Supporting Information for

Ultrasensitive detection of microbial cells by magnetic focus enhanced lateral flow sensor

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Experimental section

Chemicals and agents

Sodium citrate, sodium carbonate, $HAuCl_4$, $FeCl_2$, $FeCl_3$, casein (sodium salt type), and tetramethyl benzidine (TMB) were purchased from Sigma (St. Louis, MO). NaOH was obtained from Mallinckrodt Chemicals (Phillipsburg, NJ). Polyclonal antibodies (01-95-90) from goat against *E. coli* O157:H7 and polyclonal antibodies (01-91-99) from goat against *Salmonella* Common Structural Antigens (CSA) were purchased from KPL (Gaithersburg, MD). *E. coli* O157:H7 (50-95-90) and *Salmonella typhimurium* (50-74-01) were purchased from KPL (Gaithersburg, MD). Bacteria used as control were obtained from Bhunia's lab in the Department of Food Sciences at Purdue University. Sulfo-NHS-LC-Biotin and streptavidin Poly-HRP were purchased from Pierce (Rockford, IL).

Preparation of gold nanoparticles (GNPs) and Fe_3O_4/Au core-shell nanostructures.

All glassware used in the following experiments were cleaned in a bath of fresh 3:1 HCl: HNO_3 (aqua regia) solution and rinsed with water purified by milli-Q system. GNPs used for the probes were prepared based on the reported approach. [G. Frens, *Nature-Phys. Sci.*, 1973, **241**, 20-22] Briefly, 1 ml of 1% $HAuCl_4$ was injected into

100 ml of water and heated to boiling under strong stirring, followed by quick addition of 1 ml of 1% sodium citrate. The obtained product was kept in boiling for 10 min and cooled down to room temperature. The prepared GNPs were kept at 4^{o}C until modification.

The synthesis of Fe_3O_4 was carried out based on the reported methods [B. Unal, Z. Durmus, H. Kavas, A. Baykal and M. S. Toprak, *Mater. Chem. Phys.*, 2010, 123, 184-190; Z. Zhou and J. Irudayaraj, *Analyst*, 2015, **140**, 938-944] and modified accordingly per our experiment. Generally, in a three-neck bottle with Ar gas 3 ml of 1 M NaOH was added to 27 ml of water and heated to boiling followed by the injection of 2ml of 0.4 M sodium citrate. Under vigorous stirring 1 ml of 0.2 M $FeCl_2$ and 1ml of 0.4 M $FeCl_3$ were quickly added to the boiling mixture. Then the solution was refluxed for 4 h. The obtained Fe_3O_4 was washed with ethanol and water 3 times respectively to neutral pH and redispersed in 15 ml of water.

The Fe_3O_4/Au core-shell nanostructures were fabricated based on the approach reported by Tamer et. al.. [U. Tamer, Y. Gundogdu, I. H. Boyaci and K. Pekmez, *J. Nanopart. Res.*, 2010, **12**, 1187-1196] Typically, 80 µl of as-prepared Fe_3O_4 was added to 920 µl water. Then the solution was mixed well with 100 µl of 1% $HAuCl_4$ and sonicated for 15 min. 200 µl of 10 mM fresh and ice-cold $NaBH_4$ was rapidly injected into the mixture and sonicated for an additional 5 min. The obtained product in dark read was washed with water 3 times and kept at 4^{o}C until further use.

