Supporting Information for

Versatile biosensing toolkit using an electronic particle counter

Zhilong Wang[1,2], Jiawei Liu[1,2], Yanlian Yang[4], Ping Li[4], Kaikai Li[1], Yunlei Xianyu[3,*], Yiping Chen[1,2,*], Bin Li[1,2]

[1]College of Food Science and Technology, Huazhong Agricultural University, Shizishan Street, Hongshan District, Wuhan 430070, Hubei, China

[2]Key Laboratory of Environment Correlative Dietology, Huazhong Agricultural University, Ministry of Education, Shizishan Street, Hongshan District, Wuhan 430070, Hubei, China

[3]College of Biosystems Engineering and Food Science, Zhejiang University, 866 Yuhang Tang Road, Hangzhou, 310058 Zhejiang, China

[4]National Center for Nanoscience and Technology, No. 11 Zhongguancun Beiyitiao, Beijing 100190, China

E-mail: xianyu19@zju.edu.cn (YL Xianyu)

E-mail: chenyiping@mail.hzau.edu.cn (YP Chen)

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Methods

Activation of COOH-MNPs and covalent coupling of antibodies (probe)

For the activation of COOH-MNPs, 2 mg of COOH-MNPs were washed by the activated buffer (10 mM MEST, pH=6.0) twice. Then, COOH-MNPs were suspended in 200 μL of EDC solution (5 mg/mL, dissolved in MES) and 200 μL of NHS solution (5 mg/mL, dissolved in MES) for activation. After activation for about 0.5 h, the excess EDC, NHS, and byproduct were removed by magnetic separation using a magnetic separator. Then, 0.2 mg of antibody (probe) was added to the activated MNPs and the volume of antibody and activated MNPs was adjusted to 500 μL by the coupling buffer PBST (0.01 M, pH=7.4). The mixture was gently stirred to couple at 37 °C for at least 3 h, and it maintained the suspension state of the MNPs during coupling. After coupling, the free Ab (probe) was removed by magnetic separation and the MNPs were blocked with 1% BSA (m/v) for 0.5 h at 37 °C. Then, the conjugate was washed for four times with PBST. Finally, the conjugate was resuspended in PBST (pH=7.4, 0.01M, 0.5% BSA) and stored in 4 °C for future experiment.

Activation of COOH-PS microspheres and covalent coupling of biological ligand

First, 800 μL of MEST buffer was added into 200 μL of COOH-PS microspheres (10 mg/mL) to wash twice and centrifuged to remove impurity. 5 mg/mL of EDC and NHS solution with MES solution were prepared and added into COOH-PS microspheres to activate at 37 °C for about 0.5 h. After activation, the excess EDC, NHS, and byproduct were removed by centrifugal separation. Then, 0.2 mg of biological ligand (antibody, complete antigen, enzyme, or probe) was added to the activated PS microspheres and the volume of biological ligand and activated PS microspheres was adjusted to 500 μL with coupling buffer PBST (0.01 M, pH=7.4). The mixture was gently stirred to couple at 37 °C for at least 3 h, and it maintained the suspension state of the PS microspheres during coupling. After coupling, the unconjugated biological ligand was removed by centrifugal separation and the PS microspheres was blocked with 1% BSA (m/v) for 0.5 h at 37 °C. Then, the PS microsphere-biological ligand conjugate

was washed for four times with PBST. Finally, the conjugate was resuspended in PBST (pH=7.4, 0.01M, 0.5% BSA) and stored in 4 °C for further use.

Primer and probe design

We used NCBI nucleotide database to search the gene sequence of *Salmonella*, then the gene sequence was introduced into Primer Premier 6, and the conserved sequence was selected as a primer. We designed a pair of specific oligonucleotide probes to complementary the target sequence of *Salmonella*. The external end of the probes modified with multiple thymine residues (oligoT), with an amino group at the terminal end of the oligoT linker. The primers and probes of all oligonucleotides were custom-designed and synthesized by Tsingke Biological Technology.

