

Ultrasensitive fluorescence lateral flow assay for simultaneous detection of *Pseudomonas aeruginosa* and *Salmonella typhi-murium* via wheat germ agglutinin-functionalized magnetic quantum dot nanoprobe

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S1. Experimental section

S1.1 materials and chemicals

Water-soluble CdSe/ZnS-COOH QDs were obtained from Mesolight Inc. (Suzhou, China). Lectin from *Triticum vulgaris* (wheat germ agglutinin, WGA), glycine, polyethylenimine (PEI) branched (MW 25 kDa), polyvinylpyrrolidone (PVP, 40 K), 2-(N-morpholino) ethanesulfonic (MES), phosphate-buffered saline (PBS), N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC), fetal bovine serum (FBS), N-hydroxysulfosuccinimide sodium salt (sulfo-NHS), and Tween-20 were purchased from Sigma-Aldrich (USA). Goat anti-mouse IgG antibody were purchased from Fitzgerald (America). Mouse monoclonal anti-*P. aeruginosa* antibody was supplied by ThermoFisher (USA) and Mouse monoclonal anti-*S. typhimurium* antibody were purchased from Abcam (UK). Sample pad, absorbent pad, conjugate pad, PVC bottom plate were bought from Jieyi Biotechnology Co. (Shanghai, China) and Nitrocellulose (NC) membrane (CN95) was obtained from Sartorius (Spain). All the bacteria used in this study were supplied by Pro. Shengqi Wang's group of Beijing Key Laboratory of New Molecular Diagnosis Technologies for Infectious Diseases.

S1.2 Instruments

Transmission electron microscopy (TEM) images were taken on Hitachi H-7650 TEM. Scanning electron microscopy (SEM) images were obtained by JEOL JSM-7001F microscope (JEOL, Japan). 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 measured with Mastersizer 2000 (Malvern, UK). Fourier transform infrared (FTIR) spectroscopies were measured via FTIR spectroscopy (Thermo Fisher Scientific, Inc., Waltham, MA). The fluorescence signals of test lines were simultaneously read by using a portable FIC-S1 fluorescence reader (365 nm excitation/610 nm emission), which were purchased from Suzhou Hemai Precision Instrument Co., Ltd (China).

S1.3. Preparation of immuno-Mag@QD-LFA

The conjugation of Mag@QD and anti-*P. aeruginosa* antibodies was conducted via the carbodiimide chemistry. First, 1 mL of Mag@QD (1 mg/mL) was resuspended in 500 μ L of MES buffer (100 mM, pH 5.5) with an external magnetic field, and then mixed with 5 μ L of EDC (0.1 M) and 10 μ L of sulfo-NHS (0.1 M). After sonicating for 15 min, the mixture was washed to remove the excess activators (EDC/sulfo-NHS) and dispersed in 200 μ L of PBS buffer (10 mM, pH 7.4). Then, the activated Mag@QD solution was incubated with 20 μ g of *P. aeruginosa* antibodies for 2 h, followed by surface blocking with 100 μ L of 10% BSA solution for 1 h. The antibody-conjugated Mag@QD were magnetically collected and resuspended with 200 μ L of PBS solution (containing 0.05% Tween-20)

S1.4. Detection of bacteria by using immuno-Mag@QD-LFA

The detection assay for *P. aeruginosa* was performed in a 1.5 mL EP tube and on a test strip. First, 5 μ L of immune-Mag@QD were added into 1 mL of sample solution, and the mixture was incubated for 15 min at room temperature. Then, the mixed immune- Mag@QD-bacteria complexes were rapidly separated by using an

external magnet and resuspended in 100 μ L of running buffer (10 mM PBS, pH 7.4, 10% FBS, 0.5% milk, 1% Tween 20). Finally, the running buffer mixture were loaded onto the sample pad of tested strip to start the chromatographic reaction. After 15 min, the fluorescence intensity on the test line was measured by a portable fluorescence reader.