Preparation of GNP probes and magnetic probes

To prepare the probes for detection, antibodies and sulfo-NHS-LC-biotin were

conjugated to the obtained nanostructures. The modification approach was performed based on our previous work. [I.-H. Cho, A. Bhunia and J. Irudayaraj, Int. *J. Food Microbiol.*, 2015, **206**, 60-66] To construct GNP probes, 1 ml of as-prepared GNPs was mixed with 1 µl of 0.5 M sodium carbonate, 100 µl of 10 mM phosphate buffer (pH 7.4) and 10 µg antibody in solution, which was gently shaken for 2 h. 122 µl of 5% (w/v) casein in 10 mM phosphate buffer was added to the solution and shaken overnight to block the residual surface of GNPs. The obtained GNPs modified with antibody was centrifuged at 10,000 rpm for 10 min to remove unbound molecules, washed and redispersed in 1 ml of 10 mM phosphate buffer. 10 µg of sulfo-NHS-LC-biotin was added and shaken gently for 1h in the obtained solution to biotinylate the antibody modified GNPs. 100 µl of 5% (w/v) casein in 10 mM phosphate buffer was added. After 1h of gentle shaking, the obtained GNP probes was centrifuged at 10,000 rpm for 10 min, washed and redispersed in 100 µl of 10 mM PBS buffer with 5% (w/v) casein. The preparation of magnetic probes is similar to the approach for GNP probes. The Fe_3O_4/Au core-shell nanostructures were then modified with antibody and washed and redispersed in 100 µl of 10 mM PBS buffer with 5% (w/v) casein.

Pathogen detection

The LFIA strips were prepared by adding 0.25 µg of antibody in solution as a capture agent to the nitrocellulose membrane bearing LF strips, and then dried at 37 ^{o}C for 45 min. The LFIA detection procedure was conducted per our prior work. [I.-H. Cho, A. Bhunia and J. Irudayaraj, Int. *J. Food Microbiol.*, 2015, **206**, 60-66] To perform the detection, the sample pad of the as-prepared LFIA strip was immersed in 100 µl of

sample mixed with 1 µl of the magnetic probe, 0.25 µl of GNP probe and 0.25 µl of streptavidin Poly-HRP, while an external magnet (N52 rare earth neodymium permanent super strong magnet) was placed precisely under the detection zone of the strip. After allowing 15 min of sample flow to facilitate the immunoreaction, the strip was washed with 60 µl of water 2 times with additive conjugate and absorbent pad in the cross direction. Then 30 µl TMB was added to generate a colorimetric signal with five minutes of incubation at room temperature followed by washing with 60 µl water to stop the reaction. The obtained visual signal can be recorded by a simple photograph which can be further processed. With quantitative color intensity analysis from image analysis software, the intensity of the background from the blank part of the strip was subtracted from the intensity of the dot for a quantitative normalized color signal. To obtain a linear relation between signal and pathogen concentration, the experiments were replicated five times and the signals were normalized by subtracting the average signal from the blank obtained for the detection of *E. coli* O157:H7 in PBS. According to the linear relation shown in Fig. 2, the LOD was calculated based on the standard deviation of the signal from the samples with 0 CFU/ml.

Detection of real samples

To further demonstrate the feasibility of the proposed magnetic focus enhanced LF method in food samples, pineapple juice purchased from a local super market was purposefully inoculated with *E. coli* O157:H7 for testing in a food matrix. The pineapple juice was first added with 1 M NaOH to adjust the pH to 7. The pH-

adjusted pineapple juice was inoculated with serial amount of *E. coli* O157:H7 to obtain samples with *E. coli* O157:H7 at 0, 50, 100, 200 and 400 CFU/ml. To reduce the influence from thickening agents in the samples to the sample flow in the strip, pineapple juice was diluted in a 1:1 ratio with 10 mM PBS. Then triplicate assay were performed with the diluted samples.

Characterization

Zeta potential of the nanostructures and prepared probes were measured with a Zetasizer NanoZS (Malvern Instruments). Transmission electron microscopy (TEM) images and energy-dispersive X-ray (EDX) spectra of the samples were recorded with a FEI Tecnai G2 20 with an energy-dispersive X-ray detector operated at 100 kV. UV-vis spectra of GNP probes and magnetic probes were collected with a Jasco V570 UV/Visible/NIR spectrophotometer (Jasco, Inc., Easton, MD).

