Electronic Supplementary Material (ESI) for Nanoscale.

Supporting information

Centrifugal microfluidic chip for point-of-Care testing of *staphylococcal* enterotoxin B in complex matrixes

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Table of Content

Materials and reagents

The *Staphylococcal* Enterotoxin B (SEB) and *Staphylococcal* Enterotoxin A (SEA) were obtained from Pumai Biotechnology (Shanghai) Co., Ltd. (Shanghai, China). The primary antibody of SEB (Ab_1, clone S222) and the capture antibody of SEB (CAb, clone S643) were purchased from Abnova Corporation (Taiwan, China). The Goat anti Mouse IgG antibody (SAb) was purchased from Jackson ImmunoResearch Inc. (USA), Fluoro-max dyed carboxylate-modified microparticles (CM-EUs, 200 nm) and sulfo-NHS were received from Thermo Fisher Scientific (China) Co., Ltd. (Shanghai, China). 1-(3-dimethyl aminopropyl)-3-ethyl carbodiimide hydrochloride (EDC) and bovine serum albumin (BSA) were purchased from Sigma Aldrich Trading Co., Ltd. (Shanghai, China). 2-(N-morpholino) ethanesulfonic acid sodium salt (MES sodium salt, 50g), and phosphate buffer saline (PBS, pH 7.4) were obtained from Sangon Biotech (Shanghai) Co., Ltd. (Shanghai, China). Glycine, Trehalose Dihydrate, Ochratoxin A (OTA), Aflatoxin B1 (AFB1), Carcinoembryonic antigen (CEA) and Hemoglobin (HGB) were bought from Subosen Biotechnology Co., Ltd. (Chengdu, China). Reaction buffer (0.05 M MES, pH 6.1) and sample dilution buffer (0.1 M PBS, pH 7.4, 0.1% TX-100) were prepared in the laboratory. The urine specimens were collected from healthy people, and the orange juice (Nongfu spring, NFC) and milk (YiLi, pure milk) were purchased from the nearby supermarket. Millipore Milli-Q water (18.2 MΩ) was used in all experiments. All the other chemicals were of analytical grade and used without further purification.

Apparatus

Microchips and Dry fluorescence detection analyzer (Shanghai Suchuang Diagnostic Products Co., Ltd.) was utilized to detect the fluorescence signal of immunoassay microchip. Ad3220 Aspirate/Dispense Platform (BIODOT, USA) was used to dot the antibody on the microchip. The ultrasonic cell disruptor (Ningbo Scientz Biotechnology Co., Ltd) was used to disperse CM-EUs and CM-EUs-Ab_1. Mapping images of CM-EUs and CM-EUs-Ab1 were obtained by high resolution transmission electron microscopy (HRTEM, G2 F20 S-TWIN, Tokyo, Japan). Zeta-sizer Nano-ZS instrument (Malvern Inc., UK) was used to determine the hydrodynamic diameter and zeta potential of CM-EUs and CM-EUs-Ab_1.

Configuration of the platform

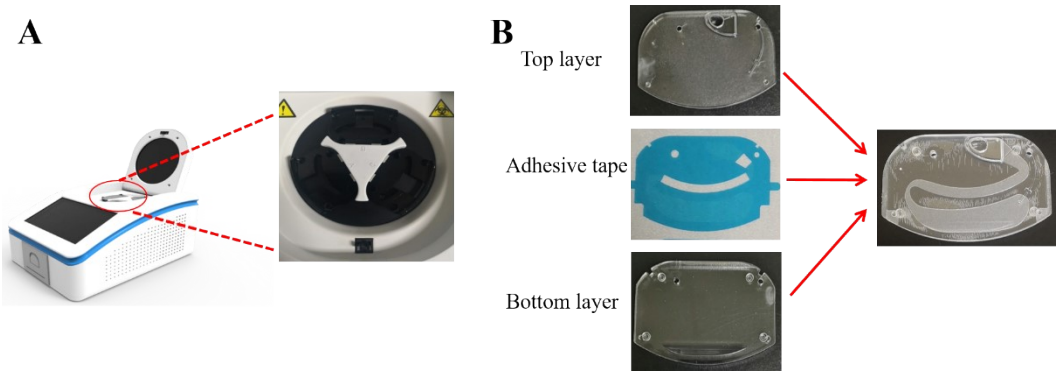

Fig. S1 The configuration of the platform. (A) The constitution of the florescence detection analyzer. (B) The structure of the microfluidic chip for immune analysis.