Pathogen culture and DNA extraction

Salmonella was seeded and cultured in suspension using the Nutrient Broth (NB) on an orbital shaker at 37 °C before use. The concentration of *Salmonella* was determined by conventional plate counting as follows: 10 μL of *Salmonella* dilutions was surface plated on the NB agar plates and incubated at 37 °C for 24 h, then *Salmonella* colonies on the plate were counted to determine the number of colony-forming units per milliliter (cfu/mL). For DNA extraction, *Salmonella* was first centrifuged (10000 rpm, 3 min), and bacterial precipitation was washed twice with deionized water. Then, bacterial precipitation was re-suspended with sterile deionized water and boiled for 15 minutes to extract DNA. After extraction of the DNA followed by centrifugation, the supernatant as a DNA template was measured by ultra-micro spectrophotometer (NanoPhotometer N60 Touch, Germany) to calculate concentration and purity.

Preparation of $MNPs_{200}$-polypeptide conjugate

The coupling of $MNPs_{200}$ with recognition polypeptide was mediated by biotin-streptavidin interaction as described in the literature[1]. Briefly, streptavidin-conjugated MNPs were incubated with biotin-conjugated recognition polypeptide at a volume ratio of 1:2 for

about 30 min in PBS at room temperature. Then, the un-conjugate part was removed by magnetic separation and the conjugates were washed with PBS for five times. Finally, the conjugates were resuspended with PBS solution and stored in 4 ℃ for future use.

Cell culture and digestion

The Caco-2 cells were routinely cultured in plastic flasks in minimum essential medium (MEM) with 15% fetal bovine serum (FBS) and 1% nonessential amino acids in the presence of 100 μg/mL penicillin and streptomycin. Then, the cells were incubated at 37 °C in a 5% CO_2 atmosphere. Mouse 3T3-L1 preadipocytes were cultured in complete medium with 10% FBS, supplemented with 50 μg/mL penicillin and streptomycinand at 37 ℃ with 5% CO_2 in a cell culture incubator. The media were replaced once every two days until the flasks reached about 80%-90% confluence before harvest. During harvest, the cells were washed twice with PBS buffer followed by trysinization with trypsin-ethylenediaminetetraacetic (EDTA) solution (0.25 w/v% trypsin, 2.5 g/L EDTA) at 37 ℃ for 5 min to detach the cells from the flask. Then, we neutralized trypsin by adding fresh medium and the harvested cells were transferred into a new centrifuge tube and centrifugal separation (1000 rpm, 5 min). The supernatant was removed and the cells were resuspended in fresh medium for assaying.

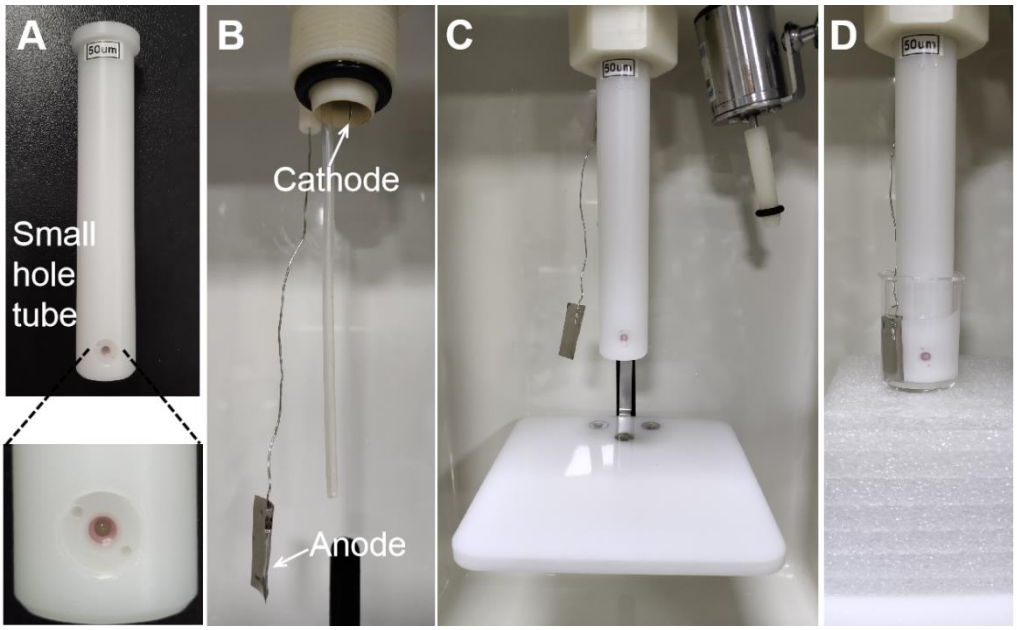

Figure S1. Physical map of the small hole tube and the process of sample measurement. (A) A PTFE small hole tube contains a gemstone and there is a 50 μm hole in the gemstone. (B) The location of the cathode and anode electrodes. (C) The position of the orifice tube and electrode before the test. (D) The anode and the small hole tube are immersed in the electrolyte containing the microspheres during the measurement.

