Supporting Information

Microfluidic Filtration Enrichment Cascade Nanozyme-Catalyzed Colorimetric Immunoassay for Rapid and Ultrasensitive Detection of *Escherichia coli* in Urine with Smartphone Readout

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Supporting Information Contents.

S1.1 Preparation of Au nanoparticles

Gold nanoparticles (AuNPs) were synthesized using a standard trisodium citrate (TSC) reduction method[1]. Briefly, 1 mL of 1% (w/w) chloroauric acid ($HAuCl_4$) solution was diluted with 100 mL of deionized water in a clean flask. The solution was heated to boiling under constant magnetic stirring (300–500 rpm) to ensure uniform temperature distribution. Upon reaching boiling, 4 mL of 1% (w/w) TSC solution was rapidly added to initiate nanoparticle formation. The reaction was maintained at 100 °C with continuous stirring for 15 min. After completion, the AuNP suspension was removed from heat and allowed to cool to room temperature under stirring to prevent aggregation. The resulting gold seed solution was stored in a cool, dark place until further use.

S1.2 Preparation of Au@Pt nanozymes

Au@Pt nanozymes were synthesized using a modified protocol based on previously reported methods[2]. Briefly, 400 μL of the pre-synthesized Au seed solution was centrifuged at 10,000 rpm for 5 min to collect the AuNPs, which were then washed twice with deionized water and redispersed in 600 μL of deionized water. To stabilize the particles, 40 μL of 10% (w/w) poly(vinylpyrrolidone) (PVP) solution was added, and the mixture was gently swirled and allowed to stand for 5 min to ensure uniform PVP coating on the Au seeds. Subsequently, 40 μL of L-ascorbic acid (L-AA, 100 mg/mL) and 40 μL of chloroplatinic acid hydrate (100 mM) were added dropwise to the mixture. The reaction was conducted at 60 °C with continuous shaking at 800 rpm for 20 min, promoting the deposition of platinum (Pt) onto the Au cores to form a porous Pt shell. The resulting Au@Pt nanozymes were collected by centrifugation at 8000 rpm for 8 min, washed twice with deionized water, and finally redispersed in 1 mL of deionized water for subsequent use.

S1.3 Antibody conjugation to Au@Pt nanozymes

The *E. coli*-specific antibody was conjugated onto the surface of Au@Pt nanozymes via the following procedure. First, 5 μL of freshly prepared 10 mM DTNB ethanol

solution was added to 500 μL of Au@Pt nanozyme suspension, followed by gentle stirring for 3 h to introduce carboxyl groups. Second, the carboxylated Au@Pt suspension was centrifuged and redispersed in 500 μL of 2 mM 2% MES buffer (pH 5.5); then 2 μL of 10 mM EDC and 4 μL of 10 mM sulfo-NHS were added to activate carboxyl groups, followed by 15 min of incubation. Third, after centrifugation, the activated Au@Pt nanozymes were resuspended in 200 μL of 2 mM PB buffer (pH 7.4), and *E. coli*-specific antibody was added and incubated for 2 h at room temperature. Fourth, 100 μL of 10% (w/w) bovine serum albumin (BSA) was added to block nonspecific binding, and the incubation was continued for 60 min. Finally, the mixture was centrifuged at 7800 r/min for 6 min and washed twice with PB buffer; the obtained antibody-conjugated Au@Pt nanozymes were resuspended in 200 μL of storage buffer (2 mM PB buffer supplemented with 0.5% sucrose, 0.02% NaN_3, and 1% BSA).

