Supplementary Information

Development of a loop mediated isothermal amplification (LAMP) – surface enhanced Raman spectroscopy (SERS) assay for the detection of *Salmonella enterica* serotype Enteritidis

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Chemicals and materials

Chloroauric acid ($HAuCl_4$), trisodium citrate dihydrate, sodium chloride, and S1 nuclease were purchased from Sigma Aldrich (St. Louis, MO). TE buffer (10mM Tris; 1 mM EDTA, pH 8.0) was obtained from Invitrogen (Carlsbad, CA). Deionized (DI) water (18.2 MΩ/cm) was prepared by using the Millipore system (Billerica, MA).

Methods

1. Characterization techniques

1.1 UV-Vis spectroscopy. Absorption spectra were measured by using a Shimadzu UV-Vis spectrophotometer. The samples were loaded in 10 mm path length quartz cuvettes and scanned at room temperature.

1.2 Transmission electron microscopy (TEM). Transmission electron microscopy (TEM) images were obtained using a JEOL model JEM-100CX microscope at an acceleration voltage of 300 kV. The specimens were prepared by dropping 10 μL of the sample onto the ultrathin Formvar-coated 200-mesh copper grids and then dried in air. The mean diameter and size distribution histogram of particles were obtained by averaging over 300 particles from the TEM images using ImageJ software (developed at the National Institutes of Health).

1.3 Gel electrophoresis. The electrophoretic mobility patterns were evaluated with a horizontal submerged gel electrophoresis apparatus (Mini-SubCell GT, Bio-Rad) using agarose gel in TAE buffer (pH 8.5). Samples aliquots (10 μL) were mixed with 10 μL 5×TAE loading buffer (5× TAE, 25% (v/v) glycerol, 0.25% (w/v) orange-G at pH 8.5), briefly vortexed, and loaded into

wells. The gel was subjected to a typical voltage of 100 V for 40 min, and then illuminated and analyzed with a digital gel documentation system (Bio-Rad).

1.4 Fluorescence spectroscopy. Fluorescence spectra of the prepared Au-nanoprobes before and after nuclease digestion step were recorded with a microplate reader (Infinite M200, TECAN) using 600 nm as excitation wavelength and 20 s as integration time.

1.5 Raman spectroscopy. SERS spectra were collected from the wet pellets, resulted from the washing and centrifugation of the products of nuclease digestion steps. Raman spectrometer was operated through a 50× objective (N.A. = 0.75). Excitation laser at 785 nm was used with 100% laser power (25 mW) and 10-s as integration time was applied for all spectral collection in a range of 500-2000 cm^{-1}. For each sample, at least 30 spectra from different hot-spot locations were collected and processed for smoothing and baseline subtraction by WiRE 3.4 software (Renishaw). The processed spectra were exported to OriginPro 2015 software (OriginLab Corp., USA) for further analysis.

2. Synthesis and characterization of citrate capped gold nanoparticles. First, all glassware were thoroughly cleaned in aqua regia (3:1 v/v concentrated HCl and HNO_3), then washed by ultrapure water and heat dried. Typically, 300-µl aliquots of 1% $HAuCl_4$ was mixed with 30 ml of H_2O in a 250 ml Erlenmeyer flask mechanically with a clean stir rod. This mixture was rapidly brought to boil and maximally stirred without splashing of the solution. Then, 900 µl of 1% sodium citrate trihydrate solution was immediately added. The flask was removed from heat after 10 min once nanoparticle maturation was completed as indicated by the formation of red color.

The prepared particles solution was kept stirring for 1 h and subsequently filtered using a 0.22 µm membrane filter. The obtained citrate capped gold nanoparticles were characterized by UV-Vis spectroscopy and transmission electron microscopy (TEM).

3. Estimation of the number of cy5-modifed DNA per Au-nanoprobes. The concentration of cy5-DNA oligonucleotides was estimated using UV-vis spectroscopy analysis (UV-245 Shimadzu). The concentration of cy5-DNA in solution of Au-nanoprobes was estimated from the absorbance value at the characteristic peak 650 nm of cy5. Then, the number of cy5-DNA on each gold nanoparticle was calculated by dividing the concentration of cy5 by the concentration of gold nanoparticles in the same solution.