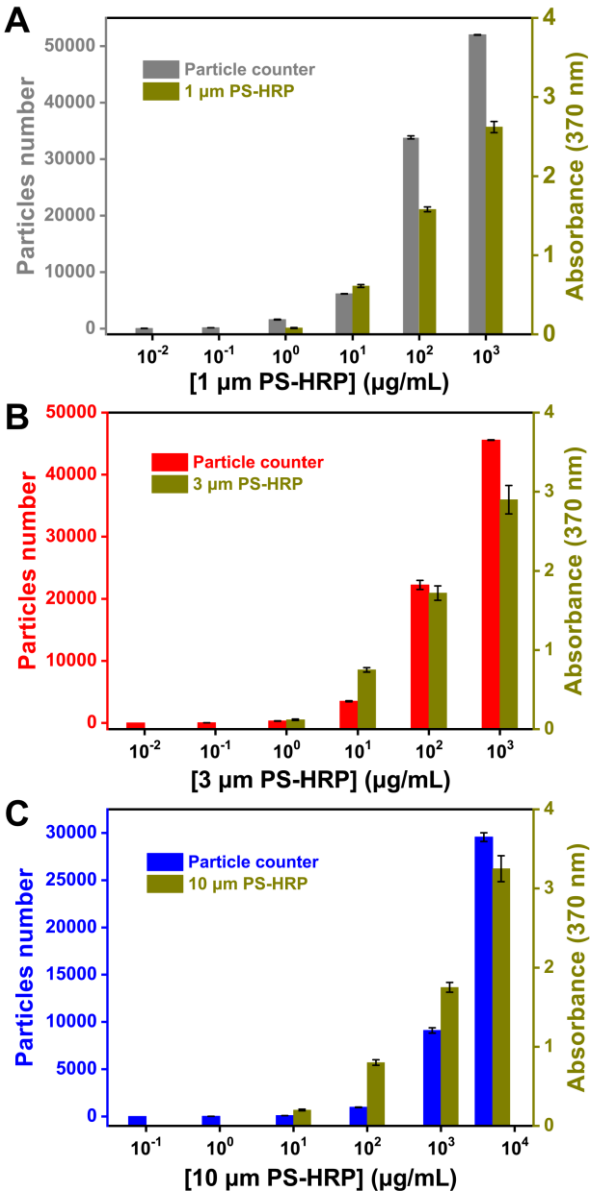

Figure S2. Comparison of the sensitivity of particle counter assay and HRP-mediated absorption method. Particle counter assay and A_{370} measurements of PS and PS-HRP at different concentrations for different sizes (A) 1 μm, (B) 3 μm, and (C) 10 μm. The error bars are the standard deviation from three measurements at each concentration (n=3).

