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SERS-Based Lateral Flow Strip Biosensor for Simultaneous Detection of *Listeria monocytogenes* and *Salmonella enterica* Serotype Enteritidis

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Supporting Information

ABSTRACT: Rapid, sensitive, point-of-care detection of bacteria is extremely important in food safety. To address this requirement, we developed a new surface-enhanced Raman scattering (SERS)-based lateral flow (LF) strip biosensor combined with recombinase polymerase amplification (RPA) for simultaneous detection of *Listeria monocytogenes* and *Salmonella enterica* serotype Enteritidis. Au^{MBA}@Ag core−shell nanoparticles were used in this SERS-LF. Highly sensitive quantitative detection is achieved by measuring the characteristic peak intensities of SERS tags. Under optimal conditions, the SERS intensities of MBA at 1077 cm^{-1} on test lines are used to measure *S.* Enteritidis ($y = 1980.6x - 539.3$, $R^2 = 0.9834$) and *L. monocytogenes* ($y = 1696.0x - 844$, $R^2 = 0.9889$), respectively. The limit of detection is 27 CFU/mL for *S.* Enteritidis and 19 CFU/mL for *L. monocytogenes*. Significantly, this SERS-LF has high specificity and applicability in the detection of *L. monocytogenes* and *S.* Enteritidis in food samples. Therefore, the SERS-LF is a feasible method for the rapid and quantitative detection of a broad range of bacterial pathogens in real food samples.

KEYWORDS: *recombinase polymerase amplification, foodborne pathogen, surface-enhanced Raman scattering, food safety*

INTRODUCTION

Foodborne illnesses caused by microorganisms have attracted worldwide attention in the food industry and scientific research.[1,2] The most common prevalent foodborne pathogens are *Salmonella enterica*, *Staphylococcus aureus*, *Listeria monocytogenes*, and *Bacillus cereus*.[3] *Salmonella* is one of the most common enteric pathogens and can cause salmonellosis in humans who consume contaminated poultry, meat, and eggs.[4] One of the most common serotypes related to salmonellosis is *S. enterica* serovar Enteritidis.[5] *L. monocytogenes* is associated with listeriosis and is more likely to lead to death.[6] Most human listeriosis cases were caused by consumption of contaminated food. Meat, poultry, milk, and vegetables are the most frequently food associated with foodborne illnesses. Therefore, feasible and accurate detection methods are urgently required for the identification and control of the two pathogens.

The current standard method for the detection of pathogens is based on culturing and includes sequential steps of pre-enrichment, selective enrichment and biochemical identification; this generally takes 2−3 days to complete.[7] Many new methods have been established for foodborne pathogen detection, such as real-time polymerase chain reaction (RT-PCR),[8] enzyme-linked immunosorbent assays (ELISAs),[9] electrochemical immunosensors,[10] and polymerase chain reaction (PCR). However, RT-PCR and electrochemical immunosensors are complicated and require skilled technicians.[11] The main disadvantage of ELISAs is a complicated washing procedure. PCR is considered a sensitive method for pathogen detection and could amplify low levels of target DNA to detectable levels in a few hours.[12] However, it requires a temperature cycling instrument and relies on a highly trained operator, which limits it use in the field.

To circumvent the limitations of PCR, several isothermal amplification techniques to amplify nucleic acids have been established to avoid the requirement for thermal cycling equipment. Recombinase polymerase amplification (RPA) is one representative isothermal amplification technique with great potential in point-of-use detection. RPA has been applied to develop detection methods for intestinal protozoa,[13] plant pathogens,[14] group B streptococcus,[15] and human noroviruses.[16] RPA is based on a recombinase enzyme to facilitate the insertion and binding of the forward and the reverse primers to their complementary sequence within the template.[17] RPA could react at a constant temperature (37−42 °C) in short time (<30 min) and eliminate the use of thermocycling equipment which makes it fit for point-of-use detection. However, RPA is mostly used in the detection of single targets, and the amplified products are detected by gel electrophoresis or through lateral flow strips (LF). Gel electrophoresis is time-consuming and laborious and requires complex and expensive instruments.[18] Traditional LF uses colloidal gold nanoparticles as the labels to visualize the results.[19,20] However, although LF methods are

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Table 1. Primers of Multiplex Recombinase Polymerase Amplification Used in This Study

target	primer name	sequence (5′-3′)
S. *enteritidis*	forward primer	GCATCCGCACAGATAAAT-digoxin
	reverse primer	TACACCACCAGATACCGAAGCCCCT-biotin
L. monocytogenes	forward primer	GTAAGTGGGAAATCTGTCTCAGGTGATGTAG-FITC
	reverse primer	ACTCCTGGTGTTTCTCGATTAAAAGTAGCA-biotin

simple, the major limitations include the lack of sensitivity[21] and the lack of quantification.[22]

To resolve these problems, a surface-enhanced Raman scattering (SERS)-based LF biosensor was established.[23−25] SERS spectroscopy is an ultrasensitive detection technique that allows trace and nondestructive detection.[26] The use of a Raman detection reader combined with LF gives a quantitative and highly sensitivity evaluation of the analyte. In SERS-LF method, the colloidal gold nanoparticles were replaced by Raman reporter-labeled SERS nanotags. The Raman signals greatly increased as the reporter molecules were absorbed onto the surface of the nanoparticles, which is known as the SERS-active point due to electromagnetic and chemical enhancement effects.[25] SERS-LF has been used to detect a single target, including HIV-1 DNA,[24] thyroid-stimulating hormone,[25] and Staphylococcal Enterotoxin B.[27] Wang et al. reported a SERS-based lateral flow biosensor for simultaneous detection of dual DNA markers.[28] In these detections, gold nanoparticles (AuNPs) with simple structure were used in SERS analysis. It was reported that modified AuNPs, such as Au@Ag NPs, could significantly further improved Raman signals.[29,30] However, Au@Ag NPs have not been used in combination with RPA to detect bacterial pathogens. Furthermore, there are great challenges to develop rapid methods for simultaneously quantitative detection of multiple pathogens in food samples.[31]

In the present study, we established a novel SERS-based RPA-LF strip for quantitative and simultaneous analysis of *L. monocytogenes* and *S.* Enteritidis. In this system, Au^{MBA}@Ag NPs were used as labels and RPA was used to amplify two target DNAs in a single tube in less than 20 min. The results were detected by multiplex lateral flow strips containing two test lines and one control line, which could give visual bands due to the accumulation of nanoparticles in the test and control zones. The specific SERS signals of MBA (4-mercaptobenzoic acid) on the test lines were measured using a confocal micro-Raman spectroscopic system (Renishaw, Gloucestershire, UK) to quantify *L. monocytogenes* and *S.* Enteritidis. To the best of our knowledge, this assay is the first report of the use of RPA for the simultaneous detection of two pathogens using a SERS-based LF biosensor in a single sample. This detection technique is a promising multiplex DNA detection biosensor for foodborne pathogen detection.

MATERIALS AND METHODS

Materials. RPA basic amplification kits were obtained from TwistDx, Ltd. (Babraham, UK). All primers (Table 1) were obtained from GENEWIZ (Suzhou, China) and suspended in water to a concentration of 10 μM. The PCR product purification kit was purchased from TAKARA (Takara Shuzo, Kyoto, Japan). Mouse anti-digoxin monoclonal antibody (mAb) and mouse anti-FITC monoclonal antibody were purchased from Abcam (Abcam Beijing Zhongyuan, Ltd., Beijing, China). Gold chloride trihydrate, trisodium citrate dihydrate, sliver nitrate ($AgNO_3$), L-ascorbic acid, streptavidin, phosphate-buffered saline (PBS, 0.01 M), Tween 20, polyethylene glycol (PEG) 20000, bovine serum albumin (BSA), agarose, 4-mercaptobenzoic acid (MBA), and ethidium bromide were obtained from Sigma-Aldrich (St. Louis, MO). The plastic backing, sample pad, absorption pad, and three types of nitrocellulose (NC) membrane (HF90, HF135, HF180) were acquired from Millipore (Billerica, MA). Water was purified with a Milli-Q system (Madrid, Spain). All solvents and other chemicals were analytical reagent grade.

Bacterial Strains and Genomic DNA Extraction. A total of four *S.* Enteritidis, four *L. monocytogenes*, and nine other bacterial strains from various sources were used to determine the specificity and sensitivity of the developed RPA-LF-SERS assay in the study (Table 2). These strains were obtained from the China Center of Industrial Culture Collection (CICC), American Type Culture Collection (ATCC), and China Microbiological Culture Collection (CMCC) and were isolated by our laboratory in chicken breast (160609). All strains were cultivated in Luria−Bertani broth (Beijing Land Bridge Technology Co., Ltd., Beijing, China) at 37 °C overnight and serially 10-fold diluted in 0.9% normal saline (NaCl). Bacterial concentration of each dilution was determined by conventional plating assay (Plate Count Agar, PCA). Simultaneously, 1 mL of each dilution was heated at 99 °C for 5 min, and the crude cell lysate was centrifuged at 10000*g* for 5 min. The supernatant was removed to a new centrifuge tube and used as an RPA template.