S1.4 Bacteria sample preparation

The bacterial strains used in this study included *Escherichia coli (E. coli), Staphylococcus saprophyticus (S. saprophyticus), Pseudomonas aeruginosa (P. aeruginosa), Enterococcus faecalis (E. faecalis), Proteus mirabilis (P. mirabilis), and Klebsiella pneumoniae (K. pneumoniae)*. The bacterial concentrations were verified using the conventional plate counting method. Briefly, each strain was inoculated onto 5% sheep blood agar plates and incubated overnight at 37 °C. Several well-isolated colonies were then collected and resuspended in 1 mL of sterile phosphate-buffered saline (PBS, 10 mM, pH 7.4). The bacterial suspensions were serially diluted 10^5–10^8 fold with sterile water, and 0.2 mL of each dilution was spread onto blood agar plates and incubated for 12 h. The colony-forming units (CFUs) were counted to determine the original bacterial concentrations. Finally, *E. coli* suspensions with defined concentrations (0–10^7 CFU/mL) were prepared by appropriate dilution of the stock cultures.

S1.5 Preparation of AuNP-based ICA

The colloidal gold (AuNP)–based ICA strip was prepared according to previously reported literature. First, AuNPs (40 nm) were fabricated through the citrate reduction

method. Briefly, 200 mL of $HAuCl_4$ solution (0.01%, w/v) was heated to the boiling point with stirring. Then, 1 mL of trisodium citrate (1%, w/v) was added rapidly to the boiling solution. The suspension was boiled for 15 min and then allowed to reach thermal equilibrium at room temperature, which yielded the Au NPs with a diameter of ~40 nm. Afterwards, the pH of 10 μg anti–bacteria antibody was adjusted to 9 with 0.2 M K_2CO_3 and incubated with 1 mL 40 nm AuNP (pH 8–9) for 15min. Then, 50 μL of 10% BSA was added to block the unreacted sites of AuNPs. The as–prepared immuno–2 AuNPs were collected by centrifugation (5000 rpm, 6 min), and resuspended with 200μL of storage solution (10 mM PB solution containing 1% BSA (w/v), 0.1% PVP (w/v), 10% sucrose (w/v), and 0.05% Tween–20 (v/v)). Finally, it was dispensed onto the glass fiber paper and dried to prepare a conjugate pad. Then, the conjugate pad was assembled on the ICA strip and cut it into a 3.2 mm strip for subsequent use.

S1.6 Statistical analysis

Statistical analysis was performed using Origin (version 2022). Experimental data points represent three independent measurements and are presented as mean ± SD. For datasets following a normal distribution, unpaired Student's t-tests were used to evaluate differences between groups. Deming regression analysis was performed by comparing bacterial concentrations measured using the microfluidic platform with those obtained by the standard quantitative urine culture method. ROC curves were generated by plotting the predicted probability values from both the standard concentrations and the validation cohort. Diagnostic performance metrics, including sensitivity and specificity, were calculated by determining the optimal threshold that maximizes the Youden index.

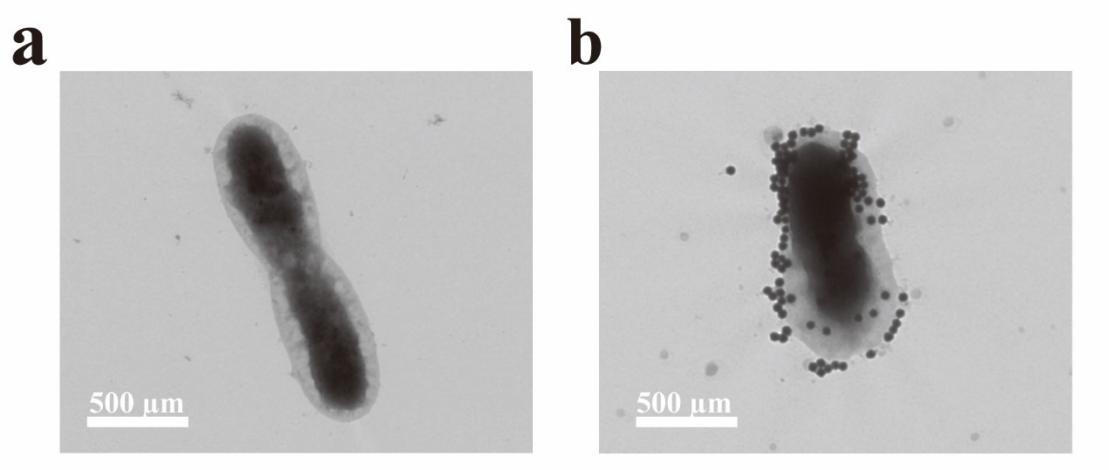

Figure S1. TEM images of **(a)** *E. coli,* and **(b)** immunocomplex of Au@Pt- *E. coli*.