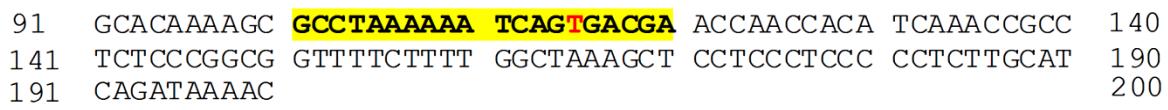

Figure S1. The sequence and position of the capturing DNA used in Au-nanoprobes preparation. The probe comprises of 20 nucleotides (highlighted) and modified at thiamine base (in red color) exist at position 6 (5’ to -3’).

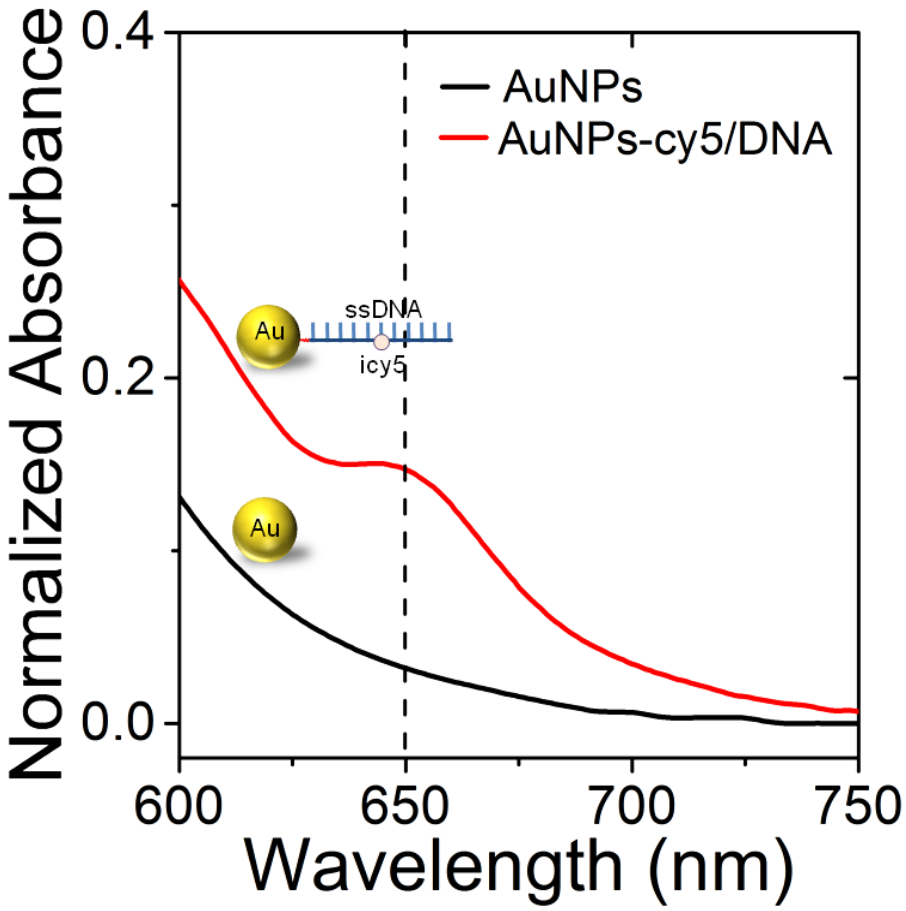

Figure S2. UV-vis analysis shows the presence of the characteristic peak of cy5 at 650 nm after the conjugation of cy5 labeled DNA oligonucleotides onto the surface of AuNPs.