Table 2. Bacterial Strains Used in Our Study

species	source
Listeria monocytogenes	CICC21633
Listeria monocytogenes	ATCC19115
Listeria monocytogenes	ATCC7644
Listeria monocytogenes	ATCC19111
Salmonella enteritidis	CMCC50041
Salmonella enterica subsp. enterica	CICC10982
Salmonella enteritidis	ATCC13076
Salmonella enteritidis	160609
Salmonella paratyphi-A	CICC21501
Salmonella cholerae-suis var. kunzendorf	CICC21494
Escherichia coli O157:H7	ATCC35150
Shigella flexneri	CICC10865
Escherichia coli	CICC10305
Staphylococcus aureus	ATCC25923
Shigella sonnei	CICC21535
Enterobacter aerogenes	CICC10293
Campylobacter jejuni subsp. jejuni	ATCC33560

Primer Design and Multiplex RPA. Two sets of primers were designed based on the sequence of the highly serovar-specific and conservative segments of *S.* Enteritidis (GenBank Accession No: L03833) and *L. monocytogenes* (GenBank Accession No: AF253320) to detect the individual target bacteria using multiplex RPA. The primers with target-specific labels were designed and tagged with digoxin and biotin or FITC and biotin (Table 1). The specificity of these primers is shown in Figure S1, indicating that the primers have good specificity.

Multiplex RPA assays were performed according to the recommended protocol from the manufacturer with slight modifications. Each reaction contained 29.5 μL of rehydration buffer, 5.9 μL of sterile water, 2.4 μL (10 μM) of each *S.* Enteritidis primer, 2.4 μL (10 μM) of each *L. monocytogenes* forward and reverse primer, and 1.25 μL of each template. After rehydrating the enzyme pellet, 2.5 μL of magnesium acetate solution was added to the tube to start the reaction. The tubes

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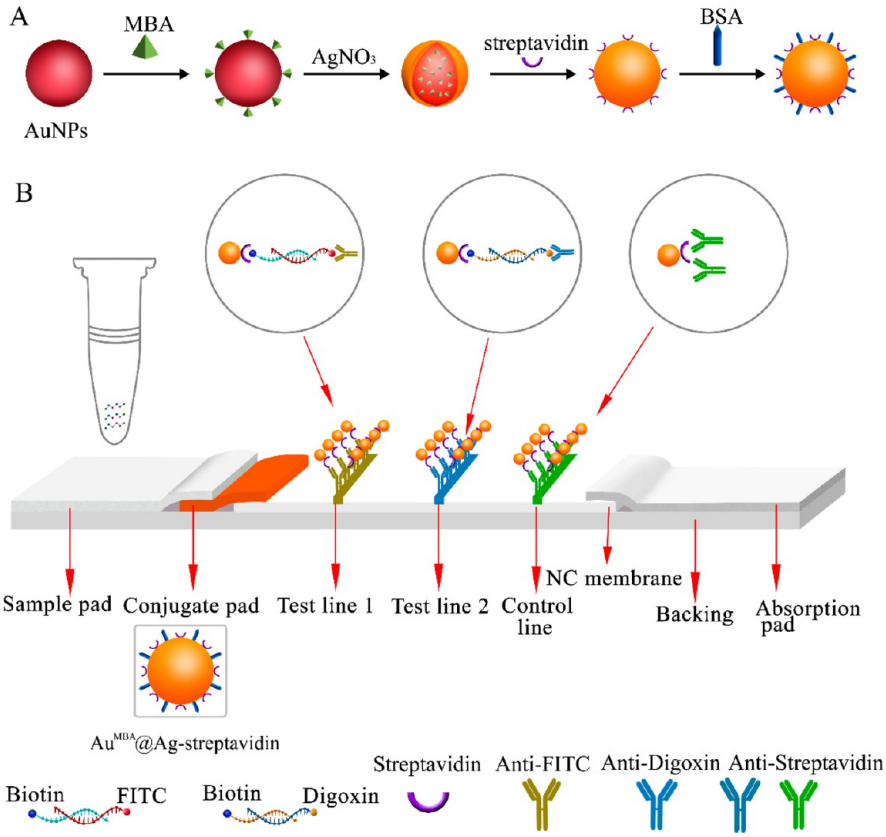

Figure 1. Schematic illustration of the preparation of Au^{MBA}@Ag-streptavidin (A) and the multiplex RPA-LF-SERS assay (B).

were briefly vortexed and centrifuged to thoroughly mix the samples. The RPA reactions were performed at 39 °C for 15 min. Finally, 5 μL of RPA products were verified by 2% gel electrophoresis.

Preparation of Streptavidin-Conjugated Au^{MBA}@Ag Nanoparticles. The fabrication of the streptavidin-conjugated Au^{MBA}@Ag SERS tags is illustrated in Figure 1A. AuNPs (nanospheres) were prepared according to previously reported methods[27] and characterized by field emission electron microscopy (JEM-2010 F, JEOL Ltd., Tokyo, Japan) and ultraviolet/visible (UV−vis) absorption spectroscopy (Evolution 300, Thermo Fisher, USA) (Figure 2A, B). The AuNPs (2 mL) were mixed with 2.5 μL of 1 M MBA and stirred at room temperature for 10 min. The MBA-modified AuNPs were purified by centrifugation at 8000*g* for 10 min and resuspended in 2 mL of ultrapure water.

Au^{MBA}@Ag core−shell nanoparticles were synthesized using an established method.[28] With slight modification, 0.4 mL of 0.1 M ascorbic acid was mixed with the above Au^{MBA} NPs under stirring for 5 min. Then, 1.2 mL of 1 mM $AgNO_3$ was dropwise added; the silver ions were reduced by the ascorbic acid to Ag metal, and continuously precipitated on Au^{MBA} NPs to form the Au^{MBA}@Ag core−shell nanoparticles. With the continuous addition of $AgNO_3$, the solution gradually changed from purple-red to orange-yellow. These resultant nanoparticles (Au^{MBA}@Ag) were purified by centrifugation and resuspended with the same volume of ultrapure water.

The pH of the suspension of Au^{MBA}@Ag was adjusted to 8 with 0.2 M K_2CO_3. In a separate tube, 10 μL of 2 mg/mL streptavidin was mixed with 390 μL of 2 mM sodium borate. Then, 1 mL of the above Au^{MBA}@Ag solution was mixed with the diluted streptavidin and reacted for 1 h at room temperature.[32] Finally, 20 μL of 20% BSA and 10 μL of 20% PEG20000 were added to the mixture and incubated for 15 min at 4 °C to prevent nonspecific binding. The resultant Au^{MBA}@Ag-streptavidin was purified by centrifugation at 10000*g* for 30 min at 4 °C. The sediment was resuspended with 200 μL of working buffer (5% bovine serum albumin, 0.025% Tween 20, 1% sodium azide, and 10% surfactant G). The Au^{MBA}@Ag-streptavidin solution was spread on the conjunction pad at 25 μL/cm and dried under vacuum overnight.

Preparation of the Lateral Flow Strip. The lateral flow strip was composed of a sample pad, a conjunction pad, an NC membrane with two test lines and one control line, an absorbent pad, and a bottom pad. The suitable NC membrane was selected from HF90, HF135, and HF180, and their capillary flow rate was 90, 135, and 180 s/4 cm, respectively. The two test lines were prepared by separately spraying them with 1 mg/mL mouse anti-digoxin monoclonal antibody and 0.6 mg/mL mouse anti-FITC monoclonal antibody. The control line was formed by spreading 1 mg/mL mouse anti-streptavidin monoclonal antibody on it. Each antibody was dispensed at a speed of 0.9 μL/cm. The NC membrane was dried at 37 °C for 6 h. To assemble the LF strips, the NC membrane was pasted on the center of the bottom pad, overlapping with absorption pad and sample pad on each side. The assembled pad was cut to a 3.7 mm width using a programmable cutter (Zeta Corporation, South Korea) and stored in a desiccator at room temperature until use.