Table S1. Zeta potential measurement of magnetic probes and GNP probes conducted at 25 ^{o}C

	Zeta potential (mV)		
	Before modification	Modified for *E. coli* O157:H7	Modified for *Salmonella typhimurium*
GNP probes	-40.7	-35.6	-29.1
Magnetic probes	-25.4	-30.4	-29.9

For particular nanoparticles, zeta potential depends on the chemicals on the surface of nanoparticles. Herein the zeta potential change of GNPs and magnetic nanostructure should be attributed to the successful modification of antibody. The opposite change of zeta potential of GNP probes and magnetic probe should be assigned to the original status of chemicals on these nanostructures. Before modification, the zeta potential of GNPs, -40.7 mV, indicates a layer of dense citrate ions on the GNP surface, subsequently the added antibody will replace some of the citrate and thus increases the zeta potential after antibody modification. Similar changes in the zeta potential of GNPs due to antibody modification was reported in our previous work. [I.-H. Cho, A. Bhunia and J. Irudayaraj, *Int. J. Food Microbiol.*, 2015, **206**, 60-66] Meanwhile, per the synthesis procedure of Fe_3O_4/Au core-shell nanostructures and a zeta potential of -25.4 mV, it is believed that almost no chemical is attached to the Fe_3O_4/Au core-shell nanostructures. The modification of antibody increased the molecules on the surface magnetic probes, resulting in a decrease in zeta potential. Similar decrease in zeta potential was used to confirm the antibody modification on Fe_3O_4/Au core-shell

nanostructures as reported. [S. Zhang, L. Zou, D. Zhang, X. Pang, H. Yang and Y. Xu, *J. Nanopart. Res.*, 2011, **13**, 3867-3876]

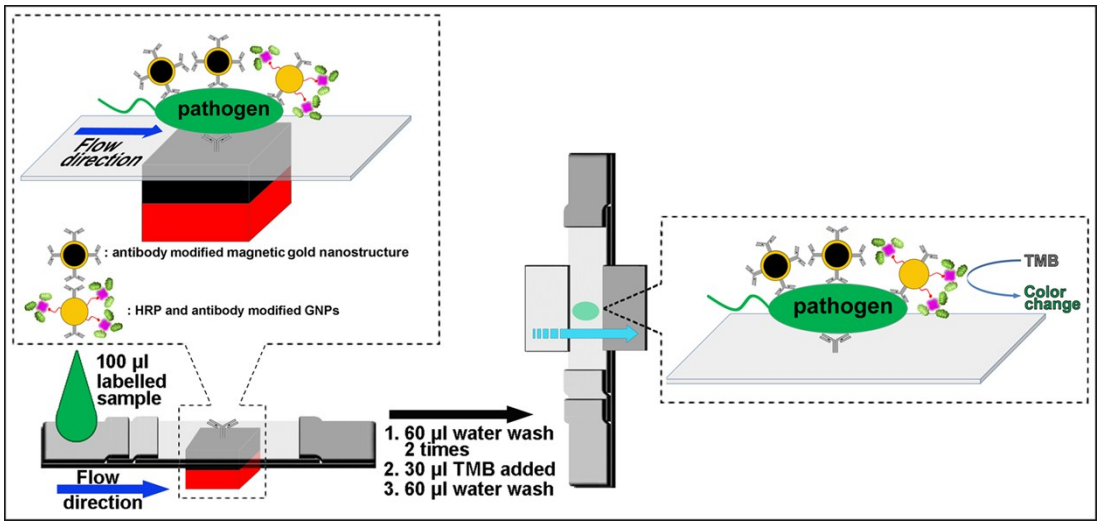

Fig. S1 Schematic of the mLFIA procedure.