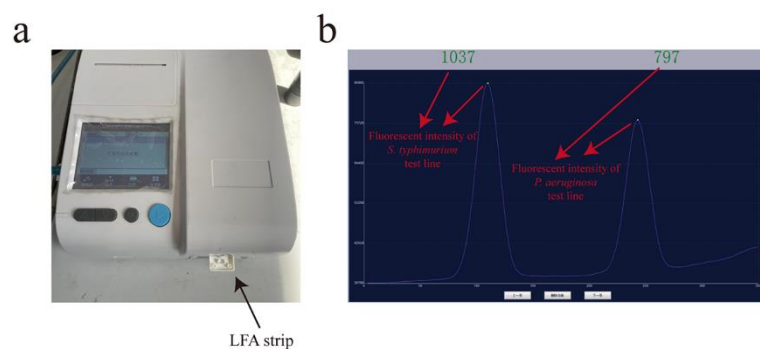


Figure S1 (a) Photograph of fluorescence reader. (b) The fluorescence intensity read by the fluorescence reader.

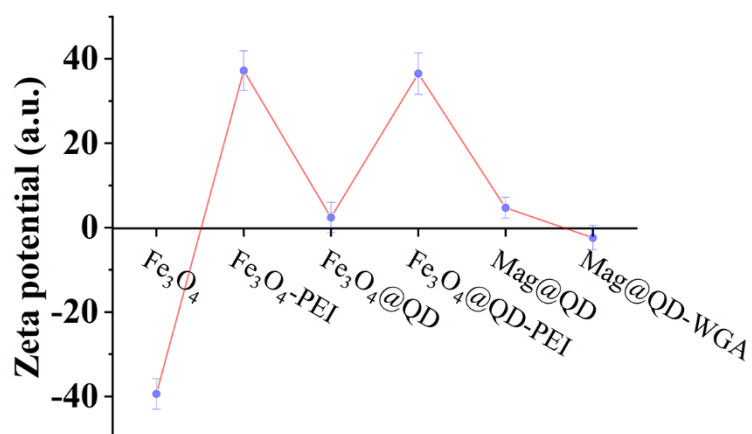


Figure S2 Zeta potentials of the products from each step.

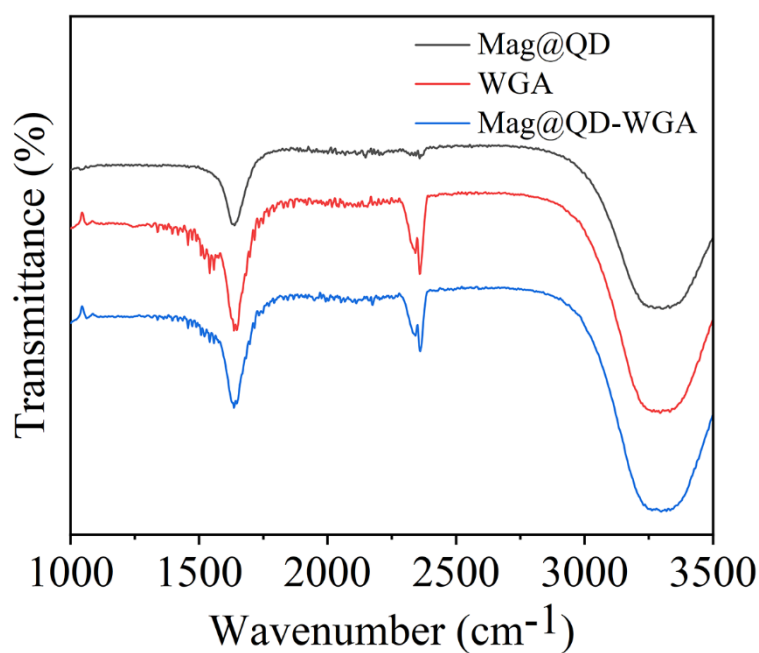


Figure S3 FTIR spectra of Mag@QD (black line), WGA (red line), and Mag@QD-WGA (blue line). The characteristic absorption peaks corresponding to protein amide bands I (2339 cm^{-1}) and II (2354 cm^{-1}) appearing in Mag@QD-WGA spectra reveal the successful coupling of WGA.