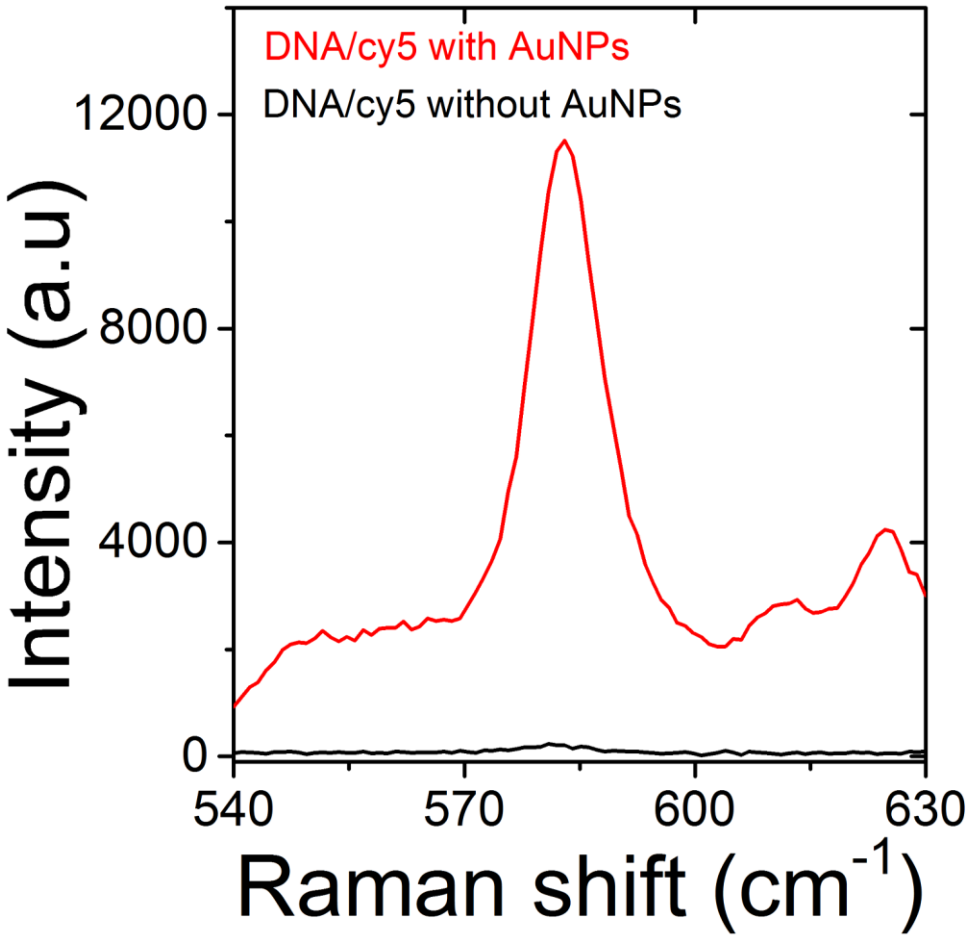

Figure S3. Raman spectra of cy5/DNA before and after conjugation onto the surface of AuNPs at 1 μM and using 100% and 10% laser power (25 mW), respectively.