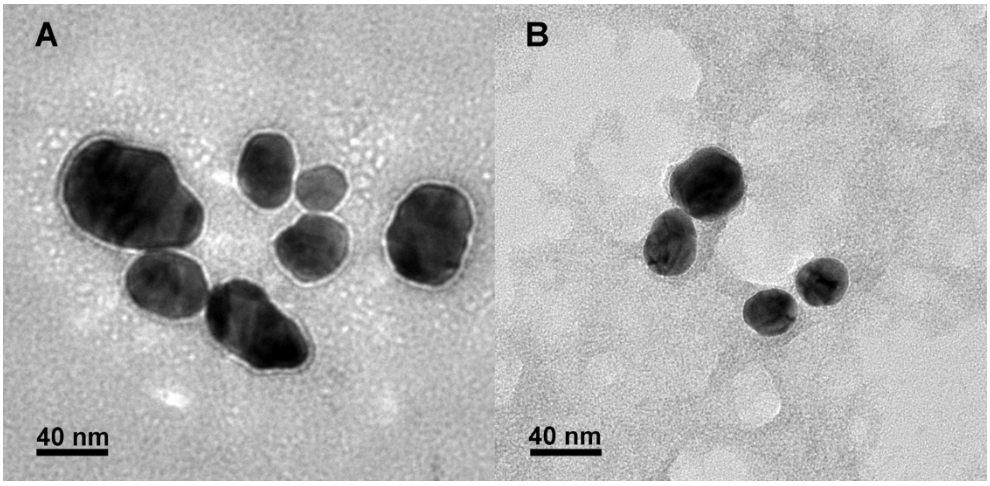

Fig. S2 TEM images of Fe_3O_4/Au core-shell magnetic probes (A) and GNP probes (B).

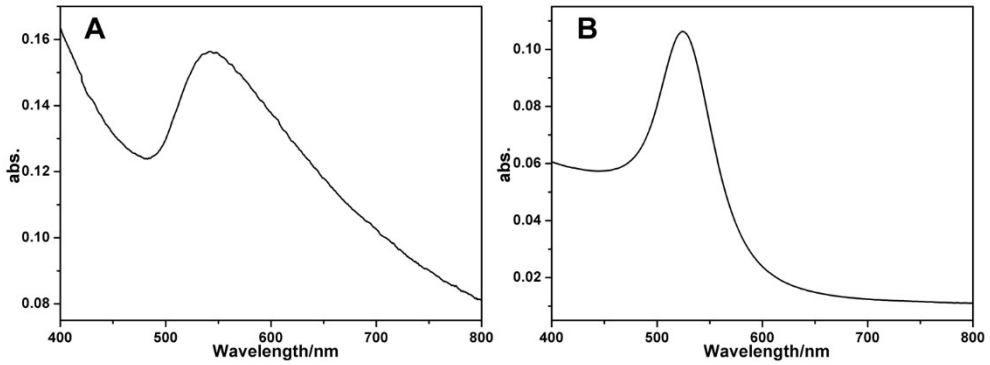

Fig. S3 UV-vis spectra of Fe_3O_4/Au core-shell magnetic probes (A) and GNP probes (B).

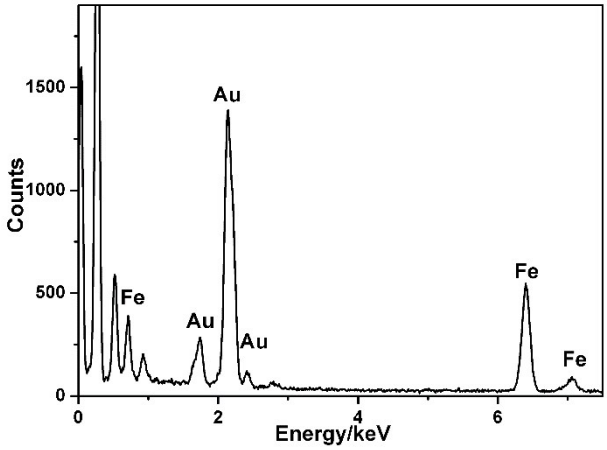

Fig. S4 EDX spectrum of Fe_3O_4/Au core-shell magnetic probes.