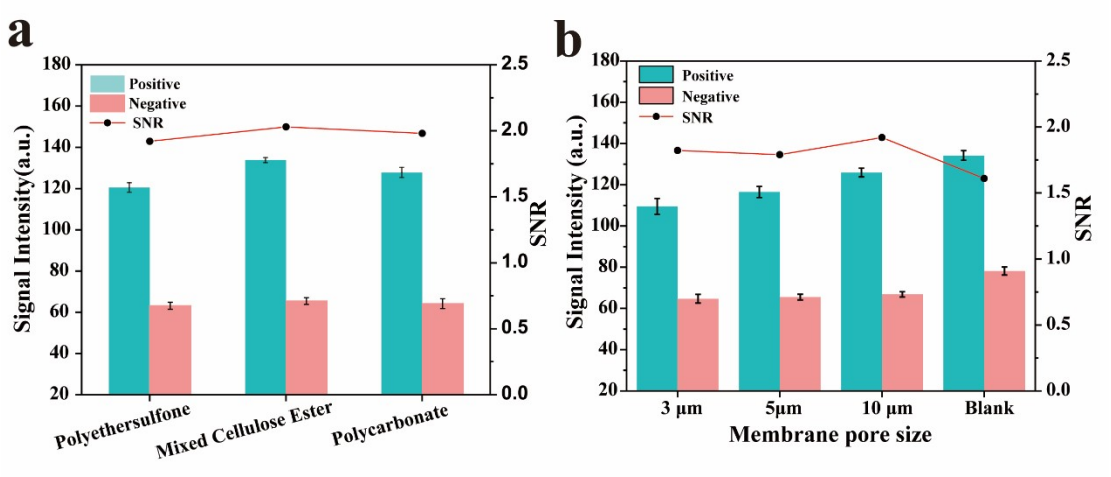

Figure S2. (a) Optimization the material of the filter membrane and **(b)** the pore size of filter i.

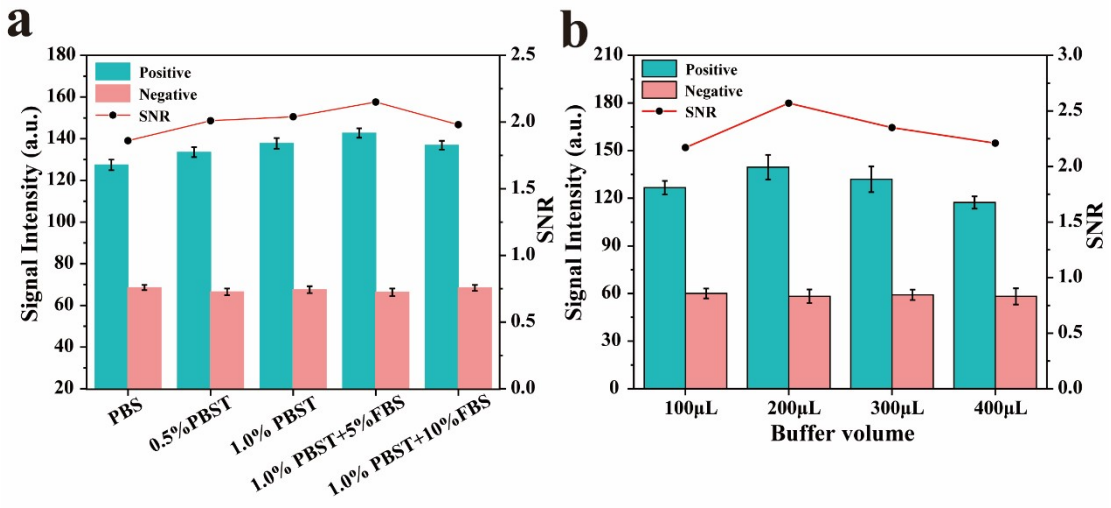

Figure S3. Optimization of **(a)** the composition ratio and **(b)** dosage of the running buffer solution.