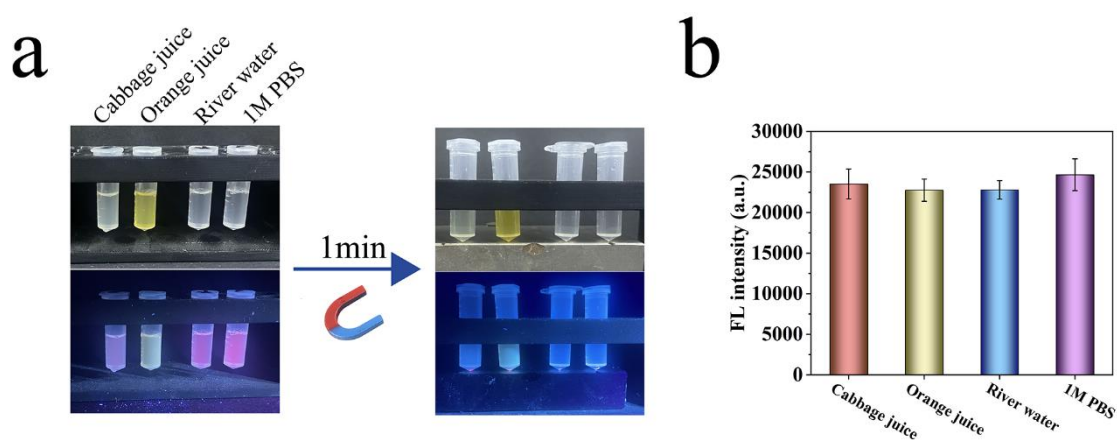


Figure S4 (a) Magnetic separation behavior and (b) fluorescence intensity of Mag@QD-WGA in different samples (cabbage juice, orange juice, river water, and PBS). The error bars are calculated from three experiments.

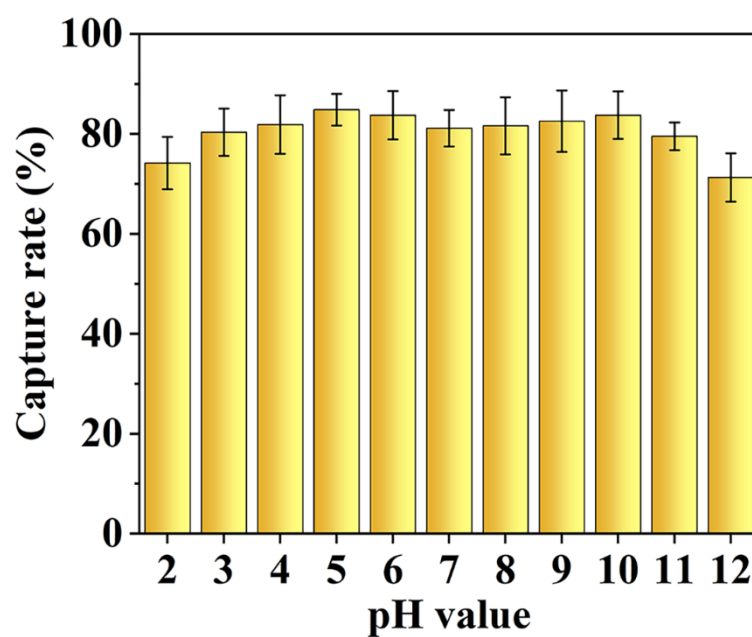


Figure S5 Capture abilities of Mag@QD-WGA for *P. aeruginosa* in aqueous solution with different pH values. The error bars are calculated from three experiments.

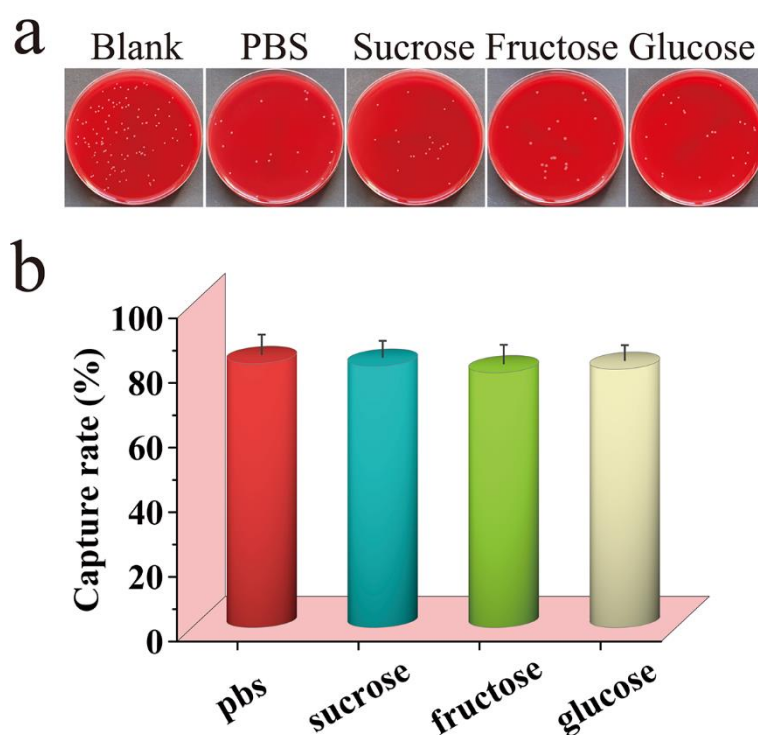


Figure S6 (a) Photographs of the colonies on blood agar plates indicating the amounts of remaining *P. aeruginosa* in the supernatant after the magnetic enrichment of Mag@QD-WGA in PBS and three different sugar solutions. (b)