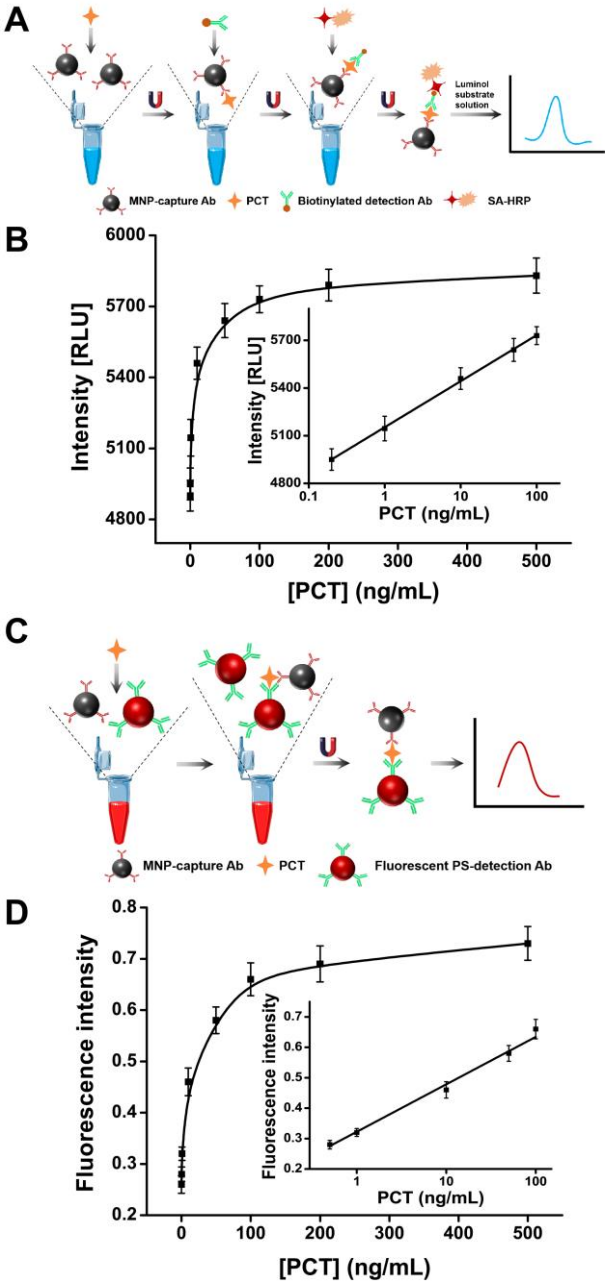

Figure S3. HRP-mediated chemiluminescence immunoassay and fluorescent microsphere-mediated immunoassay for detecting PCT. (A) Schematic illustration and (B) standard curve and linear range of HRP-mediated chemiluminescence immunoassay for detecting PCT. (C) Schematic illustration and (D) standard curve and linear range of fluorescent microsphere-mediated immunoassay for detecting PCT. The error bars are the standard deviation from three measurements at each concentration (n=3).