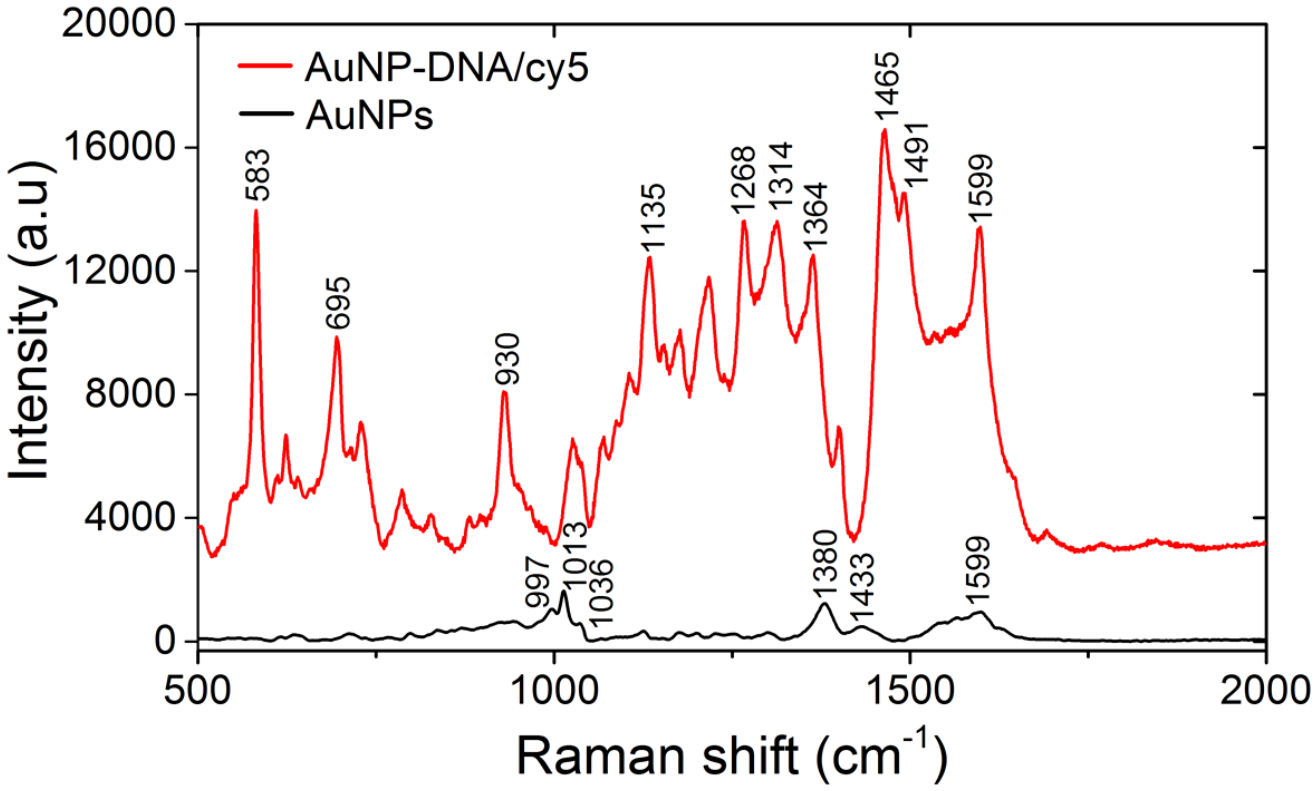

Figure S4. Raman spectra of gold nanoparticles and the prepared Au-nanoprobes (gold nanoparticles modified with cy5-DNA oligonucleotides).

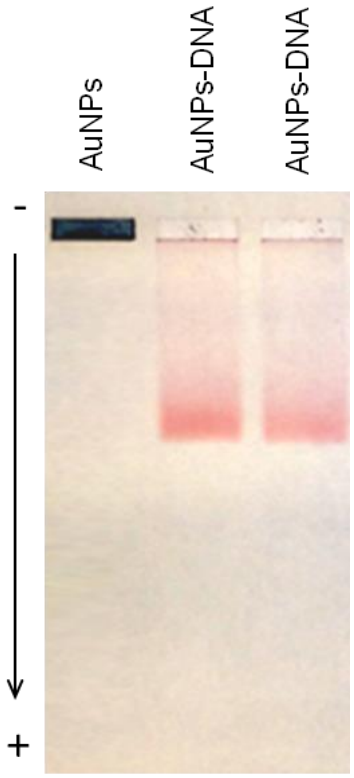

Figure S5. Gel electrophoresis of gold nanoparticles (citrate-capped particles, Lane 1), Au-nanoprobes (gold nanoparticles-modified with cy5-DNA oligonucleotides, Lane 2), and Au-nanoprobes subjected to hybridization conditions applied in the LAMP-SERS assay (gradually cooled down from 95°C to 4°C), confirming the conjugation of DNA onto the surface of gold nanoparticles to form Au-nanoprobes and the stability of the Au-nanoprobes.