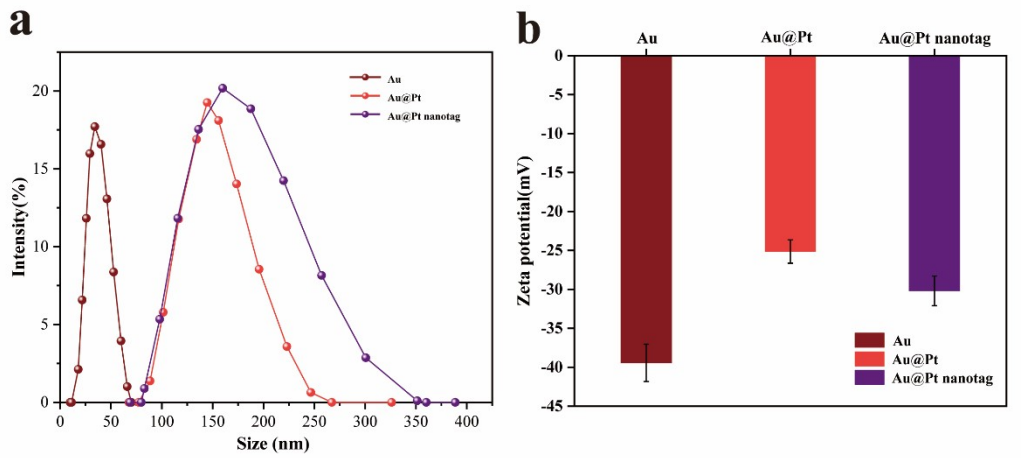

Figure S4. (a) The hydrodynamic diameters and (b) zeta potentials of Au NPs, Au@Pt and Au@Pt nanotag.

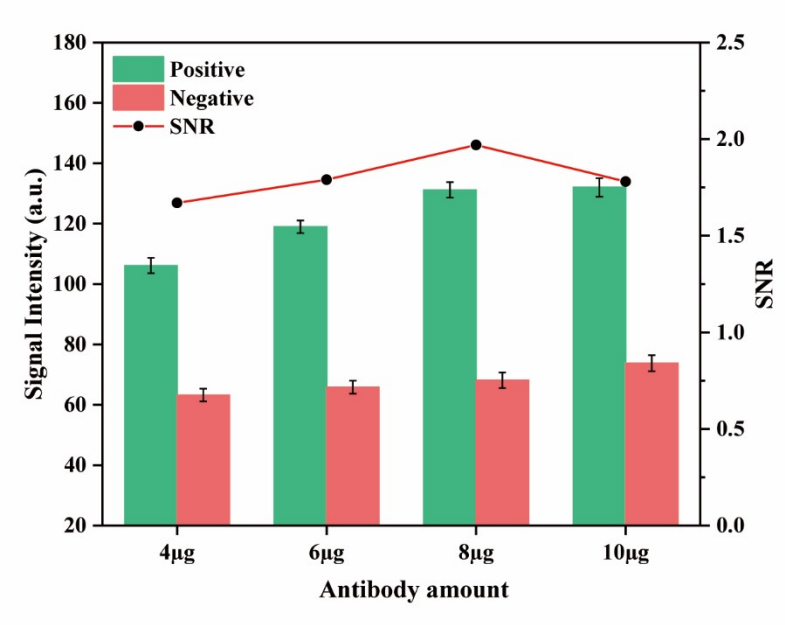

Figure S5. Optimization of *E. coli* antibody conjugation dosage on Au@Pt nanozymes.