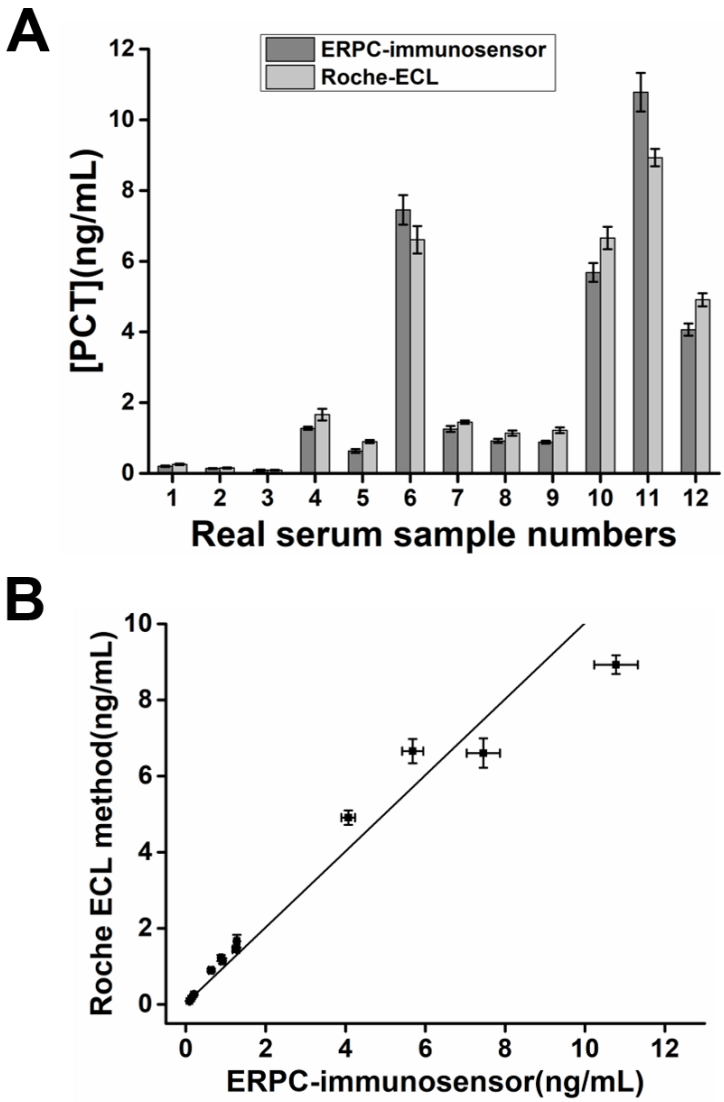

Figure S4. Results of PCT detection in real serum samples. (A) The results of the ERPC biosensor and Roche-ECL for PCT detection in real serum samples. (B) The comparison of PCT levels measured by the ERPC biosensor and Roche-ECL. The error bars are the standard deviation from three measurements at each concentration (n=3).

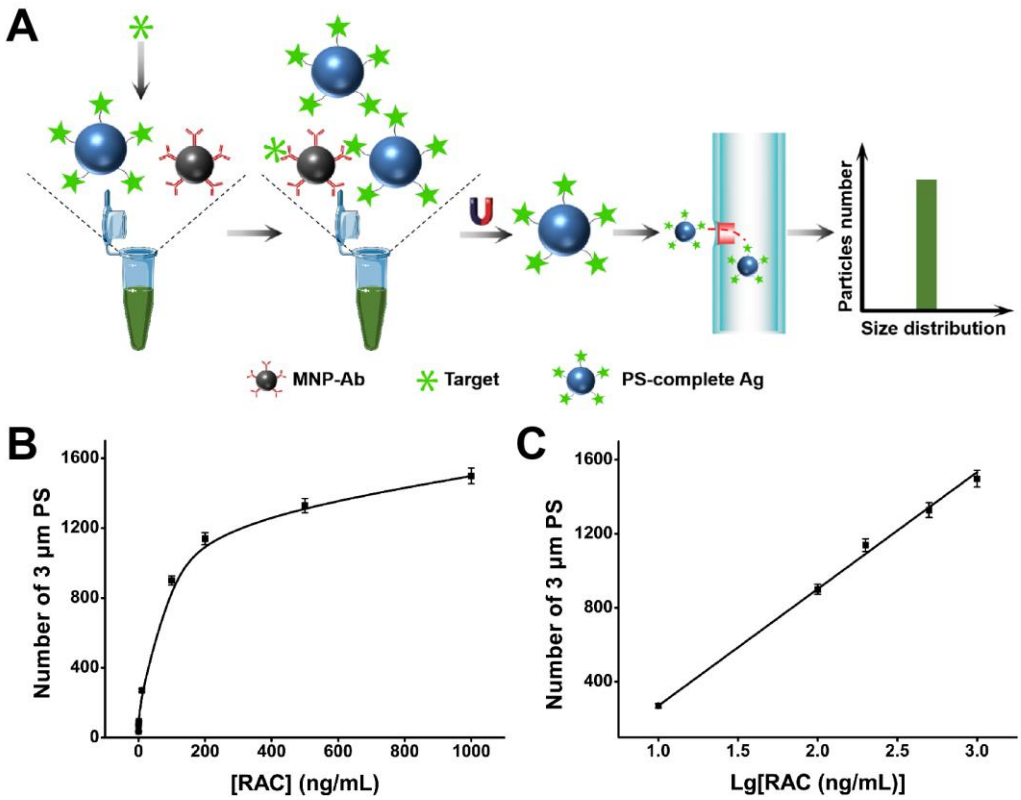

Figure S5. ERPC biosensor for small molecule detection. (A) Scheme of one-step competitive immunoassay for detection of RAC. (B) Standard curve, and (C) linear range for detection of RAC. The error bars are the standard deviation from three measurements at each concentration (n=3).