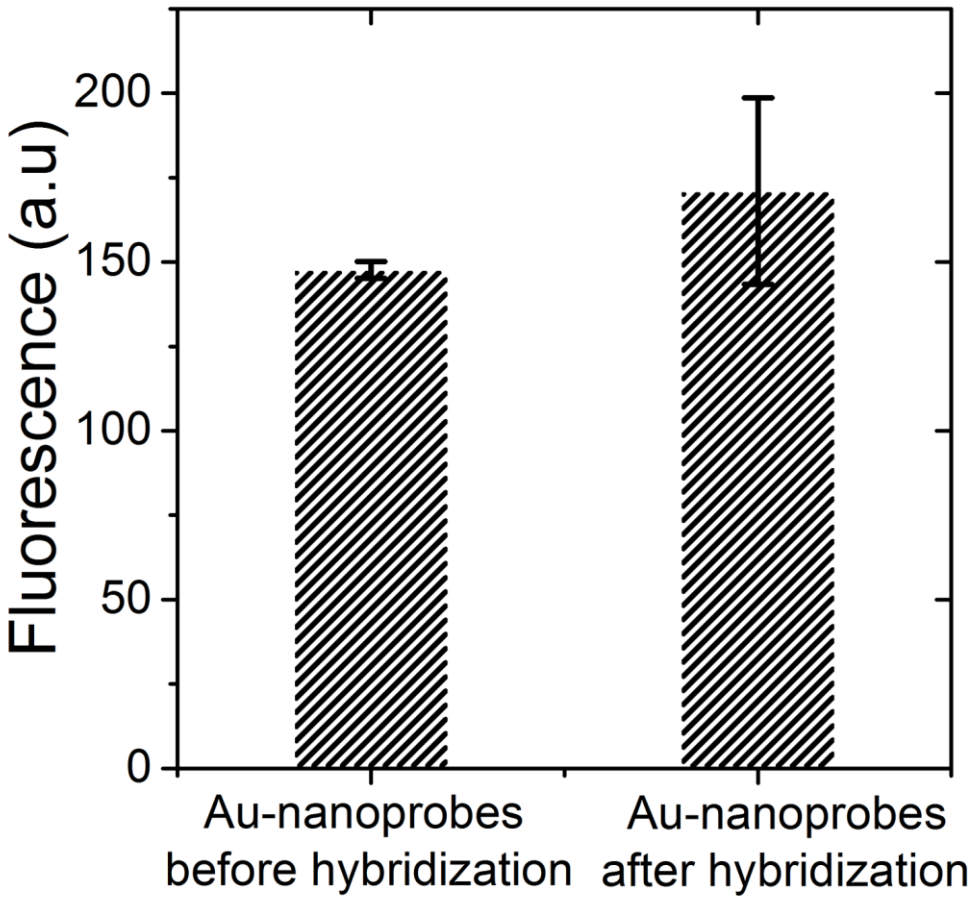

Figure S6. Fluorescence analysis of Au-nanoprobes solution before and after the hybridization step. The fluorescence intensity was measured at the maximum emission beak for cy5 at 670 nm for all treatments. Each data point represents an average of three measurements and error bars are the standard deviations.