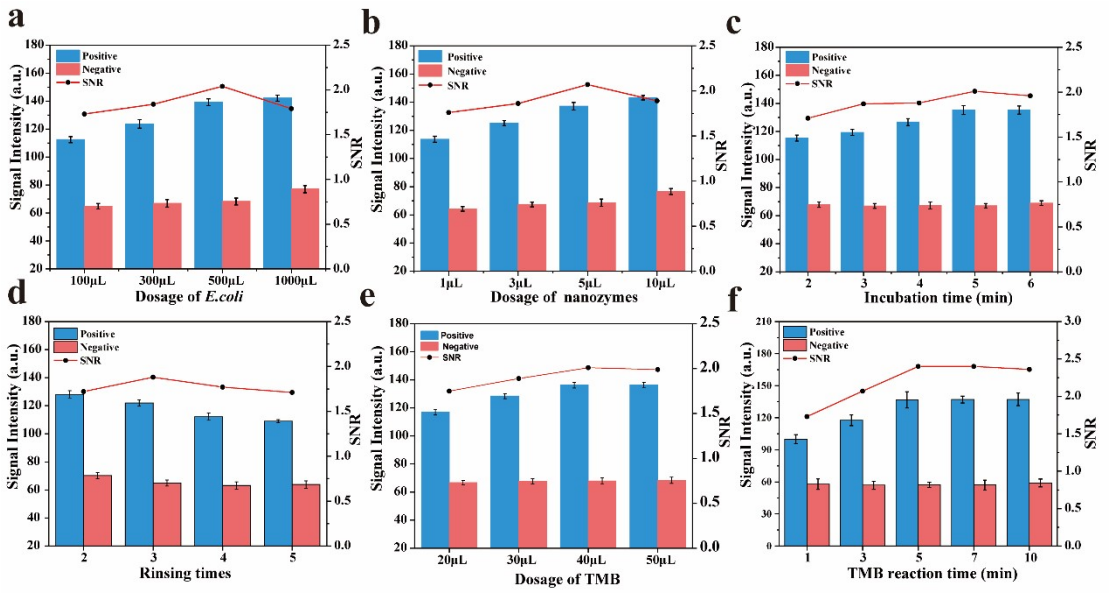

Figure S6. Optimization of the dosage of *E. coli* with concentration of 10^4 cfu/mL **(a)**, dosage of Au@Pt nanozyme with concentration of 30mg/mL **(b)**, incubation time **(c)**, the number of filter membrane washes with 0.05%PBST solution **(d)**, dosage of TMB **(e)**, and TMB reaction time **(f)**.