Procedure of the RPA-LF-SERS Assay. The principle of RPA-LF-SERS prepared in this work is shown in Figure 1B. Five different types of running buffer including PBS (pH = 7.4), PBS (pH = 5.7), PBS (pH = 8.5), PBS with 0.05%Tween 20, Tris-HCl buffer (pH = 7.4) were used to optimize the diffusion of streptavidin-conjugated Au^{MBA}@Ag nanoparticles. The RPA reaction product was purified using the Takara miniBEST DNA Fragment Purification Kit. The purified RPA product (10 μL) was mixed with 90 μL of running buffer and pipetted onto the sample pad. The mixture together with the Au^{MBA}@Ag-streptavidin probes moved from the sample pad to the absorbent pad. The orange-yellow color of Au^{MBA}@Ag on the test lines and control line gradually appeared and could be observed by the naked eye. The entire LF procedure took approximately 5 min. The Raman signals of MBA integrated in the Au^{MBA}@Ag-streptavidin probes on the two test lines were detected using a confocal micro-Raman spectroscopic system (Renishaw, Gloucestershire, UK). The excitation source was tuned at 785 nm with a laser power of 25 mW,

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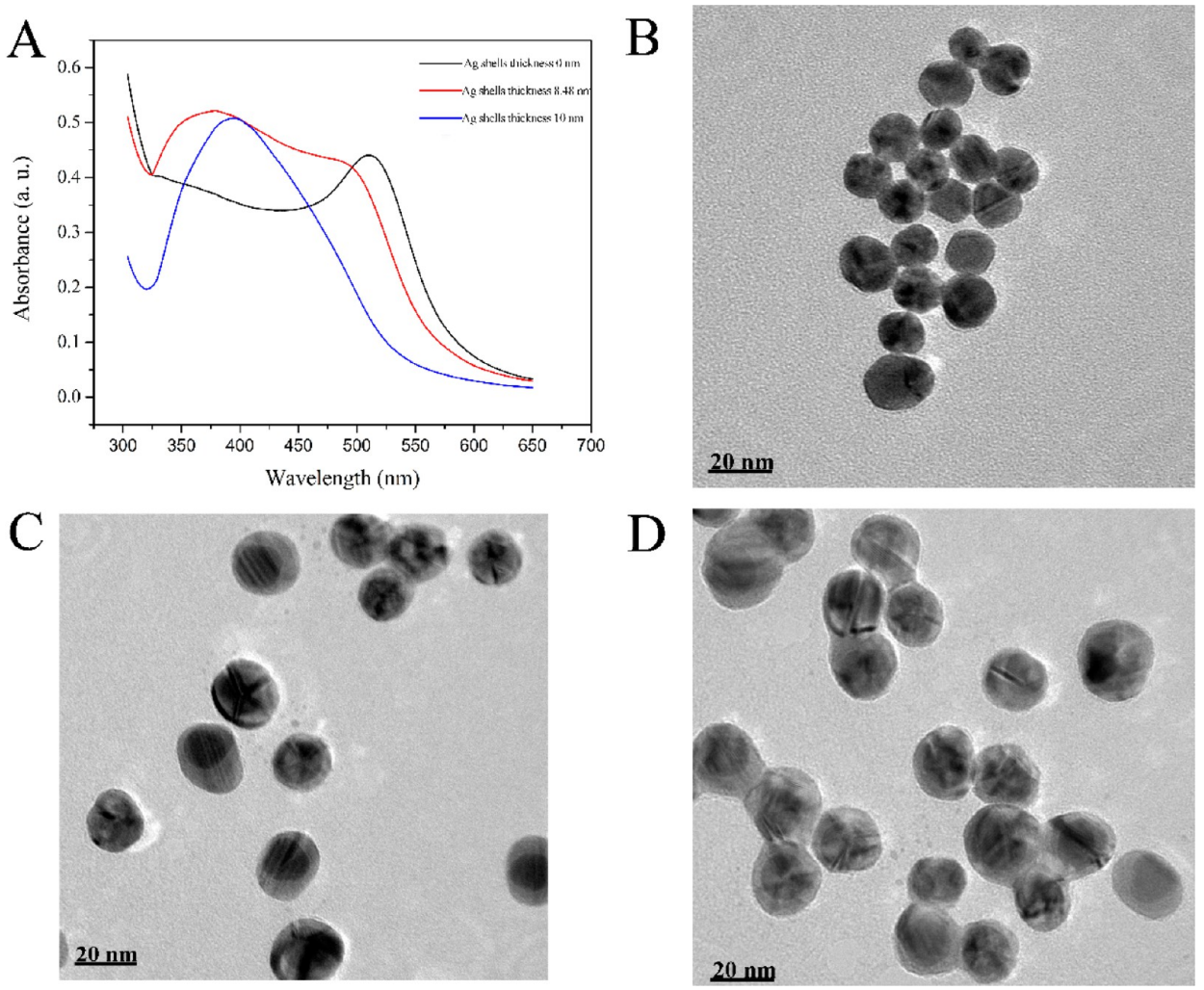

Figure 2. UV−vis (A) and TEM of Au@Ag with different Ag shell thicknesses of 0 nm (B), 8.48 nm (C), and 10 nm (D).

and the integration time was 10 s. The SERS intensity from MBA at 1077 cm^{-1} was collected at 10 different spots on the test lines and used for quantification.

Specificity and Sensitivity of RPA-LF-SERS. The genomic DNAs of four strains of *S.* Enteritidis, four strains of *L. monocytogenes*, and nine strains of other foodborne strains were extracted by boiling at 99 °C for 5 min. A total of 1.25 μL of each template was added to the RPA reaction to determine the specificity of the RPA-LF-SERS assay. The sensitivity of the RPA-LF-SERS assay was tested using 10-fold serial dilutions of *S.* Enteritidis (CMCC 50041) and *L. monocytogenes* (CICC21633) genomic DNA (10^6 to 10^0 CFU/mL). All test reactions were performed at least in triplicate.

Application of RPA-LF-SERS to Artificially Contaminated Food Samples. Milk, chicken breast, and beef were purchased from a local supermarket (Tianjin, China) and verified to be free of *Salmonella* and *L. monocytogenes* according to the National Standard GB/4789.4-2010 and the National Standard GB/4789.30-2010, respectively. *S.* Enteritidis and *L. monocytogenes* were individually cultured in LB enrichment broth at 37 °C overnight, and the cultures were used to prepare various concentrations of S. Enteritidis and *L. monocytogenes* (S. Enteritidis broth and *L. monocytogenes* broth at a 1:1 volume ratio were mixed). Each sample was inoculated with 10^4, 10^3, 10^2, and 10^1 CFU/mL or CFU/g of *S.* Enteritidis and *L. monocytogenes*. Immediately, 1 mL of the inoculated samples was collected, and the DNA of each sample was extracted by boiling at 99 °C for 5 min. The extracted DNA solution was stored at 4 °C and then used in a multiplex RPA reaction. Noninoculated samples were used as a negative control.

Data Analysis. SERS spectra were acquired using a confocal micro-Raman spectroscopic system (Renishaw, Gloucestershire, UK) and was baseline corrected and placed in a same horizontal line (*y*-axis is about 0). All data were processed using Origin 8.5 (Origin lab corporation, Northampton, MA) to smooth the spectra for the removal of background signals.

RESULTS

Characterization of Au^{MBA}@Ag. The morphologies and size distribution of AuNPs were characterized by TEM and UV−vis. As shown in Figure 2A, B, the AuNPs were homogeneous, with an average size of 15.1 nm. Au^{MBA} was prepared by simply adding MBA solution to the colloidal gold nanoparticles, and MBA was attached to the AuNPs via Au−S bonds. The Au^{MBA}@Ag was synthesized by ascorbic acid reduction of silver nitrate, and Ag continuously adhere to the surface of the Au^{MBA}. With the addition of $AgNO_3$, the color of Au^{MBA}@Ag gradually changed from purple-red to orange-yellow. As characterized by TEM (JEM-2010 F, JEOL Ltd., Tokyo, Japan) and UV−vis (Evolution 300, Thermo Fisher, USA), Au^{MBA}@Ag with an 8.48 nm shell thickness was monodisperse, with an average diameter of 23.58 nm (Figure 2C). However, when the shell thickness increased to 10 nm (Figure 2D), the monodispersity of the nanoparticles was destroyed.