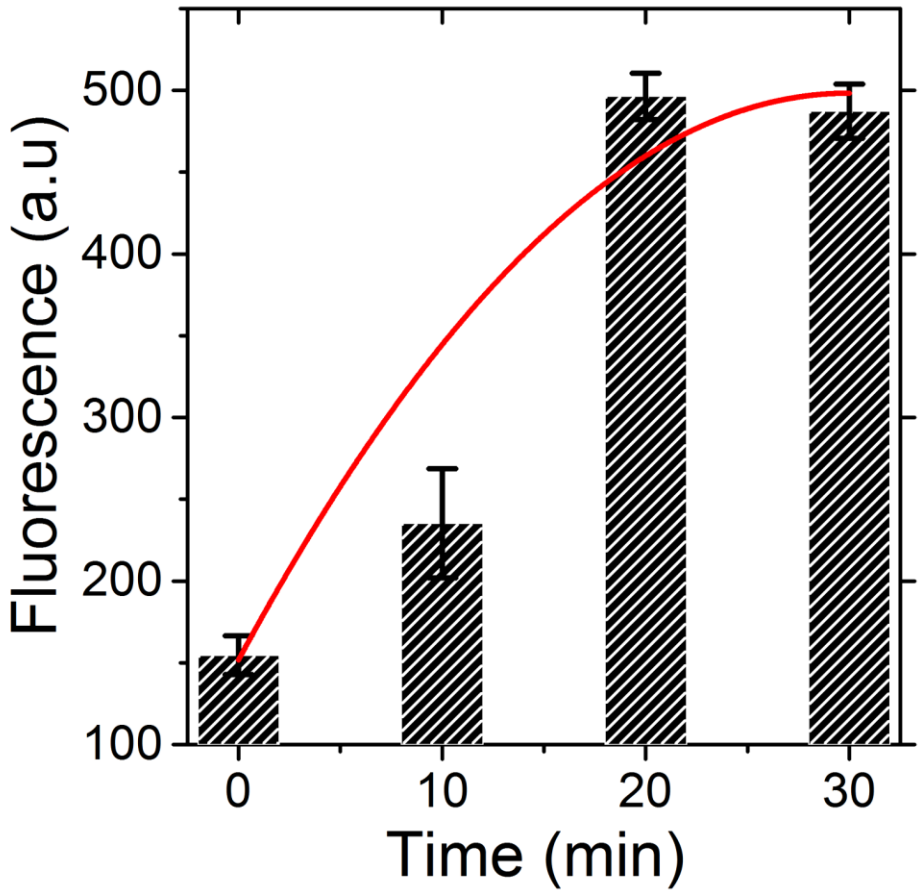

Figure S7. Fluorescence analysis of the Au-nanoprobes solution digested by S1 nuclease enzyme at different time points. Fluorescence intensity was measured at the maximum emission peak for cy5 at 670 nm for all treatments. The data were fit by nonlinear curve fit using OriginPro 2015 software. Each data point represents an average of three measurements and error bars are the standard deviations.

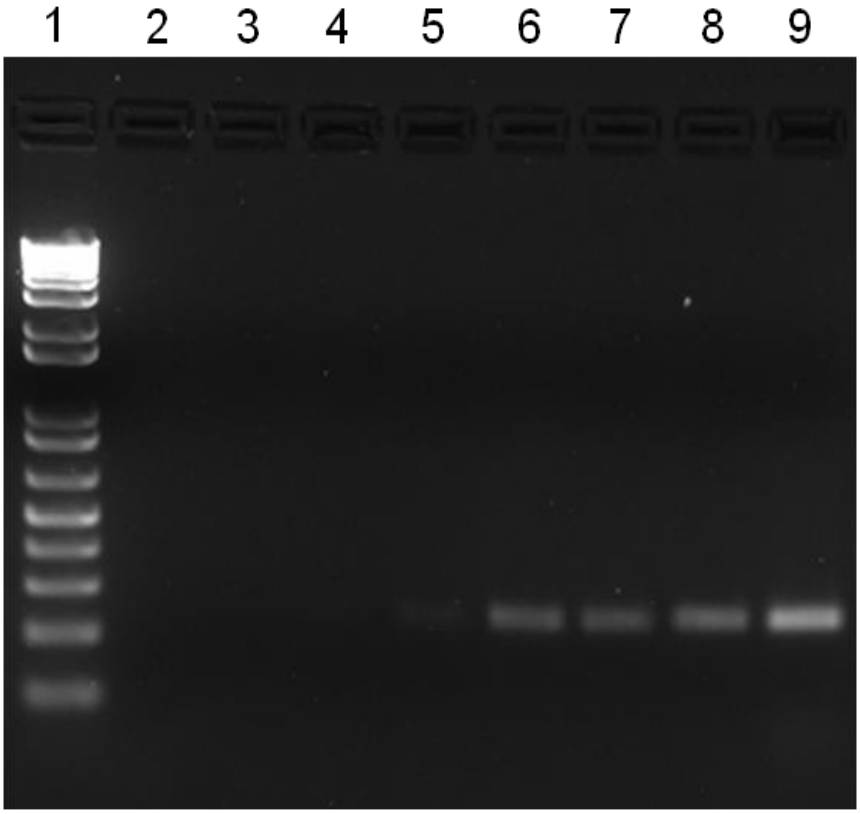

Figure S8. Gel electrophoresis of the conventional PCR performed using 10-fold serial dilutions of *S*. Enteritidis DNA template (6.6×10^{0} CFU/mL-6.6×10^{6} CFU/mL). Lane 1: 1-kb DNA ladder marker; Lane 2: negative control (without target DNA template); Lane 3: 6.6×10^{0} CFU/mL; Lane 4: 6.6×10^{1} CFU/mL; Lane 5: 6.6×10^{2} CFU/mL; Lane 6: 6.6×10^{3} CFU/mL; Lane 7: 6.6×10^{4} CFU/mL; Lane 8: 6.6×10^{5} CFU/mL; Lane 9: 6.6×10^{6} CFU/mL