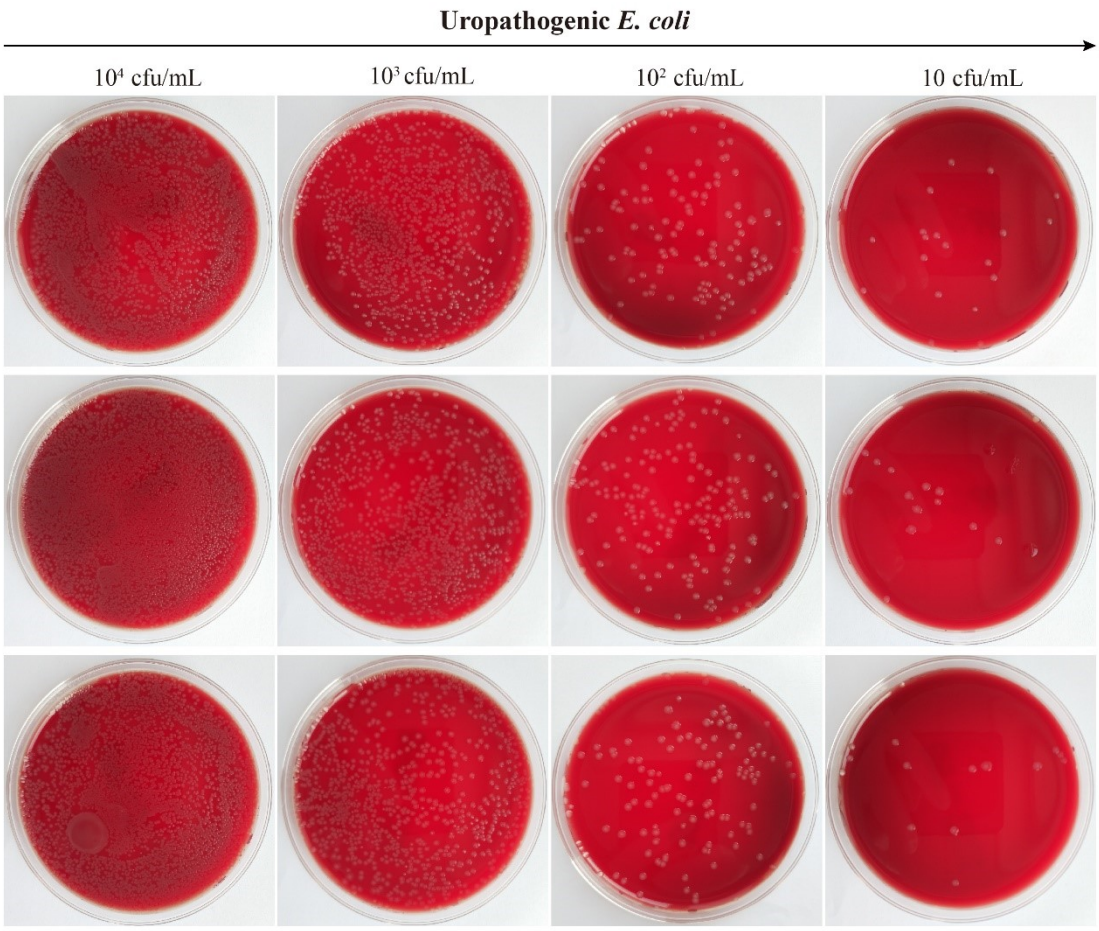

Figure S7 Standard plate counting method for quantitative determination of *E. coli.*