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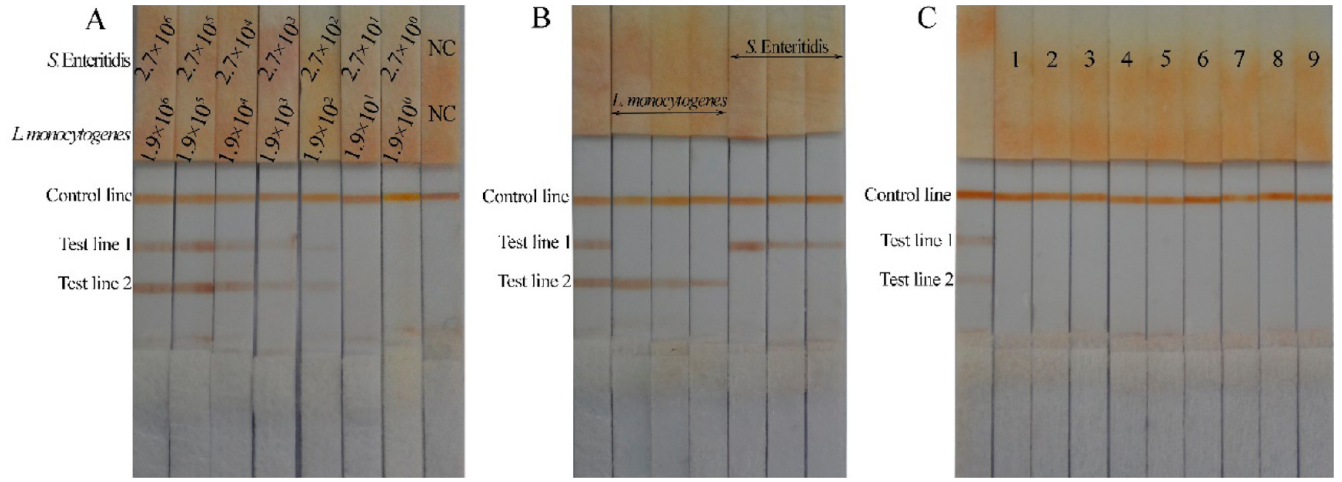

Figure 3. (A) Sensitivity results of multiplex lateral flow strips for the detection of *S.* Enteritidis (test line 1) and *L. monocytogenes* (test line 2). Test lines could be observed by the naked eye at a concentration of 1.9×10^2 CFU/mL of *L. monocytogenes* and 2.7×10^2 CFU/mL of *S.* Enteritidis. The SERS signal of the test lines could be obtained at a concentration of 1.9×10^1 CFU/mL of *L. monocytogenes* and 2.7×10^1 CFU/mL of *S.* Enteritidis. Molecular specificity of the RPA-LF assay for three *L. monocytogenes*, three *S.* Enteritidis bacteria (B), and nine other bacteria (C). (1) *Salmonella paratyphi-A*, (2) *Salmonella cholerae-suis* var. kunzendorf, (3) *Escherichia coli* O157:H7, (4) *Shigella flexneri*, (5) *Escherichia coli*, (6) *Staphylococcus aureus*, (7) *Shigella sonnei*, (8) *Enterobacter aerogenes*, and (9) *Campylobacter jejuni* subsp. jejuni.

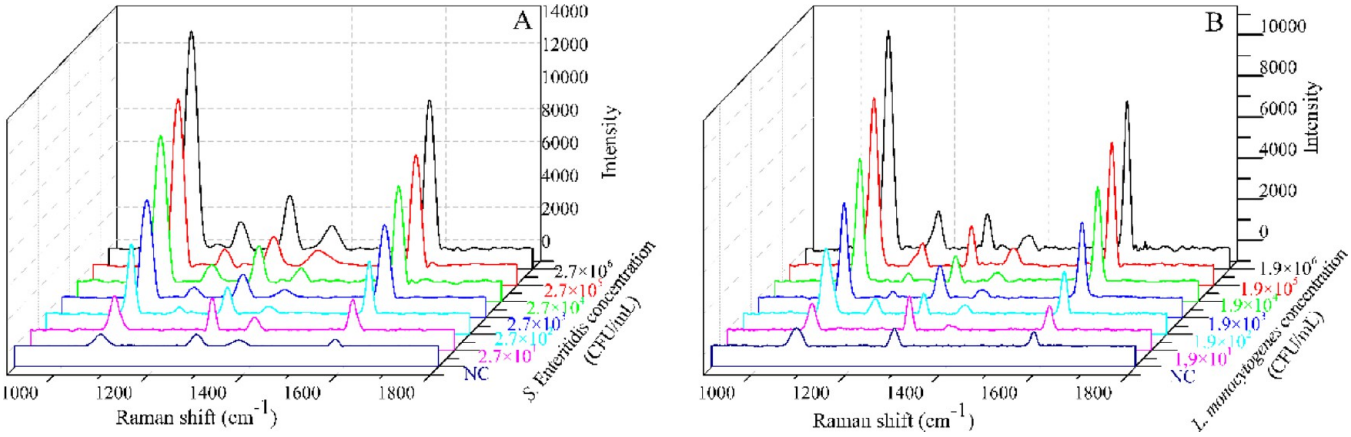

Figure 4. Raman spectra from MBA on test line 1 (A for detection of *S.* Enteritidis) and test line 2 (B for detection of *L. monocytogenes*) with RPA products of different concentrations of *S.* Enteritidis and *L. monocytogenes*. The SERS peak intensity centered at 1077 cm^{-1} was used for quantitative analysis of *S.* Enteritidis and *L. monocytogenes*. The bacterial concentration of *L. monocytogenes* and *S.* Enteritidis ranged from 1.9×10^0 to 1.9×10^6 CFU/mL and from 2.7×10^0 to 2.7×10^6 CFU/mL, respectively.

Optimization of the Lateral Flow Strip. Three types of NC membrane, HF90, HF135, and HF180, were tested to determine which gave the satisfied orange-yellow bands. As shown in Figure S2A, the color of the orange-yellow band on HF135 is brighter than HF90 and HF180. Therefore, HF135 is selected in this SERS-LF assay. In addition, the running buffer solution strongly affects the migration of Au^{MBA}@Ag nanoparticles. Therefore, five different types of running buffer solution: Tris-HCl buffer (pH = 7.4), PBS in three different pH (pH = 5.7, pH = 7.4, pH = 8.5), and PBS with 0.05% Tween 20. As shown in Figure S2B, the color of the visual band in the LF using PBS (pH = 7.4) as running buffer was significantly brighter than those using other buffer solutions. Based on these results, PBS (pH = 7.4) was selected as the optimum running buffer solution.

Sensitivity and Quantitative Ability of RPA-LF-SERS. The RPA reaction was performed according to the manufacturer's quick guide. The RPA products were first analyzed by agarose gel electrophoresis (AGE). The reactions for the detection of *L. monocytogenes* and *S.* Enteritidis produced two visual bands at 180 bp and 267 bp, respectively (Figure S3). The brightness of the bands depended on the bacterial concentration. The concentration of *L. monocytogenes* and *S.* Enteritidis ranged from 1.9×10^0 to 1.9×10^6 CFU/mL and from 2.7×10^0 to 2.7×10^6 CFU/mL. The sensitivity of RPA-AGE for the detection of *L. monocytogenes* and *S.* Enteritidis was 1.9×10^4 and 2.7×10^4 CFU/mL, respectively (Figure S3).

The RPA-LF assay was performed under the optimal conditions, and 10 μL of the purified multiplex RPA product was diluted with 90 μL of PBS (pH 7.4) and pipetted onto the sample pad. The color of the test and control lines was visible to the naked eye after 5 min. Figure 3A shows digital photographic images of LF for the simultaneous detection of digoxin and FITC on multiplex RPA products. With increasing bacterial concentration, more sandwich complexes are formed, and the color of the test lines increases proportionally. Furthermore, the color of the test lines was not visible to the

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naked eye below bacterial concentrations of *L. monocytogenes* and *S.* Enteritidis of 1.9×10^2 and 2.7×10^2 CFU/mL, respectively. Therefore, 1.9×10^2 and 2.7×10^2 CFU/mL were considered the visual detection limit of the RPA-LF for the simultaneous detection of *L. monocytogenes* and *S.* Enteritidis.