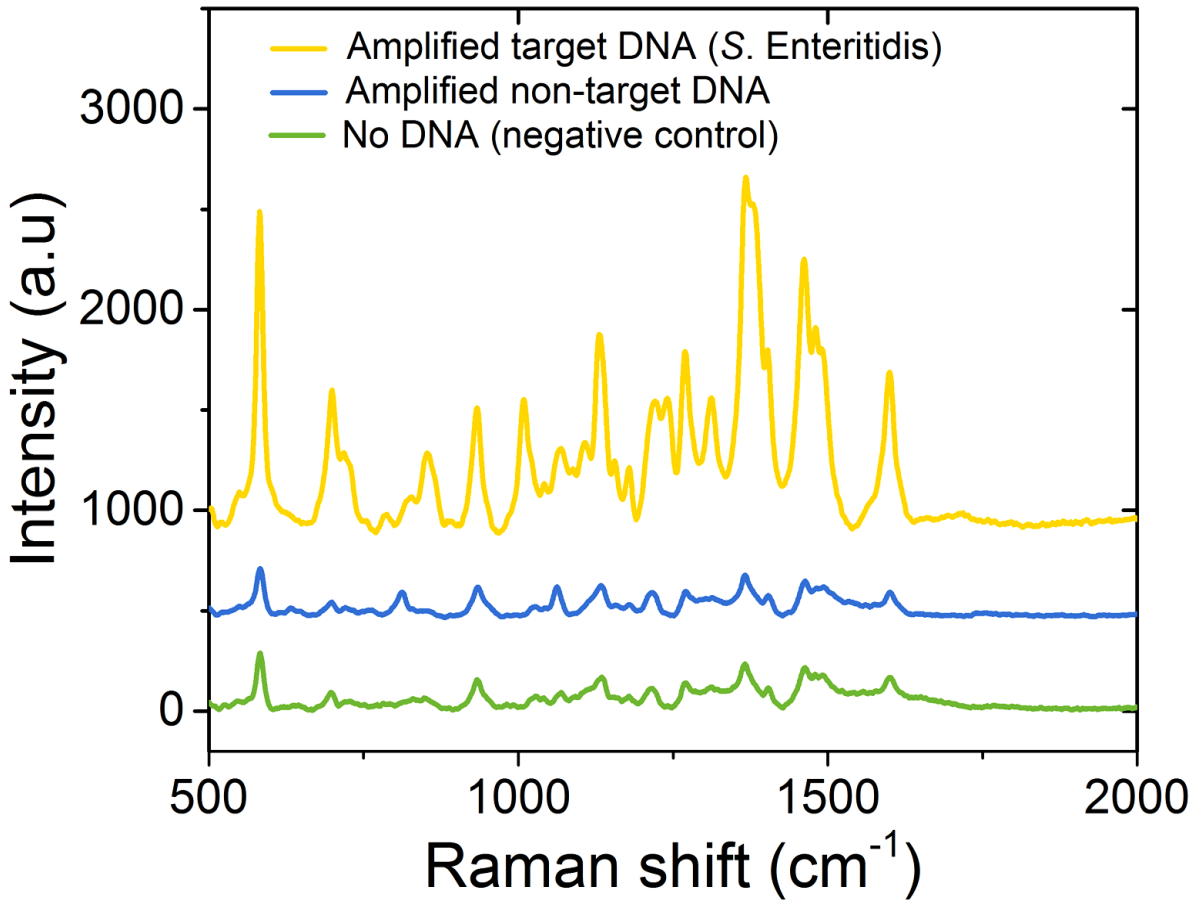

Figure S9. Raman spectroscopy confirms the ability of the LAMP-SERS assay to differentiate the target DNA even in the presence of non-specific amplification products generated by LAMP.

