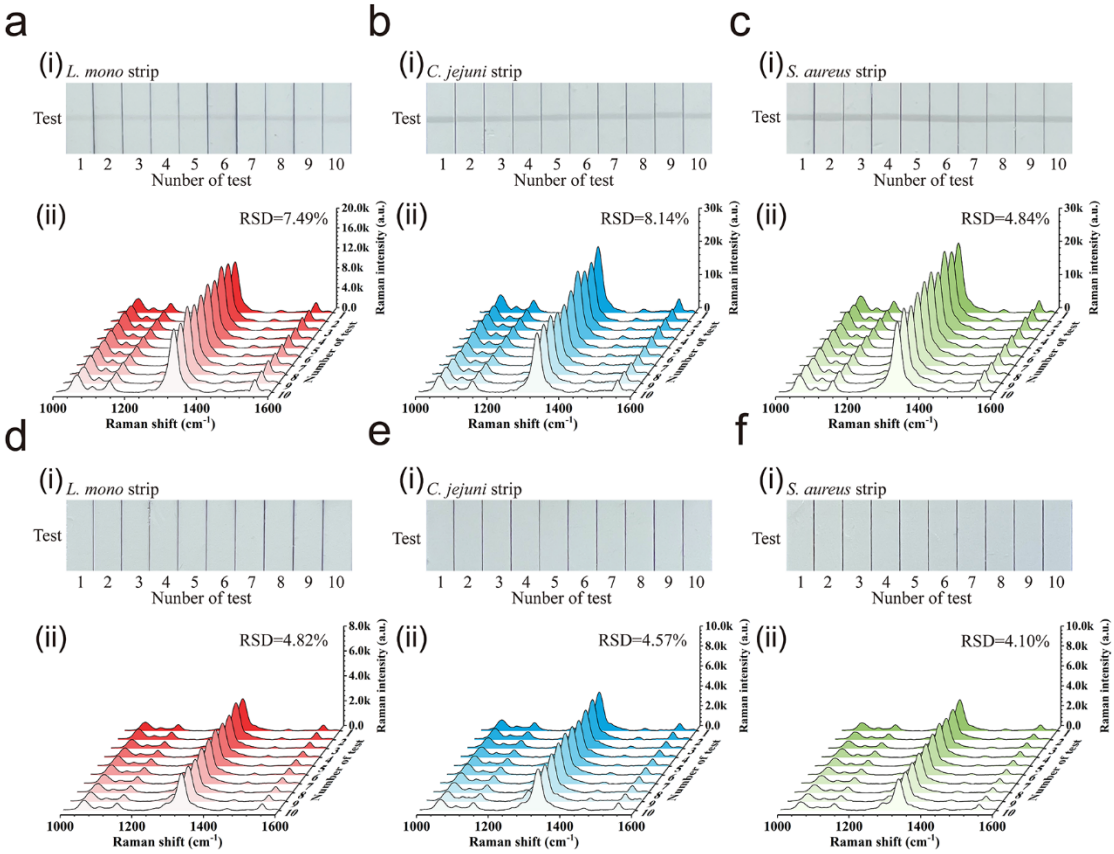

Fig. S8 Reproducibility of Au@MNP-WGA-based SERS-LFA for 10^4 cells mL^{-1} (a) and 10^2 cells mL^{-1} (b) of *L. mono*, 10^4 cells mL^{-1} (b) and 10^2 cells mL^{-1} (e) of *C. jejuni*, and 10^4 cells mL^{-1} (c) and 10^2 cells mL^{-1} (f) of *S. aureus*: (i) Digital photographic images, (ii) corresponding Raman spectra on test lines of tested strips.

Table. S1 The capture efficiency of Au@MNP-WGA for different bacteria (10^2-10^3 cells mL^{-1}).

Bacteria	Capture rate (%)
Staphylococcus aureus	94.26
Campylobacter jejuni	91.43
Listeria monocytogenes	94.52
Shigella sonnei	62.34
Shigella flexneri	92.38
Vibrio parahemolyticus	53.25
Staphylococcus epidermidis	93.83
Streptococcus pyogenes	86.45
Klebsiella pneumoniae	46.01
Acinetobacter baumannii	54.04
Bacillus anthracis	76.90
Vibrio cholera	85.56

References

[1] C. Wang, C. Wang, X. Wang, K. Wang, Y. Zhu, Z. Rong, et al., Magnetic SERS Strip for Sensitive and Simultaneous Detection of Respiratory Viruses, ACS applied materials & interfaces, 11(2019) 19495-505.