Using a SERS-based LF strip, it is possible to achieve quantitative results by recording the Raman intensity of the MBA in Au^{MBA}@Ag core−shell nanoparticles, as MBA has characteristic Raman peaks at 1077 and 1583 cm^{-1} (Figure 4). Quantitative analysis of *L. monocytogenes* and *S.* Enteritidis was performed by monitoring the Raman peak intensity of test line 1 and test line 2 at 1077 cm^{-1}, which is a stronger signal than that at 1583 cm^{-1}. The SERS spectra intensity of the corresponding test lines is shown in Figure 4, and the SERS intensity increased with increasing DNA template concentrations of *L. monocytogenes* and *S.* Enteritidis. The resulting standard curve of SERS intensity versus the logarithm of target bacterial concentration is displayed in Figure 5. The linear

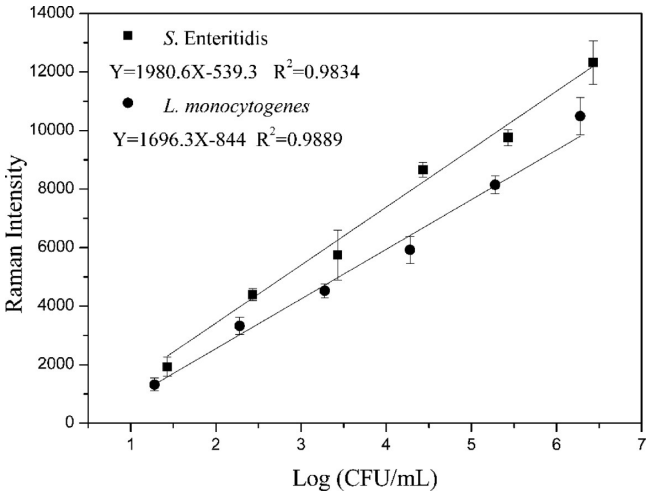

Figure 5. Standard curve of Raman intensity on a SERS-based lateral flow strip for the detection of *S.* Enteritidis (■) and *L. monocytogenes* (●). Error bars indicate standard deviations calculated from three parallel measurements.

curves have correlation coefficients of determination for *L. monocytogenes* and *S.* Enteritidis of 0.9834 and 0.9889, respectively. The limit of detection was defined as the Raman signals intensity is 2-fold higher $(S/N > 2)$[4] than the average signal of negative control. At the concentrations of 1.9×10^1 CFU/mL of *L. monocytogenes* and 2.7×10^1 CFU/mL of *S.* Enteritidis, the SERS intensity of test line 1 and test line 2 was 1857.6 and 1362.2, respectively, 3.4- and 2.5-fold higher than the negative control, with a SERS intensity of 549.0 calculated at 1077 cm^{-1}. Therefore, the detection limit of RPA-LF-SERS was 1.9×10^1 CFU/mL of *L. monocytogenes* and 2.7×10^1 CFU/mL of *S.* Enteritidis.

Specificity of the RPA-LF-SERS Assay. To evaluate the specificity of the SERS-based strip assay, four strains of *L. monocytogenes*, four strains of *S.* Enteritidis, and nine other foodborne strains at OD0.6 were applied to the RPA-LF-SERS assay; the results are displayed in Figure 3. As expected, only the *L. monocytogenes* and *S.* Enteritidis strains displayed positive results (Figure 3B), and no obvious color changes were observed when testing other bacterial strains (Figure 3C).

Application to Selected Food Samples. Milk, chicken breast, and beef were collected for *L. monocytogenes* and *S.* Enteritidis detection. The samples were spiked with different concentrations of *L. monocytogenes* and *S.* Enteritidis, and the genomic DNAs of each tested samples were directly extracted without enrichment and tested with our RPA-LF-SERS. As shown in Table 3 and Figure 6, the recoveries of *L.*

Table 3. Recoveries of *S.* Enteritidis and *L. monocytgenes* in Selected Food Samples by SERS-LF

sample	*S.* Enteritidis concn in spiked samples (CFU/mL)	recovery (%, $n = 3$)	*L. monocytegenes* concn in spiked samples (CFU/mL)	recovery (%, $n = 3$)
milk	7.6×10^4	91.34	8.5×10^4	105.63
	8.2×10^3	90.46	6.9×10^3	107.68
	9.8×10^2	109.90	5.6×10^2	113.86
	3.1×10^1	95.63	3.6×10^1	103.55
chicken breast	1.9×10^5	106.16	9.2×10^4	104.44
	1.49×10^4	91.76	1.5×10^4	96.80
	8.5×10^2	92.76	7.3×10^2	94.65
	3.5×10^1	93.27	2.9×10^1	95.30
beef	1.2×10^5	104.09	1.6×10^5	100.94
	1.3×10^4	96.78	8.6×10^3	103.83
	2×10^3	105.57	1×10^3	97.21
	3.5×10^1	95.27	2.2×10^1	96.21

monocytogenes in milk, chicken breast, and beef were 94.65−113.86%. Meanwhile, the recoveries of *S.* Enteritidis in milk, chicken breast, and beef were 90.46−109.90%. The RPA-LF-SERS biosensor has a detection limit of *S.* Enteritidis in milk, chicken breast, and beef of 31, 35, and 35 CFU/mL, respectively. The detection limit of *L. monocytogenes* in milk, chicken breast, and beef is 36, 29, and 22 CFU/mL, respectively. The detection limit of bacteria in food samples is slightly higher than that in a pure bacterial solution.

Reproducibility of the RPA-LF-SERS Assay. Two LF strips with *L. monocytogenes* and *S.* Enteritidis concentrations at 1.9×10^6 and 2.7×10^6 CFU/mL or at 1.9×10^2 and 2.7×10^2 CFU/mL were tested. The SERS intensities at 1077 cm^{-1} from 10 parallel spots on the middle of the test lines were measured and displayed in Figure 7. Within each strip, the RSD values of the 10 different spots on each test lines were 5.99%, 4.26%, 5.81%, and 11.07%, indicating the high precision of the SERS signal.

■ DISCUSSION

A SERS-based lateral flow strip composed of two test lines and one control line was developed for simultaneous detection of two pathogens, *S.* Enteritidis and *L. monocytogenes*. The capture antibodies (McAb-digoxin and McAb-FITC) and control antibodies (McAb-streptavidin) were dispensed on the test lines (test line 1 and test line 2) and control line of the NC membrane, respectively. In the three tested NC membranes, HF135 was found to be the appropriate one in SERS-LF detection and this might because HF135 could provide a suitable capillary flow rate giving a suitable antigen−antibody interaction time. The antibodies were immobilized on the NC membrane via electrostatic interactions, achieved by interactions between the dipoles of the nitrate group in the NC membrane and the dipoles of the peptide bonds in the antibody.[33] In the conjunction pad, the streptavidin with positive charges is passively attached to Au^{MBA}@Ag via electrostatic interaction[34] to form monodisperse Au^{MBA}@Ag-

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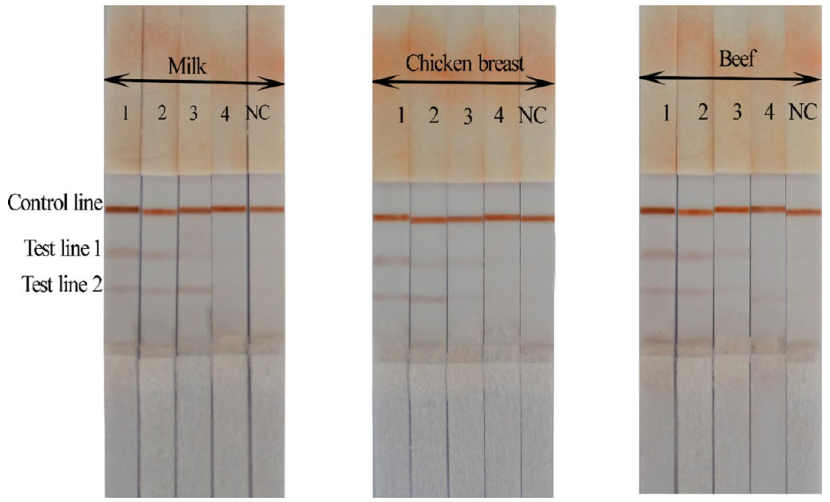

Figure 6. Detection of different concentrations of *S.* Enteritidis and *L. monocytogenes* in simulated food samples using a multiplex lateral flow strip: 1, 2, 3, and 4 represent milk inoculated with 7.6×10^4, 8.2×10^3, 9.8×10^2, and 3.1×10^1 CFU/mL *S.* Enteritidis and 8.5×10^4, 6.9×10^3, 5.6×10^2, and 3.6×10^1 CFU/mL *L. monocytogenes*; 1, 2, 3, and 4 represent chicken breast inoculated with 1.9×10^5, 1.49×10^4, 8.5×10^2, and 3.5×10^1 CFU/mL *S.* Enteritidis and 9.2×10^4, 1.5×10^4, 7.3×10^2, and 2.9×10^1 CFU/mL *L. monocytogenes*; 1, 2, 3, and 4 represent beef inoculated with 1.2×10^5, 1.3×10^4, 2×10^3, and 3.5×10^1 CFU/mL *S.* Enteritidis and 1.6×10^5, 8.6×10^3, 1×10^3, and 2.2×10^2 CFU/mL *L. monocytogenes*; NC represents the negative control.