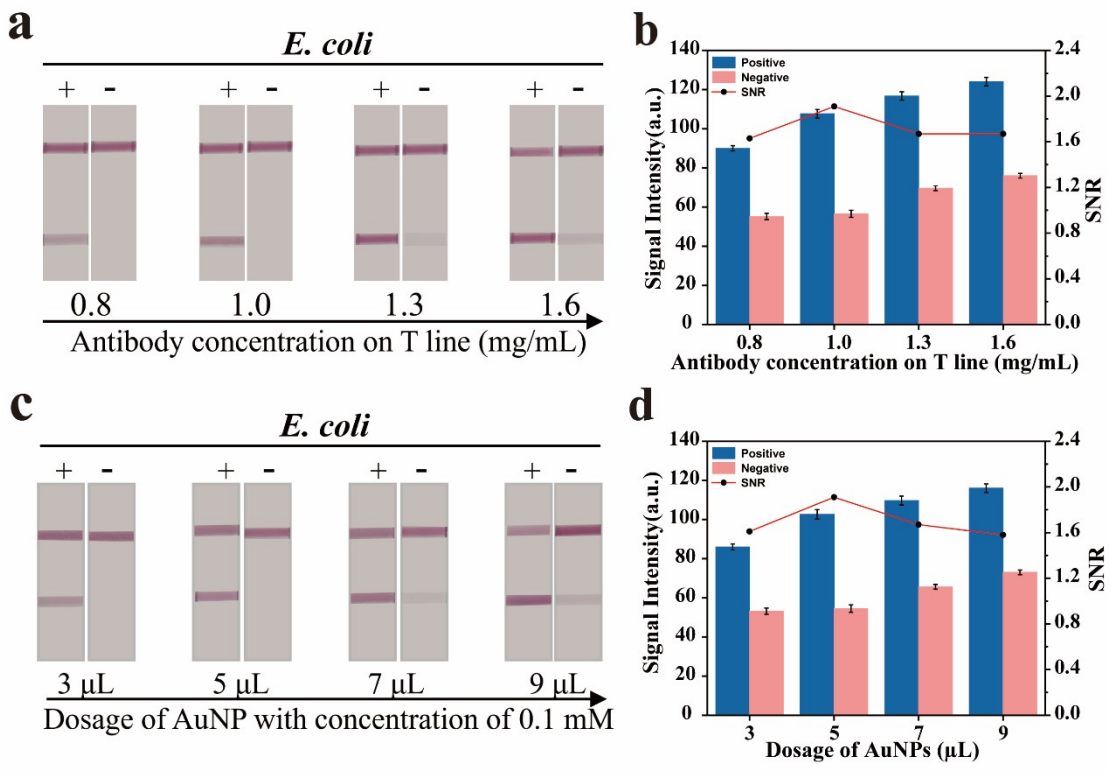

Figure S8 Optimization of (a) antibody concentration on the T lines and (b) the detailed colorimetric signal intensities and corresponding signal-to-noise ratios of different antibody concentration. (c) the dosage of AuNPs (25mg/mL) for AuNP-based ICA strips to detect *E. coli*.(d) the detailed colorimetric signal intensities and corresponding signal-to-noise ratios of different dosage of AuNPs.

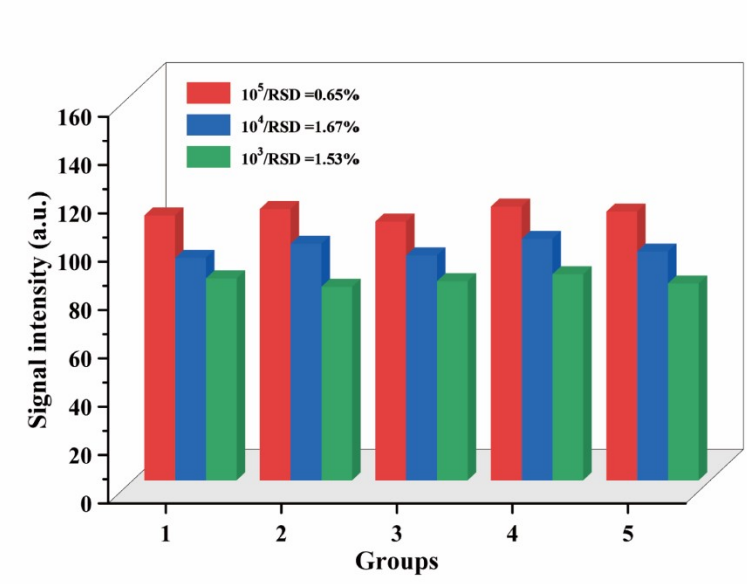

Figure S9. Evaluation of the reusability of the microfluidic chip for *E. coli* of 10^3, 10^4, and 10^5 cfu/mL.