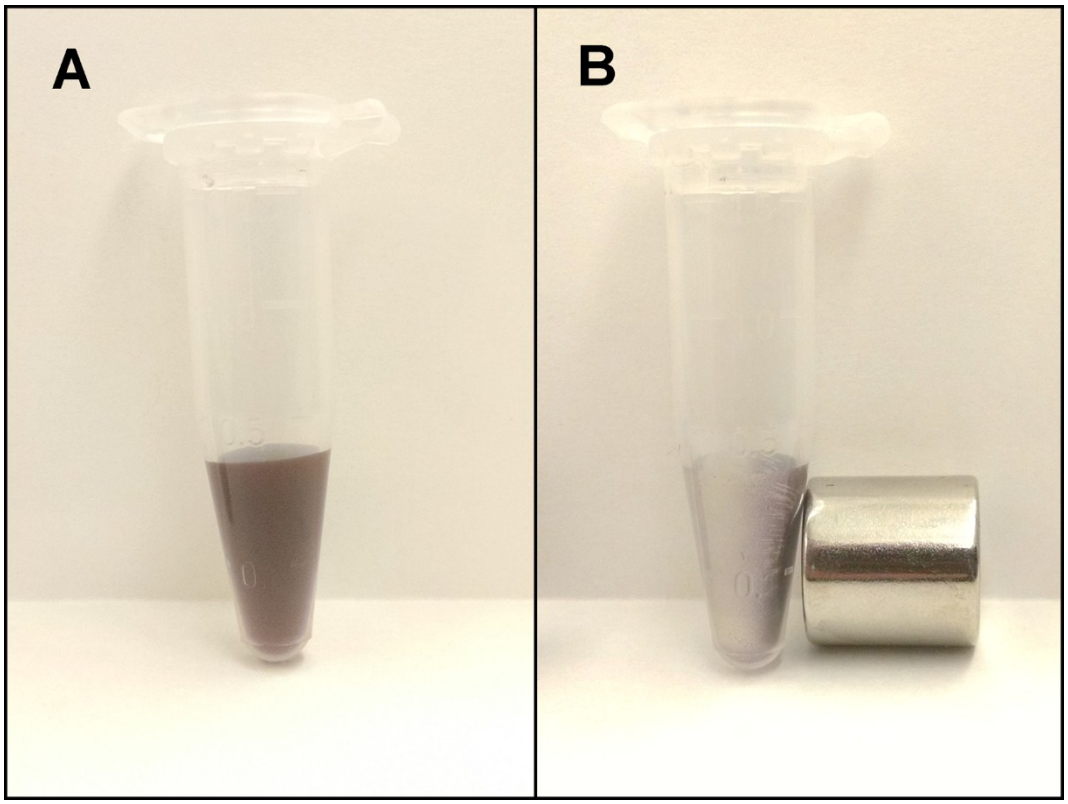

Fig. S5 Response of magnetic probes to an external magnet.