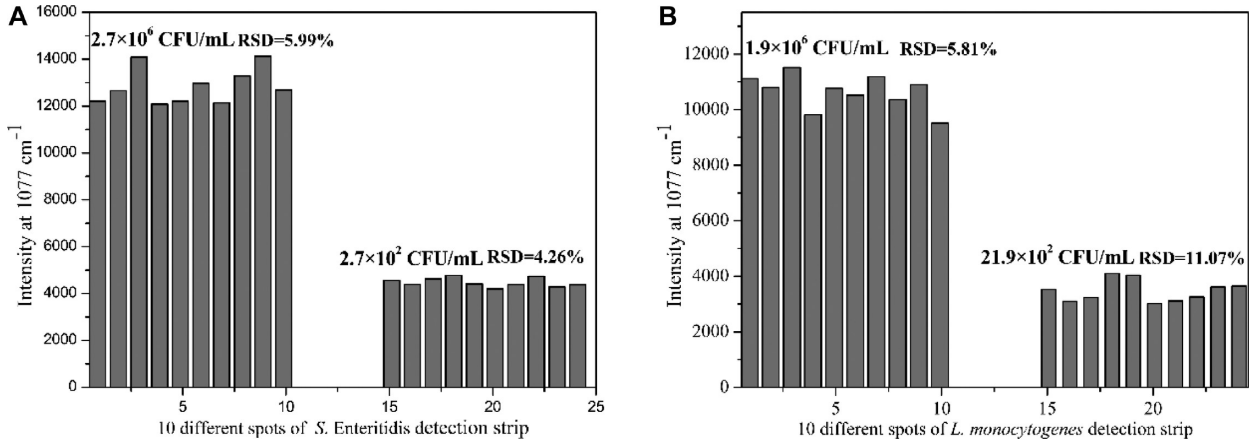

Figure 7. SERS intensities of MBA at 1077 cm^{-1} from 10 different spots in the middle of test line 1 (A, for detection of *S.* Enteritidis) and middle of test line 2 (B, for detection of *L. monocytogenes*).

streptavidin. The multiplex RPA was used to generate numerous biotin- and digoxin-labeled or biotin- and FITC-labeled duplex DNAs. The product solution migrated toward the absorption pad by capillary force,[32] passed through the conjunction pad, and rehydrated the streptavidin-coated Au^{MBA}@Ag. The biotin on the duplex DNA reacted with the streptavidin on the surface of the Au^{MBA}@Ag to form digoxin-duplex DNA-biotin-streptavidin-Au^{MBA}@Ag or FITC-duplex DNA-biotin−streptavidin-Au^{MBA}@Ag, which was then captured by the antidigoxin antibody on test line 1 or the anti-FITC antibody on test line 2. Two visual bands appeared in the test zone because the immunoreaction led to increased Au^{MBA}@Ag. Excess Au^{MBA}@Ag- streptavidin complex continued to migrate and was then captured by the mouse antistreptavidin monoclonal antibody on the control line, which was used as a control to determine whether the LF strip worked properly. Regardless of the presence of target bacteria, the control line always appeared. In the presence of *S.* Enteritidis and *L. monocytogenes*, two visible orange-yellow lines in the test zone appeared (Figure 3A). If only *S.* Enteritidis or *L. monocytogenes* is present in the sample, one orange-yellow line forms (Figure 3B). Moreover, the addition of various concentrations of RPA products could result in corresponding amounts of Au^{MBA}@Ag captured by the test line. Consequently, the Raman intensity of MBA can be expressed as different concentrations of RPA product, proportional to the concentration of target DNA.[4,35] Therefore, it is possible to achieve a quantitative result by measuring the characteristic SERS peak intensities of the test lines.[24]

Raman active Au^{MBA}@Ag nanoparticles were used in the SERS-based LF strip, and the Raman enhancement effect of Au@Ag colloids is stronger than that for pure Au or Ag.[28] MBA absorbed on Au serves as a SERS donor, and the Raman signal of MBA was intensified due to the formation of SERS "hot spots" in the gap between Au and Ag. Therefore, Au@Ag greatly enhanced the sensitivity of the LF-SERS assay. Uniform Au@Ag NPs were synthesized by a two-step reaction. The AuNP colloid was first prepared, and ascorbic acid reduced silver nitrate to Ag metal. Because of the similarity of the crystalline lattice between Au and Ag, the Ag shell continuously

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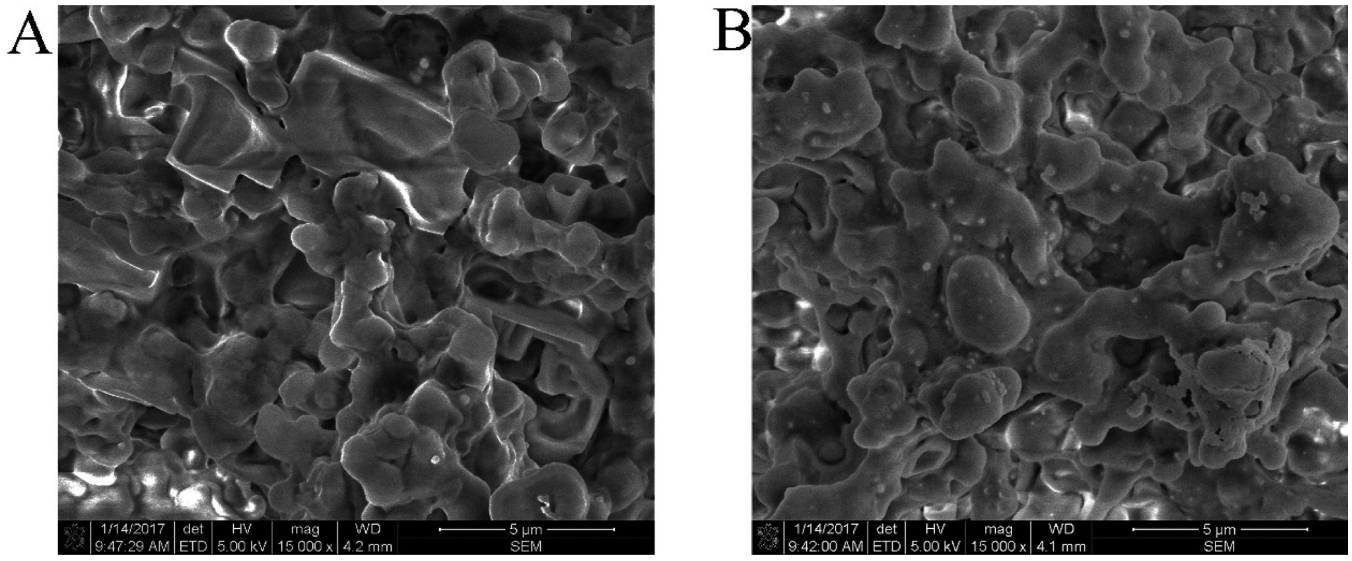

Figure 8. SEM images of SERS-based lateral flow strips in the absence (A) and presence (B) of target bacteria.

adhere to the surface of Au core. Core−shell Au@Ag with an obvious color change from the wine red of AuNPs to orange-yellow was synthesized and characterized by TEM and UV−vis (Figure 2). Au@Ag NPs with 8.48 nm silver shells are highly monodisperse and possess two plasmon resonance frequencies of Au and Ag (Figure 2A red line). Au@Ag with 8.48 nm silver shells has a wide, strong plasmon resonance that is responsible for the maximum Raman enhancement effect. With an increased Ag shell thickness of 10 nm (Figure 2D), the plasmon resonance of the Au core decreased and the two resonances merged into a single peak at a wavelength similar to that for the AgNPs (Figure 2A blue line).

Numerous methods have been established to detect pathogenic bacteria in food samples, such as the use of electrochemical immunosensors,[10] immunomagnetic separation,[38] and surface plasmon resonance.[36−40] However, these methods employ complicated operations, requiring time-consuming procedures that severely limit their suitability for point-of-use detection. DNA amplification methods such as PCR can amplify trace DNA to detectable levels.[13] Although PCR is regarded as a sensitive and specific method for the detection of pathogenic bacteria, it requires specialized thermal cycling equipment, complicated sample pretreatment and trained technicians.[40] A number of isothermal amplification methods have been developed to eliminate the use of thermal cycling equipment, including helicase-dependent amplification, rolling circle amplification, and loop-mediated isothermal amplification. Among these methods, RPA shows significant advantages such as a short incubation time, lower and single incubation temperatures (35−45 °C), and high tolerance to sample impurities. However, the established methods combined with RPA have mostly been used in the detection of a single target. In this study, we exploit the potency of RPA for the simultaneous amplification of multiple DNA of pathogenic bacteria. According to our study, RPA can successfully amplify *S.* Enteritidis and *L. monocytogenes* DNA in one tube to detectable levels, decreasing sample consumption, reducing the cost per assay and improving throughput of detection. To the best of our knowledge, this is the first time that simultaneous detection of *S.* Enteritidis and *L. monocytogenes* has given a quantification result.