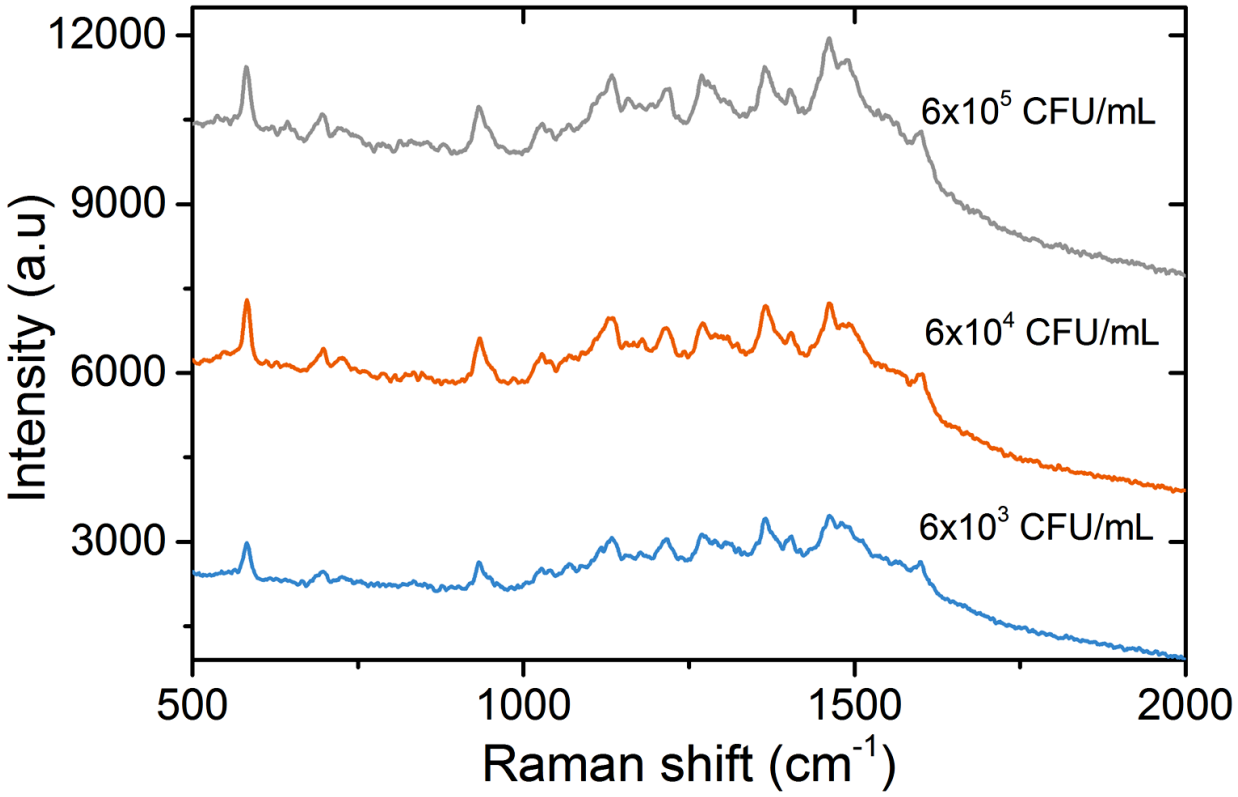

Figure S10. Raman spectral features of milk samples spiked with different concentrations of *S.* Enteritidis.

Table S1. List of bacterial strains tested in this study

Species	Strain No.
Salmonella enterica serotype Enteritidis	ATCC43353
Salmonella enterica serotype Enteritidis	0EA2669
Salmonella enterica serotype Enteritidis	3512H
Salmonella enterica serotype Enteritidis	ME-14
Salmonella enterica serotype Typhimurium	PS-23
Escherichia coli O157:H7	ATCC35150
Pseudomonas aeruginosa	ATCC 9721
Staphylococcus aureus	clinical isolate 10
Listeria monocytogenes	ATCC19114

Table S2. Comparison of LAMP-SERS assay with other molecular assays used for the detection of *Salmonella*

Method	**Detection limit**	**Time***	**Procedures**
PCR[1]	300 cells per reaction	3-4 h	Enrichment required; Two-steps protocol
Real-time PCR[2]	0.04 CFU/g	3-4 h	Enrichment required; Two-steps protocol
Reverse-transcriptase PCR[3]	40 copies of *invA* mRNA per reaction	4-5 h	Enrichment required; Three-steps protocol
LAMP[4]	10^2 CFU/ml	<2 h	Enrichment required; Two-steps protocol
Real-time LAMP[5, 6]	10^2 CFU/ml (turbidity); 100 fg DNA/tube (fluorescence)	1-2 h	Enrichment required; One-step protocol
Reverse-transcriptase LAMP[7]	10^6 CFU/25 mL	2-3 h	Enrichment required; Two-steps protocol
LAMP-SERS	66 CFU/mL	<2 h	Enrichment not required; Two-steps protocol

* Without enrichment time

References

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