Corresponding capture ability of Mag@QD-WGA in PBS and three different sugar solutions. The error bars are calculated from three experiments.

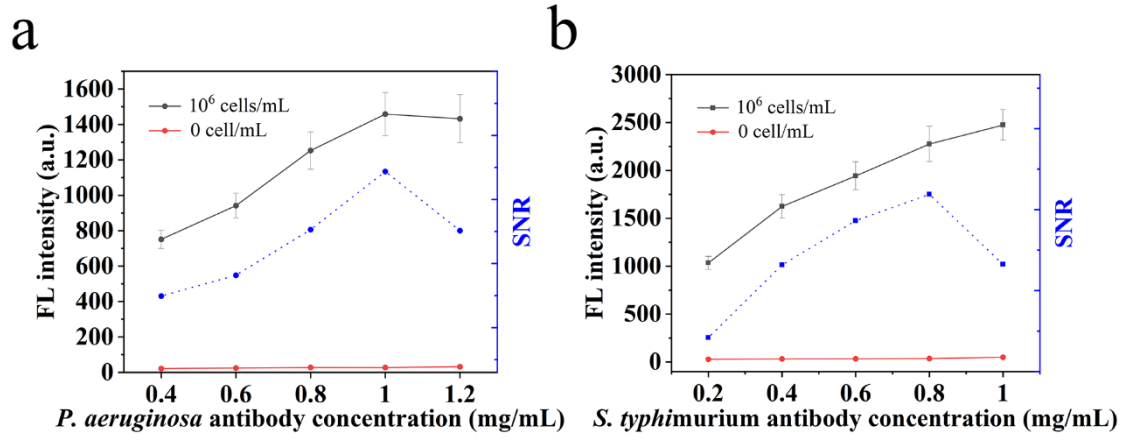


Figure S7 Optimization of capture antibody concentration on test line for (a) *P. aeruginosa* and (b) *S. typhimurium*. The error bars are calculated from three experiments.

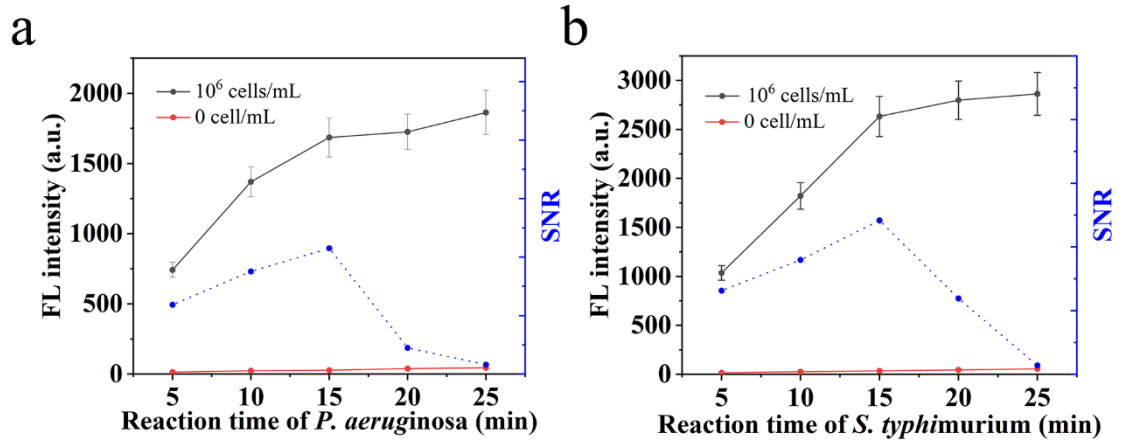


Figure S8 Optimization of (a) *P. aeruginosa* and (b) *S. typhimurium* reaction time on the test line for the LFA detection system. The error bars are calculated from three experiments.