The RPA-LF-SERS strip was developed for the highly sensitive and quantitative detection of pathogenic bacteria. This assay can simultaneously detect as few as 1.9×10^1 CFU/mL *L. monocytogenes* or 2.7×10^1 CFU/mL *S.* Enteritidis with Raman intensity of 1362.2 and 1857.5, which is at least 2-fold higher than ($S/N > 2$) the average SERS signal for negative control with SERS signal intensity of 549 calculated at 1077 cm^{-1}. This detection limit is equivalent to that of LAMP-SERS for the quantification detection of *S.* Enteritidis,[4] lower than that of a flow colloidal gold immunoassay strip for simultaneous detection of *Shigella boydii* and *E. coli*,[41] and lower than that of multiplexed RPA for the detection of protozoa.[13] This high detection sensitivity can be attributed to the high amplification efficiency of RPA, the Raman enhancement of Au@Ag and the single-molecule level of SERS detection. According to the SEM (SEM, JEOL-IT300, Akishima, Japan) image of the positive result on the test line (Figure 8B), Au^{MBA}@Ag clusters in the fiber pores created many interparticle gaps and produced a strong SERS signal.[42] Furthermore, the detection limit of LF with two test lines was almost the same as that of the LF with only one test line (Figure S4), demonstrating that the sensitivity of RPA-LF was not affected by the addition of the second test line. In addition, quantitative analysis could be obtained by measuring the SERS signal intensity at 1077 cm^{-1}. In the absence of target bacteria, immunocomplexes did not appear on the test line (Figure 8A), and the SERS intensity was extraordinarily weak. However, in the presence of target bacteria, immunocomplexes captured by the test line produced strong SERS signals.

The RPA-LF-SERS biosensor is suitable for point-of-use detection by the naked eye since the entire detection result could be obtained within 30 min. Furthermore, quantitative analysis was achieved by detection of the SERS intensity of the test lines. Although this additional SERS analysis leads to slightly greater complexity compared to the naked-eye-based LF strip and requires more operation time, SERS detection improved the detection sensitivity, eliminated the enrichment step, and gave quantifiable results.

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■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jafc.7b03957.

Electrophoresis images of primers specific for *S.* Enteritidis and *L. monocytogenes* using four *S.* Enteritidis (A S1−S4), four *L. monocytogenes* (A L1−L4) and nine other bacteria; optimization the type of NC membrane and type of running buffer; sensitivity of RPA-AGE for simultaneous detection of *S.* Enteritidis and *L. monocytogenes*; sensitivity results of RPA-LF for the detection of *L. monocytogenes* and *S.* Enteritidis (PDF)

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Notes

The authors declare no competing financial interest.

■ ABBREVIATIONS USED

SERS, surface-enhanced Raman scattering; LF, lateral flow; RPA, recombinase polymerase amplification; TL, test line; RT-PCR, real-time polymerase chain reaction; ELISAS, enzyme-linked immunosorbent assays; DNA, DNA; MBA, 4-mercapto-benzoic acid; FITC, fluorescein isothiocyanate; $AgNO_3$, sliver nitrate; PBS, phosphate-buffered saline; PEG, polyethylene glycol; BSA, bovine serum albumin; mAb, monoclonal antibody; K_2CO_3, potassium carbonate; CFU, colony-forming unit; LB broth, luria bertani broth; AGE, agarose gel electrophoresis; RSD, relative standard deviation

■ REFERENCES

(1) Jin, B. R.; Wang, S. R.; Lin, M.; Jin, Y.; Zhang, S. J.; Cui, X. Y.; Gong, Y.; Li, A.; Xu, F.; Lu, T. J. Upconversion nanoparticles based FRET aptasensor for rapid and ultrasenstive bacteria detection. *Biosens. Bioelectron.* **2017**, *90*, 525−533.

(2) Shan, S.; Lai, W.; Xiong, Y.; Wei, H.; Xu, H. Novel strategies to enhance lateral flow immunoassay sensitivity for detecting foodborne pathogens. *J. Agric. Food Chem.* **2015**, *63*, 745−753.

(3) Alhogail, S.; Suaifan, G. A. R. Y.; Zourob, M. Rapid colorimetric sensing platform for the detection of Listeria monocytogenes foodborne pathogen. *Biosens. Bioelectron.* **2016**, *86*, 1061−1066.

(4) Draz, M. S.; Lu, X. N. Development of a Loop Mediated Isothermal Amplification (LAMP) - Surface Enhanced Raman spectroscopy (SERS) Assay for the Detection of Salmonella Enterica Serotype Enteritidis. *Theranostics* **2016**, *6*, 522−532.

(5) Li, F.; Li, F.; Chen, B.; Zhou, B.; Yu, P.; Yu, S.; Lai, W.; Xu, H. Sextuplex PCR combined with immunomagnetic separation and PMA treatment for rapid detection and specific identification of viable Salmonella spp., Salmonella enterica serovars Paratyphi B, Salmonella Typhimurium, and Salmonella Enteritidis in raw meat. *Food Control* **2017**, *73*, 587−594.

(6) Alhogail, S.; Suaifan, G.; Zourob, M. Rapid colorimetric sensing platform for the detection of Listeria monocytogenes foodborne pathogen. *Biosens. Bioelectron.* **2016**, *86*, 1061−1066.

(7) Zhao, Y.; Wang, H.; Zhang, P.; Sun, C.; Wang, X.; Wang, X.; Yang, R.; Wang, C.; Zhou, L. Rapid multiplex detection of 10 foodborne pathogens with an up-converting phosphor technology-based 10-channel lateral flow assay. *Sci. Rep.* **2016**, *6*, 21342.

(8) Koskinen, M. T.; Holopainen, J.; Pyorala, S.; Bredbacka, P.; Pitkala, A.; Barkema, H. W.; Bexiga, R.; Roberson, J.; Solverod, L.; Piccinini, R.; Kelton, D.; Lehmusto, H.; Niskala, S.; Salmikivi, L. Analytical specificity and sensitivity of a real-time polymerase chain reaction assay for identification of bovine mastitis pathogens. *J. Dairy Sci.* **2009**, *92*, 952−959.

(9) Song, X.; Shukla, S.; Lee, G.; Park, S.; Kim, M. Detection of Cronobacter Genus in Powdered Infant Formula by Enzyme-linked Immunosorbent Assay Using Anti-Cronobacter Antibody. *Front. Microbiol.* **2016**, *7*, 1124.

(10) Pandey, C. M.; Tiwari, I.; Singh, V. N.; Sood, K. N.; Sumana, G.; Malhotra, B. D. Highly sensitive electrochemical immunosensor based on graphene-wrapped copper oxide-cysteine hierarchical structure for detection of pathogenic bacteria. *Sens. Actuators, B* **2017**, *238*, 1060−1069.

(11) Zhao, Y.; Wang, H. R.; Zhang, P. P.; Sun, C. Y.; Wang, X. C.; Wang, X. R.; Yang, R. F.; Wang, C. B.; Zhou, L. Rapid multiplex detection of 10 foodborne pathogens with an up-converting phosphor technology-based 10-channel lateral flow assay. *Sci. Rep.* **2016**, *6*, 21342.

(12) Fan, Q.; Xie, Z.; Xie, Z.; Deng, X.; Xie, L.; Huang, L.; Luo, S.; Huang, J.; Zhang, Y.; Zeng, T.; Wang, S.; Liu, J.; Pang, Y. Development of a GeXP-multiplex PCR assay for the simultaneous detection and differentiation of six cattle viruses. *PLoS One* **2017**, *12*, e0171287−e0171287.

(13) Crannell, Z.; Castellanos-Gonzalez, A.; Nair, G.; Mejia, R.; White, A. C.; Richards-Kortum, R. Multiplexed Recombinase Polymerase Amplification Assay To Detect Intestinal Protozoa. *Anal. Chem.* **2016**, *88*, 1610−1616.

(14) Lau, H. Y.; Wang, Y.; Wee, E. J. H.; Botella, J. R.; Trau, M. Field Demonstration of a Multiplexed Point-of-Care Diagnostic Platform for Plant Pathogens. *Anal. Chem.* **2016**, *88*, 8074−8081.

(15) Clarke, C.; O'Connor, L.; Carre-Skinner, H.; Piepenburg, O.; Smith, T. J. Development and performance evaluation of a recombinase polymerase amplification assay for the rapid detection of group B streptococcus. *BMC Microbiol.* **2016**, *16* (1), 221.