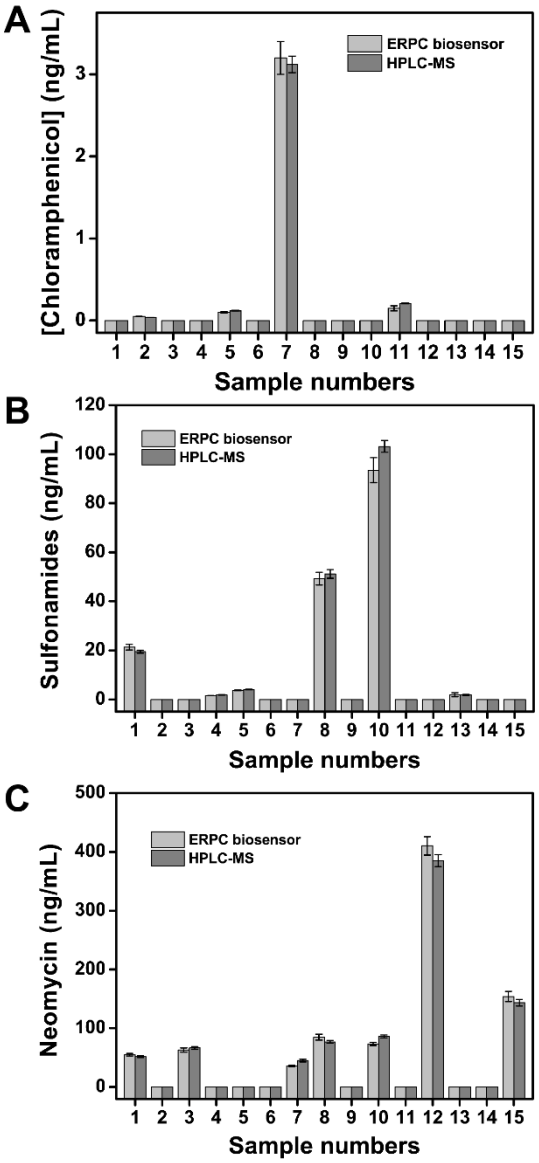

Figure S6. Comparison with HPLC-MS for the quantitative detection of antibiotics in real samples. (A) Chloramphenicol detection using ERPC biosensor based on 10 μm PS microspheres. (B) Sulfonamides detection using ERPC biosensor based on 3 μm PS microspheres. (C) Neomycin detection using ERPC biosensor based on 1 μm PS microspheres. The error bars are the standard deviation from three measurements at each concentration (n=3).