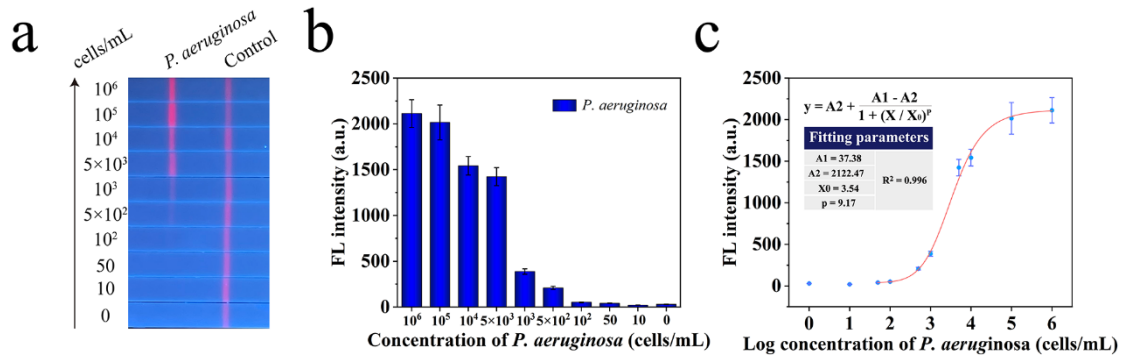


Figure S9 (a) Photograph of LFA strip with C line for *P. aeruginosa*. (b) Corresponding fluorescence intensity of test lines for different concentrations of *P.*

aeruginosa. (c) Corresponding calibration line of *P. aeruginosa*. The error bars are calculated from three experiments.

Table S1. Recovery results of the bacteria in river water, cabbage juice, and orange juice.

Sample	Spiked/Detected (cells/mL)		Recovery (%)		RSD (%)	
	<i>P. aeruginosa</i>	<i>S. typhimurium</i>	<i>P. aeruginosa</i>	<i>S. typhimurium</i>	<i>P. aeruginosa</i>	<i>S. typhimurium</i>
River water	10 ⁶ /1.07×10 ⁶	10 ⁶ /0.99×10 ⁶	107.35	98.91	7.99	4.82
	10 ⁴ /0.94×10 ⁴	10 ⁴ /0.95×10 ⁴	93.7	95.48	6.51	8.13
	10 ² /1.06×10 ²	10 ² /1.01×10 ²	105.8	101.03	7.05	8.52
Cabbage juice	10 ⁶ /0.94×10 ⁶	10 ⁶ /0.92×10 ⁶	93.91	91.64	8.12	6.6
	10 ⁴ /0.97×10 ⁴	10 ⁴ /0.90×10 ⁴	97.49	90.19	9.23	10.39
	10 ² /1.00×10 ²	10 ² /0.99×10 ²	99.66	99.19	7.3	9.98
Orange juice	10 ⁶ /0.97×10 ⁶	10 ⁶ /0.95×10 ⁶	96.57	94.71	6.29	6.23
	10 ⁴ /1.05×10 ⁴	10 ⁴ /1.10×10 ⁴	104.96	109.51	9.51	6.57
	10 ² /1.03×10 ²	10 ² /0.96×10 ²	102.75	95.64	9.78	8.92

Table S2 Comparison of Mag@QD-WGA-LFA with other reported multi LFA.

Detection method	Bacteria	Recognition molecules	LODs (cells/mL)	Reference
Fluorescent-LFA	<i>Salmonella</i> spp./ <i>E. coli</i> O157:H7	Dual antibodies sandwich complex	10 ⁵ /10 ⁵	[1]
Colorimetric-LFA	<i>S. boydii</i> / <i>E. coli</i> O157:H7	Dual antibodies sandwich complex	10 ⁶ /10 ⁶	[2]
Colorimetric-LFA	<i>Klebsiella</i> / <i>Raoultella</i>	Dual antibodies sandwich complex	10 ⁴ /10 ⁵	[3]
Fluorescent-LFA	<i>E. coli</i> O157:H7/ <i>S. typhimurium</i>	Dual antibodies sandwich complex	50/50	[4]
Fluorescent-LFA	<i>S. pneumoniae</i> / <i>S. aureus</i>	Dual antibodies sandwich complex	13/20	[5]
Fluorescent-LFA	<i>P. aeruginosa</i> / <i>S. typhimurium</i>	WGA and antibody	25/28	This work

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