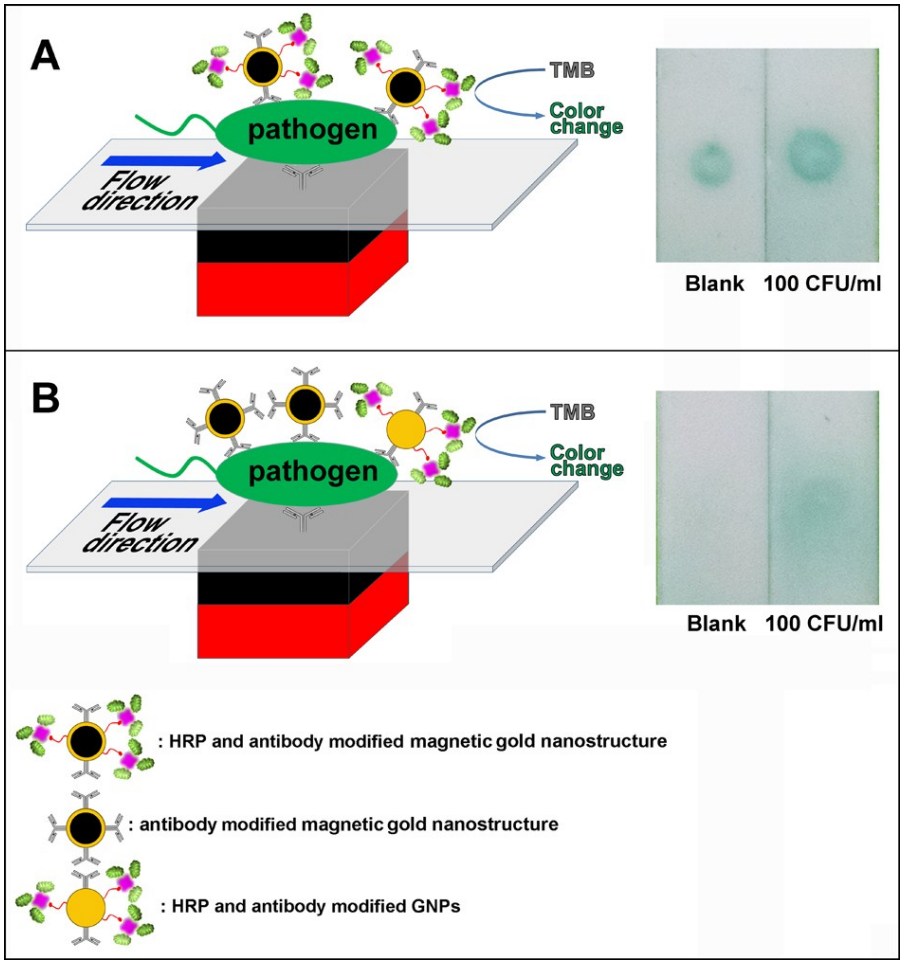

Fig. S6 Detection results of 100 CFU/ml *E. Coli* O157:H7 with (A) 1 µl biotinylated magnetic gold nanostructures modified with antibodies and (B) 1 µl magnetic probes and 0.25 µl GNP probes. It is noted that to label pathogen with enough magnetic gold nanostructures for the slowdown of labeled pathogens in the strip, the amount of used biotinylated magnetic gold nanostructures modified with antibodies will result in strong signal with the blank samples. In proposed detection method, two probes are used: i) magnetic probes control the movement of the labeled pathogens; ii) GNP probes generate signal. By optimizing the amount of these two probes, the blank signal can be suppressed while achieving the highest detection sensitivity.

Fig. S7 Optimization of the amount of magnetic probes and GNP probes used in the reaction: magnetic focus LFIA detection of 100 CFU/ml *E. Coli* O157:H7 performed with 0.25 µl of streptavidin Poly-HRP and (a) 1 µl magnetic probes and 0.25 µl GNP probes, (b) 0.5 µl magnetic probes and 0.5 µl GNP probes and (c) 0.25 µl magnetic probes and 1 µl GNP probes. It should be noted that the best result was achieved with 1 µl magnetic probes and 0.25 µl GNP probes.

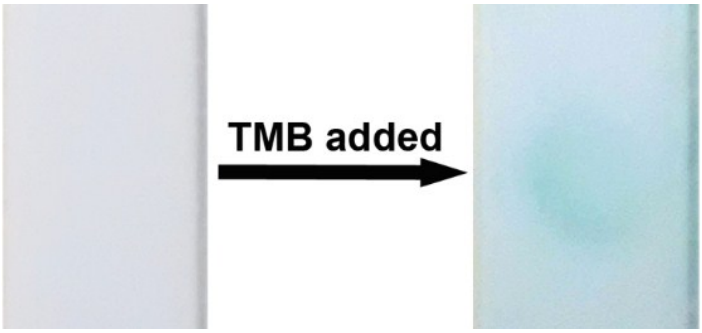

Fig. S8 Signal generation of the strip with TMB amplification from the sample with 100 CFU/ml *E. coli* O157:H7.