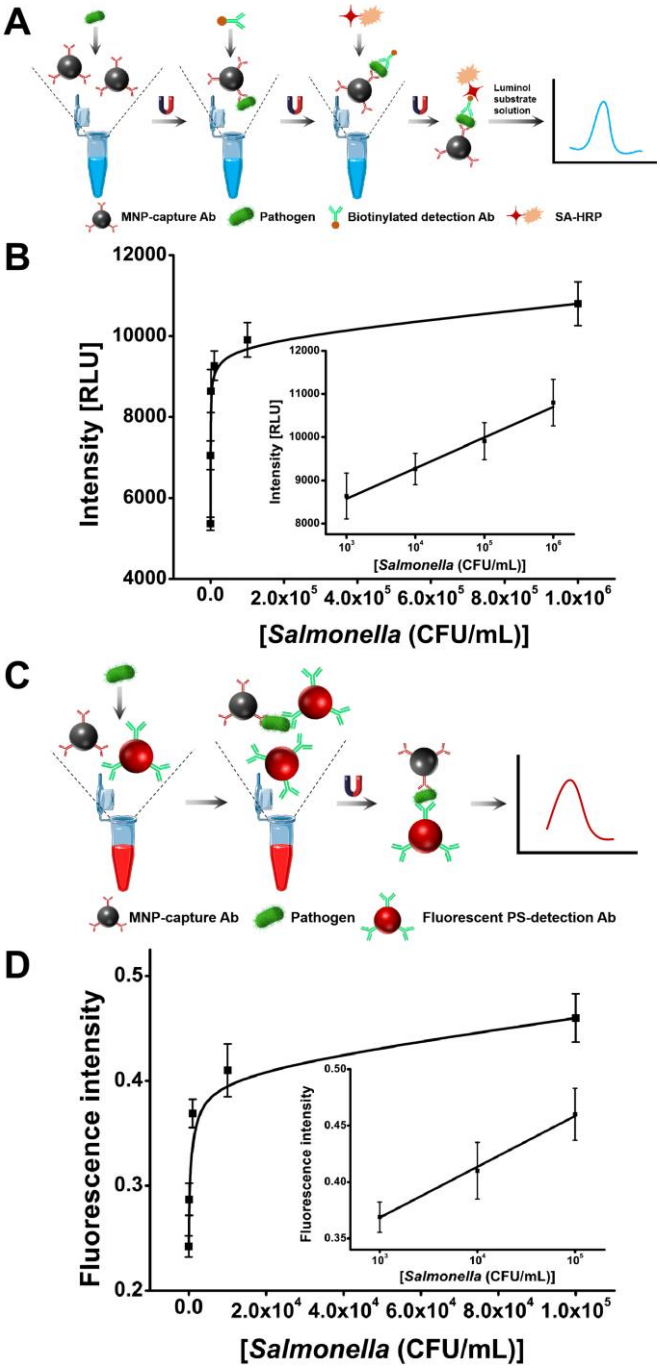

Figure S7. Chemiluminescence method and immunofluorescent microspheres-based method for detecting *Salmonella.* (A) Schematic illustration and (B) standard curve and linear range of chemiluminescence method for detecting *Salmonella.* (C) Schematic illustration and (D) standard curve and linear range of immunofluorescent microspheres-based method for detecting *Salmonella.* The error bars are the standard deviation from three measurements at each concentration (n=3).

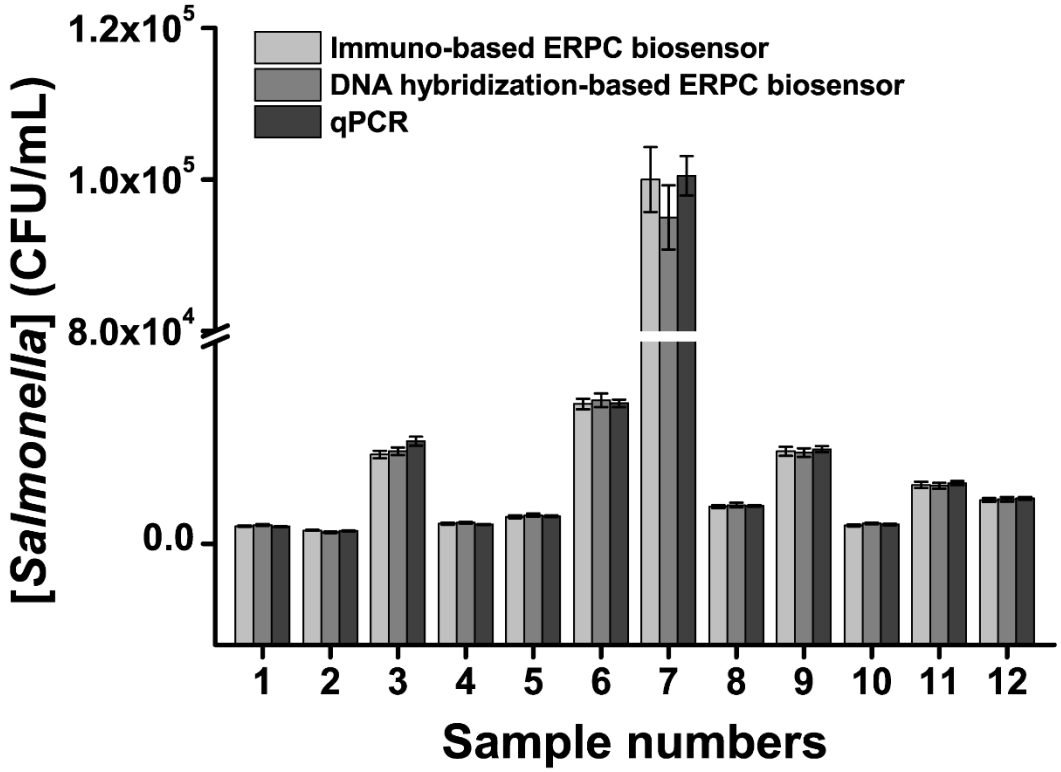

Figure S8. Comparison of the ERPC biosensor and qPCR for detecting *Salmonella.* All the milk samples purchased from different local supermarkets are proved to be positive by traditional culture method.

Table S1. Sensitivity of different apertures (50 μm, 100 μm and 200 μm) for 1 μm PS microspheres at the same sampling volume (100 μL) (n=3). "-" represents no data.