The platform consists of two parts, the first part is the dry fluorescence detection analyzer, and the second part is the microfluidic chip for immune analysis. Dry fluorescence detection analyzer is a portable fluorescence detection instrument integrating centrifugation, detection and data reading. The instrument can detect three chips simultaneously. The microchip mainly includes three parts, the upper cover, the middle channel layer, and the bottom cover. The upper cover includes a sample loading cell and a sample inlet, which is mainly used for adding samples; the middle channel layer is a fluid channel layer designed with double-sided tape, the double-sided tape can not only form a reaction channel, but also connect the upper cover and the lower cover; and the lower cover, which contains a waste area. The entire reaction channel connects the sample adding region, capture area, T-area, C-area and waste liquid region. The added sample could flow along the fluid channel through capillary-driven and finally into the waste reservoir.

Microchip assembly

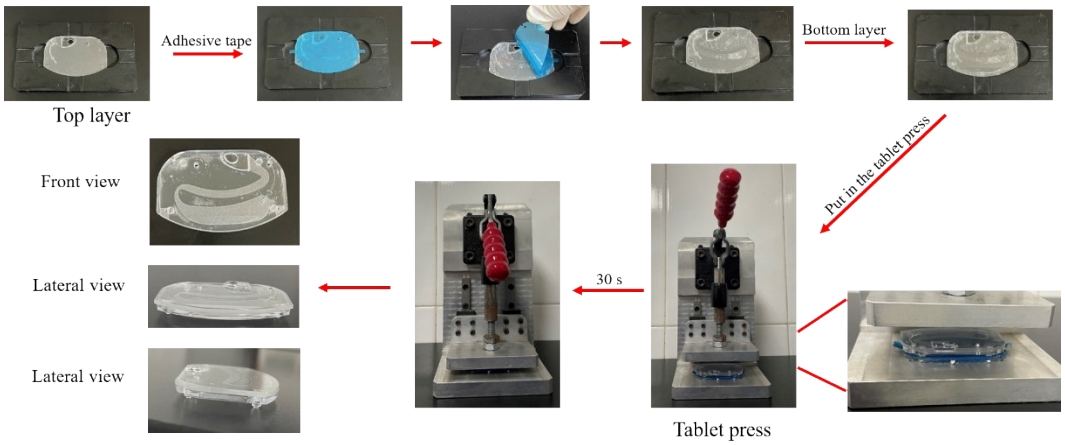

Fig. S2 The detailed procedure of tablet pressing.

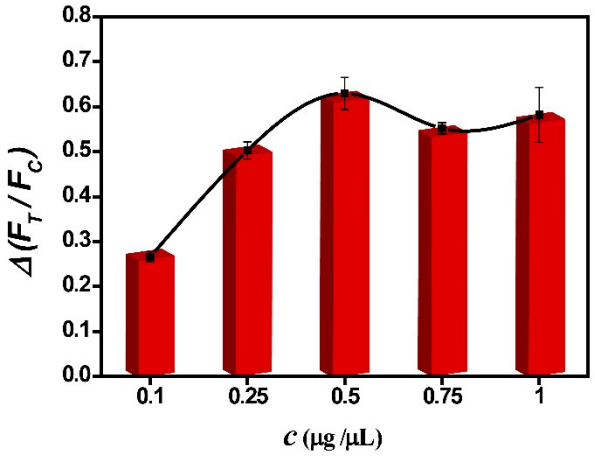

Fig. S3 The effect of the concentration of the CAb and SAb.

