Electronic Supporting Information

Highly efficient enrichment and identification of pathogens by herringbone microfluidic chip and MALDI-TOF mass spectrometry

Yueqing Shen^{a,b,#}, Jia Yi^{b,#}, Minghui Song^{a,*}, Dandan Li^b, Yi Wu^b, Yanjun Liu^b,

Meicheng Yang^{a,*} and Liang Qiao^{b,*}

^a NMPA Key Laboratory for Testing Technology of Pharmaceutical Microbiology,

Shanghai Institute for Food and Drug Control, Shanghai 201203, China

^b Department of Chemistry, and Institutes of Biomedical Sciences, Fudan University, Shanghai 200433, China

These authors contribute equally.

*: Correspondence should be addressed to Dr. Meicheng Yang

(<u>yangmeicheng@vip.sina.com</u>), Dr. Liang Qiao (<u>liang_qiao@fudan.edu.cn</u>) or Dr. Minghui Song (<u>sminghui88@163.com</u>)



Fig. S1 Mask patterns of two layers of the herringbone microchip. A multilayer mold was prepared by stacking two patterned layers on a silicon wafer.



Fig. S2 Detailed patterns of one periodic unit of the herringbone microchip.



Fig. S3 Comparison of bacterial capture efficiency by the VMBs after different days of storage under 4 °C. 200 μ L of 10⁶ CFU/mL *S. aureus* were used for investigation of capture efficiency. Error bars indicate standard deviation from three replicates.



Fig. S4 Bacterial concentration-absorbance curve of *S. aureus*, *S. hominis*, *E. gallinarum* and *S. epidermidis*.



Fig. S5 Optical microscope picture of the dispersed VMBs in the herringbone microchip.



Fig. S6 SEM images of (A) GL-NH₂ magnetic beads, (B) VMBs, (C) VMB@S. aureus, (D) VMB@S. hominis, (E) VMB@E. gallinarum, and (F) VMB@S. epidermidis. The scale bar is 100 nm in (A) and (B) and 200 nm in (C)-(F).



Fig. S7 MALDI-TOF mass spectrum of pure VMBs.



Fig. S8 MALDI-TOF mass spectra of the VMB@bacteria after the herringbone-VMB microchip extraction of bacteria from urine samples (10^6 , 10^5 , 10^4 , 10^3 CFU/mL, 2.5 mL) compared to the reference spectra of the pure isolates of the corresponding strain. (A) *S. aureus*, (B) *S. hominis*, (C) *E. gallinarum*, and (D) *S. epidermidis*.



Fig. S9 MALDI-TOF mass spectra of the VMB@bacteria after in-tube bacteria extraction from urine samples (10⁶ CFU/mL, 2.5 mL) compared to the reference spectra of the pure isolates of the corresponding strain. (A) *E. gallinarum* and (B) *S. epidermidis*.

Table S1	Capture	efficiency	and	identificati	on results	of	bacteria	in	urine	sampl	les 1	by :	in-
tube enrie	chment c	oupled MA	LDI	-TOF MS.									

Samula	Capture	Identified	Identification	Confidence		
Sample	efficiency (%)	strain	score ^b	level		
	27.5% Nocardia		22.2	Madium		
<i>E.gaunarum</i> in urine		yamanashiensis	23.3	wiedium		
S anidoumidia in unino a	45.6%	Mycobacterium	20.0	Madium		
S.epidermials in urine		tuberculosis	20.9	wiedlum		

a: 10^6 CFU/mL bacteria in urine, 2.5 mL.

b: The identification score is from the Clin-TOF II MALDI-TOF MS system. A score larger than 25 indicates confident identification.