(16) Moore, M. D.; Jaykus, L. A. Development of a Recombinase Polymerase Amplification Assay for Detection of Epidemic Human Noroviruses. *Sci. Rep.* **2017**, *7*, 40244.

(17) Piepenburg, O.; Williams, C. H.; Stemple, D. L.; Armes, N. A. DNA detection using recombination proteins. *PLoS Biol.* **2006**, *4*, e204.

(18) Wu, W.; Zhao, S.; Mao, Y.; Fang, Z.; Lu, X.; Zeng, L. A sensitive lateral flow biosensor for Escherichia coli O157:H7 detection based on aptamer mediated strand displacement amplification. *Anal. Chim. Acta* **2015**, *861*, 62−68.

(19) Zhang, X.; Yu, X.; Wen, K.; Li, C.; Mujtaba Mari, G.; Jiang, H.; Shi, W.; Shen, J.; Wang, Z. Multiplex Lateral Flow Immunoassays Based on Amorphous Carbon Nanoparticles for Detecting Three Fusarium Mycotoxins in Maize. *J. Agric. Food Chem.* **2017**, *65*, 8063−8071.

(20) Jawaid, W.; Meneely, J. P.; Campbell, K.; Melville, K.; Holmes, S. J.; Rice, J.; Elliott, C. T. Development and validation of a lateral flow immunoassay for the rapid screening of okadaic acid and all dinophysis toxins from shellfish extracts. *J. Agric. Food Chem.* **2015**, *63*, 8574−8583.

(21) Foubert, A.; Beloglazova, N. V.; Gordienko, A.; Tessier, M. D.; Drijvers, E.; Hens, Z.; De Saeger, S. Development of a rainbow lateral flow immunoassay for the simultaneous detection of four mycotoxins. *J. Agric. Food Chem.* **2017**, *65*, 7121−7130.

(22) Joung, H.-A.; Oh, Y. K.; Kim, M.-G. An automatic enzyme immunoassay based on a chemiluminescent lateral flow immunosensor. *Biosens. Bioelectron.* **2014**, *53*, 330−335.
(23) Wang, X.; Choi, N.; Cheng, Z.; Ko, J.; Chen, L. X.; Choo, J. Simultaneous Detection of Dual Nucleic Acids Using a SERS-Based Lateral Flow Assay Biosensor. *Anal. Chem.* **2017**, *89*, 1163−1169.
(24) Fu, X.; Cheng, Z.; Yu, J.; Choo, P.; Chen, L.; Choo, J. A SERS-based lateral flow assay biosensor for highly sensitive detection of HIV-1 DNA. *Biosens. Bioelectron.* **2016**, *78*, 530−537.
(25) Choi, S.; Hwang, J.; Lee, S.; Lim, D. W.; Joo, H.; Choo, J. Quantitative analysis of thyroid-stimulating hormone (TSH) using SERS-based lateral flow immunoassay. *Sens. Actuators, B* **2017**, *240*, 358−364.
(26) Li, J. F.; Huang, Y. F.; Ding, Y.; Yang, Z. L.; Li, S. B.; Zhou, X. S.; Fan, F. R.; Zhang, W.; Zhou, Z. Y.; Wu, D. Y.; Ren, B.; Wang, Z. L.; Tian, Z. Q. Shell-isolated nanoparticle-enhanced Raman spectroscopy. *Nature* **2010**, *464*, 392−395.
(27) Hwang, J.; Lee, S.; Choo, J. Application of a SERS-based lateral flow immunoassay strip for the rapid and sensitive detection of staphylococcal enterotoxin B. *Nanoscale* **2016**, *8*, 11418−11425.
(28) Wang, X.; Choi, N.; Cheng, Z.; Ko, J.; Chen, L.; Choo, J. Simultaneous Detection of Dual Nucleic Acids Using a SERS-Based Lateral Flow Assay Biosensor. *Anal. Chem.* **2017**, *89*, 1163−1169.
(29) Liu, B.; Han, G.; Zhang, Z.; Liu, R.; Jiang, C.; Wang, S.; Han, M.-Y. Shell Thickness-Dependent Raman Enhancement for Rapid Identification and Detection of Pesticide Residues at Fruit Peels. *Anal. Chem.* **2012**, *84*, 255−261.
(30) Karn-orachai, K.; Sakamoto, K.; Laocharoensuk, R.; Bamrungsap, S.; Dharakul, T.; Miki, K. SERS-based immunoassay on 2D-arrays of Au@ Ag core−shell nanoparticles: influence of the sizes of the SERS probe and sandwich immunocomplex on the sensitivity. *RSC Adv.* **2017**, *7*, 14099−14106.
(31) Yang, L.; Li, Y. Simultaneous detection of Escherichia coli O157: H7 and Salmonella Typhimurium using quantum dots as fluorescence labels. *Analyst* **2006**, *131*, 394−401.
(32) Toubanaki, D. K.; Christopoulos, T. K.; Ioannou, P. C.; Flordellis, C. S. Identification of Single-Nucleotide Polymorphisms by the Oligonucleotide Ligation Reaction: A DNA Biosensor for Simultaneous Visual Detection of Both Alleles. *Anal. Chem.* **2009**, *81*, 218−224.
(33) Low, S. C.; Shaimi, R.; Thandaithabany, Y.; Lim, J. K.; Ahmad, A. L.; Ismail, A. Electrophoretic interactions between nitrocellulose membranes and proteins: Biointerface analysis and protein adhesion properties. *Colloids Surf., B* **2013**, *110*, 248−253.
(34) Lai, Y. H.; Koo, S.; Oh, S. H.; Driskell, E. A.; Driskell, J. D. Rapid screening of antibody-antigen binding using dynamic light scattering (DLS) and gold nanoparticles. *Anal. Methods* **2015**, *7*, 7249−7255.
(35) de la Escosura-Muñiz, A.; Baptista-Pires, L.; Serrano, L.; Altet, L.; Francino, O.; Sánchez, A.; Merkoçi, A. Magnetic Bead/Gold Nanoparticle Double-Labeled Primers for Electrochemical Detection of Isothermal Amplified Leishmania DNA. *Small* **2016**, *12*, 205−213.
(36) Pandey, A.; Gurbuz, Y.; Ozguz, V.; Niazi, J. H.; Qureshi, A. Graphene-interfaced electrical biosensor for label-free and sensitive detection of foodborne pathogenic E. coli O157:H7. *Biosens. Bioelectron.* **2017**, *91*, 225−231.
(37) Suaifan, G. A. R. Y.; Alhogail, S.; Zourob, M. Paper-based magnetic nanoparticle-peptide probe for rapid and quantitative colorimetric detection of Escherichia coli O157:H7. *Biosens. Bioelectron.* **2017**, *92*, 702−708.
(38) Yang, X.; Zhou, X.; Zhu, M.; Xing, D. Sensitive detection of Listeria monocytogenes based on highly efficient enrichment with vancomycin-conjugated brush-like magnetic nano-platforms. *Biosens. Bioelectron.* **2017**, *91*, 238−245.
(39) Vaisocherová-Lísalová, H.; Víšová, I.; Ermini, M. L.; Špringer, T.; Song, X. C.; Mrázek, J.; Lamačová, J.; Lynn, N. S.; Šedivák, P.; Homola, J. Low-fouling surface plasmon resonance biosensor for multi-step detection of foodborne bacterial pathogens in complex food samples. *Biosens. Bioelectron.* **2016**, *80*, 84−90.
(40) Eriksson, E.; Aspan, A. Comparison of culture, ELISA and PCR techniques for salmonella detection in faecal samples for cattle, pig and poultry. *BMC Vet. Res.* **2007**, *3*, 21−21.
(41) Song, C. M.; Liu, C.; Wu, S. Y.; Li, H. L.; Guo, H. Q.; Yang, B.; Qiu, S.; Li, J. W.; Liu, L.; Zeng, H. J.; Zhai, X. Z.; Liu, Q. Development of a lateral flow colloidal gold immunoassay strip for the simultaneous detection of Shigella boydii and Escherichia coli O157:H7 in bread, milk and jelly samples. *Food Control* **2016**, *59*, 345−351.
(42) Sun, Y.; Han, Z.; Liu, H.; He, S.; Yang, L.; Liu, J. Three-dimensional hotspots in evaporating nanoparticle sols for ultrahigh Raman scattering: solid-liquid interface effects. *Nanoscale* **2015**, *7*, 6619−6626.

DOI: 10.1021/acs.jafc.7b03957
J. Agric. Food Chem. 2017, 65, 10290−10299