1 μm PS (μg/mL)	50 μm aperture	100 μm aperture	200 μm aperture
0.01	50 ± 4	-	-
0.05	83.6 ± 5.2	-	-
0.1	164.3 ± 6.0	-	-
0.5	807.7 ± 43.5	152 ± 8.2	-
1	1605.0 ± 72.3	321 ± 16.5	260 ± 12
10	6182.3 ± 29.2	548 ± 29.3	452 ± 23.6
50	24974.8 ± 711.7	2205 ± 112.5	1630 ± 77.4
100	33856.8 ± 268.8	3050 ± 148.6	2050 ± 126.5
500	49420.7 ± 42.2	3508 ± 168.9	2451 ± 157.5

Table S2. Sensitivity of different apertures (50 μm, 100 μm and 200 μm) for 10 μm PS microspheres at the same sampling volume (100 μL) (n=3). "-" represents no data.

10 μm PS (μg/mL)	50 μm aperture	100 μmaperture	200 μm aperture
0.1	1	-	-
0.5	5 ± 1	3.3 ± 1.1	1.6 ± 0.2
1	9 ± 1	7.9 ± 1.5	3.1 ± 0.2
10	94 ± 13	77.9 ± 6.5	31.1 ± 1.4
50	484 ± 13.2	319.8 ± 42.7	158 ± 11.5
100	969 ± 27.5	717.3 ± 31	309 ± 7.3
500	4475.1 ± 121.7	2885.1 ± 64.3	1424.5 ± 20.7
1000	9106.8 ± 283.9	4814.7 ± 125.4	1819.7 ± 7.8
2000	16303 ± 408.8	6986.3 ± 23	2275.8 ± 3.8
5000	29561.4 ± 456.6	8848.32 ± 53.3	2425.6 ± 1.1

Table S3. Recoveries of ractopamine detection from spiked swine urine at different concentration levels (n=3).

Matrix	Spiked ractopamine (ng/mL)	Detected concentration (ng/mL)	Recovery rate (%)	Coefficient of variation (%)
Swine urine	10	9.61 ± 0.79	96.1	8.2
	100	83.7 ± 8.54	83.7	10.2
	1000	1057.4 ± 75.08	105.7	7.1

Table S4. Recoveries of chloramphenicol, sulfonamides and neomycin from spiked milk samples at different concentration levels (n=3).

Target	Spiked concentration (ng/mL)	Detected concentration (ng/mL)	Recovery rate (%)	Coefficient of variation (%)
Chloramphenicol	0.01	0.0084 ± 0.001	84	11.9
	1	1.03 ± 0.066	103	6.4
	50	47.6 ± 4.43	95.2	9.31
Sulfonamides	0.5	0.54 ± 0.048	108	8.9
	10	9.48 ± 0.56	94.8	5.9
	500	489.6 ± 37.2	97.9	7.6
Neomycin	50	49.3 ± 2.32	98.6	4.7
	1000	993.7 ± 57.63	99.4	5.8
	50000	50154 ± 1805.5	100.3	3.6

Table S5. Recoveries of *Salmonella* detection from spiked milk at different concentration levels using ERPC biosensor (n=3).

Matrix	Spiked concentration (CFU/mL)	Detected concentration (CFU/mL)	Recovery rate (%)	Coefficient of variation (%)
Milk	10^3	$(0.87\pm0.11) \times 10^3$	87.2	12.6
	10^5	$(0.83\pm0.062) \times 10^5$	83.4	7.5
	10^7	$(0.95\pm0.093) \times 10^7$	95.3	9.8

Table S6. Recoveries of *Salmonella* detection from spiked milk at different concentration levels using DNA hybridization-based ERPC biosensor (n=3).

Matrix	Spiked concentration (CFU/mL)	Detected concentration (CFU/mL)	Recovery rate (%)	Coefficient of variation (%)
Milk	10^3	$(0.87\pm0.081) \times 10^3$	87.2	9.3
	10^5	$(0.86\pm0.057) \times 10^5$	86.7	6.6
	10^7	$(1.13\pm0.12) \times 10^7$	113.6	10.6

References

(1) Bai, L. L.; Du, Y. M.; Peng, J. X.; Liu, Y.; Wang, Y. M.; Yang, Y. L.; Wang, C. Peptide-based isolation of circulating tumor cells by magnetic nanoparticles. *J. Mater. Chem. B* **2014**, *2*, 4080-4088.