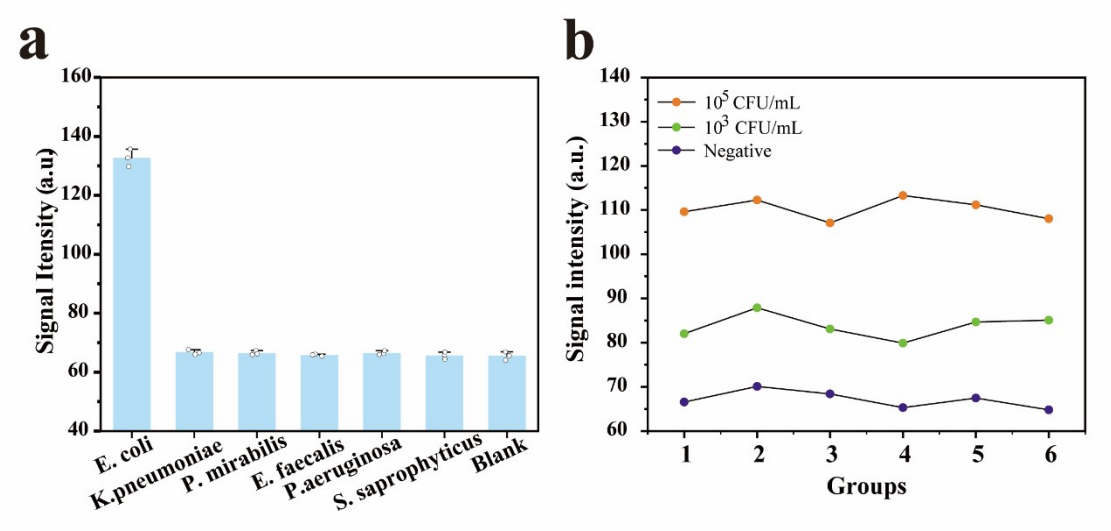

Figure S10 (a) Validation of the cross-reactivity between *E. coli* detection and other common uropathogens(*S. saprophyticus*, *P. aeruginosa*, *E. faecalis*, *P. mirabilis*, and *K. pneumoniae*) **(b)** The repetitive detection results of *E. coli* at the concentrations of 0, 10^3, 10^5 CFU/mL.

Table S1. Main performance of microfluidic platform compared with other approaches for bacteria detection.

Bacterial	Analytical method	LOD	Assay time	Reference
E. coli	Microfluidic chip	5×10^3 CFU/mL	45 min	[3]
E. coli	graphene quantum dots-based AI	3.38×10^2 CFU/mL	40–60 min	[4]
E. coli	LAMP	10^3 CFU/mL	35 min	[5]
E. coli	Engineered Reporter Phages	<100 CFU/mL	5 hour	[6]
P. aeruginosa	LVSi	10^3 CFU/mL	10 min	[7]
P. aeruginosa	Colorimetric LFA	3.3×10^2 CFU/mL	30min	[8]
E. coli	Paper based LFA	10^2–10^6 CFU/mL	20 min	[9]
E. coli	Microfluidic electrochemical	10^2 CFU/mL	30-60 min	[10]
E. coli	Microfluidic Platform	10^2 CFU/mL	15 min	This work

Table S2. Recovery efficiency of Microfluidic platform for *E. coli* in urine samples.

Sample	Add (cfu/mL)	Detected (cfu/mL)	Recovey (%)	RSD (%)
Urine	10^6	9.67×10^5	96.7	6.49
	10^5	1.06×10^5	106.0	4.12
	10^3	9.36×10^2	93.6	4.68

Table S3. Quantification of 20 *E. coli* positive urine samples determined by Microfluidic platform and Plate cultrue method.

Clinical Samples	Microfluidic platform		Plate cultrue method	
	Bacteria	Quantification(cfu/mL)	Bacteria	Quantification(cfu/mL)
1	*E. coli*	8.76×10^4	*E. coli*	7.85×10^4
2	*E. coli*	6.98×10^3	*E. coli*	7.96×10^3
3	*E. coli*	7.38×10^4	*E. coli*	6.89×10^4
4	*E. coli*	3.74×10^5	*E. coli*	3.37×10^5
5	*E. coli*	4.63×10^4	*E. coli*	4.23×10^4
6	*E. coli*	1.46×10^4	*E. coli*	1.43×10^4
7	*E. coli*	4.39×10^3	*E. coli*	4.45×10^3
8	*E. coli*	4.61×10^4	*E. coli*	4.12×10^4
9	*E. coli*	1.19×10^5	*E. coli*	1.08×10^5
10	*E. coli*	5.32×10^5	*E. coli*	4.95×10^5
11	*E. coli*	2.86×10^4	*E. coli*	3.21×10^4
12	*E. coli*	1.34×10^5	*E. coli*	1.19×10^5
13	*E. coli*	2.02×10^4	*E. coli*	1.89×10^4
14	*E. coli*	1.46×10^6	*E. coli*	1.29×10^6
15	*E. coli*	8.36×10^4	*E. coli*	7.86×10^4
16	*E. coli*	6.15×10^4	*E. coli*	7.01×10^4
17	*E. coli*	3.64×10^4	*E. coli*	4.02×10^4
18	*E. coli*	3.12×10^4	*E. coli*	3.34×10^4
19	*E. coli*	5.46×10^4	*E. coli*	5.19×10^4
20	*E. coli*	2.42×10^4	*E. coli*	2.83×10^4

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