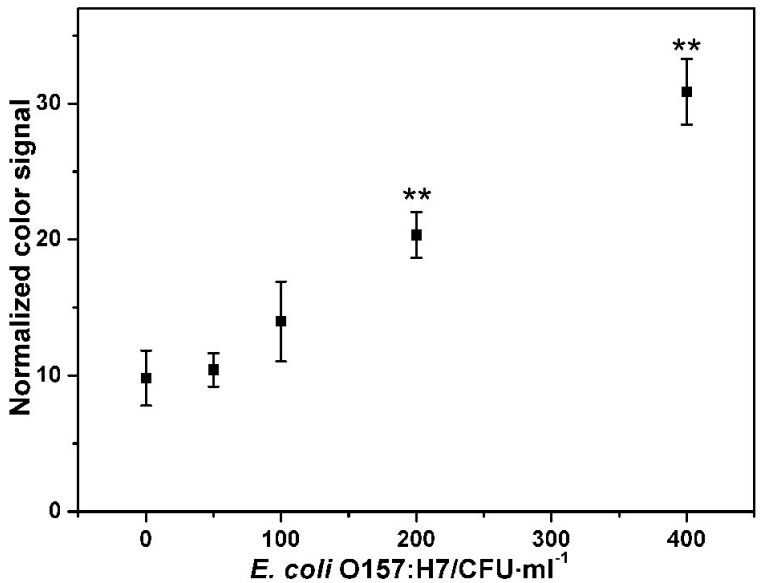

Fig. S9 Detection results from pineapple juice. [mean ± SD, n=3] **p<0.01 vs. blank. It can be noted that the detection sensitivity in pineapple juice is not as high as that in PBS, which should be attributed to the influence from other chemicals in pineapple juice such as thickening agents. The recovery in inoculated pineapple juice was also calculated to be 102.4 % for 200 CFU/ml samples and 100.3 % for 400 CFU/ml samples.

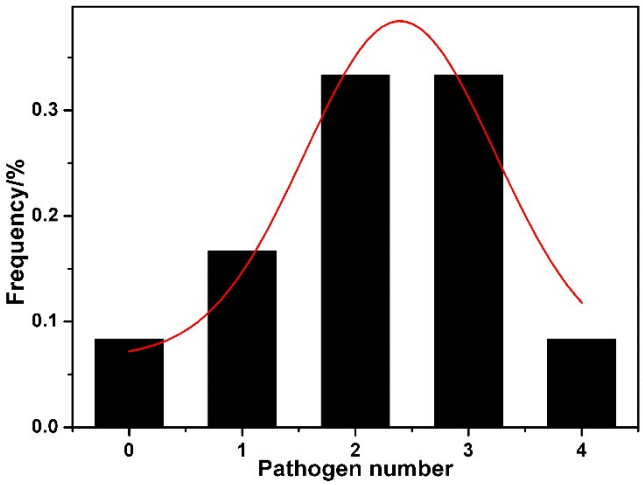

Fig. S10 Histogram of cell number of *E. coli* O157:H7 in 100 µl of 25 CFU/ml samples [n=12]. The data are fitted with a Gaussian distribution (the red curve). The number of *E. coli* O157:H7 was calculated as: $N_{cell} = I_{normalized\ color\ signal}/2.81$, in which N_{cell} is the rounding number of *E. coli* O157:H7 cells, $I_{normalized\ color\ signal}$ is the signal obtained for the detection of 25 CFU/ml of *E. coli* O157:H7 and 2.81 is 1/10 of the average normalized color signal from 100 CFU/ml of *E. coli* O157:H7 as shown in Fig. 2. The red curve shows the Gaussian fit to the experimental data.