Table S1 Recovery tests of SEB detection in milk.

Sample	Added (nM)	Found (ng/mL) Mean[a] ± SD[b]	Recovery (%, *n*=3)	RSD (%, *n*=3)
1	150	149.2±2.22	98.3-101.1	1.45
2	50	51.8±1.50	100.4-106.4	2.91
3	10	9.7±0.81	91.0-106.0	8.42

[a] The mean of three determinations. [b] SD = standard deviation.

Table S2 Recovery tests of SEB detection in juice.

Sample	Added (nM)	Found (ng/mL) Mean[a] ± SD[b]	Recovery (%, *n*=3)	RSD (%, *n*=3)
1	150	152.1±3.56	98.9-103.6	2.33
2	50	51.0±0.76	100.4-103.4	1.50
3	10	10.2±0.60	96.0-108.0	5.93

[a] The mean of three determinations. [b] SD = standard deviation.

Table S3 The comparison of LOD with another method.

Methods	Time	Pretreatment (YES or NO)	LOD	Linear range	Ref.
Fluorescence anisotropy	-	YES	92 pg/mL	0.1~100ng/mL	1
Magnetic Nanoparticles based LFA	30min or 240min	YES	54 pg/mL or 8 pg/mL	0.01~100ng/mL or 0.003~30ng/mL	2
SERS-Based Immunoassay	-	YES	1.3pg/mL	2~100 pg/mL	3
Colorimetric detection	-	YES	120 pg/mL	0.4~20 pg/mL	4
Electrical based LOC chip	-	YES	5000 pg/mL	5~100 ng/mL	5
Centrifugal microfluidic	12 min	NO	68 pg/mL	0.1~250 ng/mL	This work

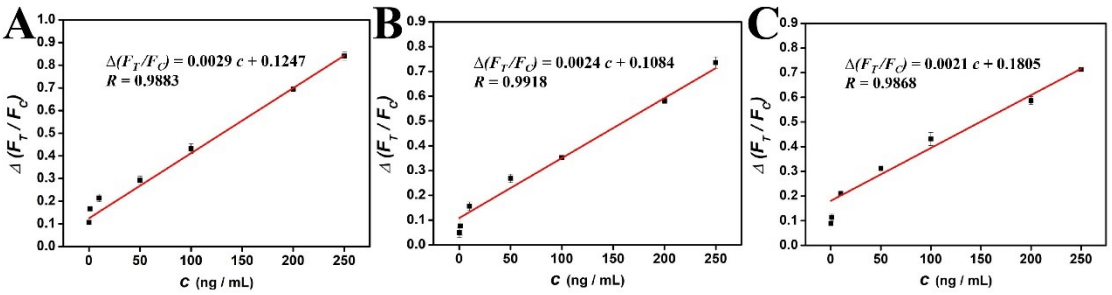

Fig. S4 The linear relationship between $\Delta(F_T/F_C)$ and SEB concentration in (A) urine, (B) milk, (C) orange juice. Experimental conditions: the concentrations of SEB was from 0.1 ng/mL to 250 ng/mL; CM-EUs-Ab_1: 20 μg/mL; reaction time: 10 min; the CAb and SAb: 0.5 mg/mL. The error bars represented the standard deviation of three replicate determinations.

Table S4 The comparisons of LOD and LOL to detection SEB in real situations

Sample	Linear regression equation	*R*	Linear range	LOD (3σ/k)
Urine	$\Delta(F_T/F_C) = 0.0029\ c + 0.1247$	0.9883	0.1-250ng/mL	35 pg/mL
Milk	$\Delta(F_T/F_C) = 0.0024\ c + 0.1084$	0.9918	0.1-250ng/mL	63 pg/mL
Orange juice	$\Delta(F_T/F_C) = 0.0021\ c + 0.1805$	0.9868	0.1-250ng/mL	22 pg/mL

As shown in Fig.S4 and Table S4, our platform has good sensitivity for detection of SEB in the complex matrixes of urine, milk and orange juice.

Reference:

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