An Integrated Microfluidic Device Utilizing Dielectrophoresis and Multiplex Array PCR for Pointof-Care Detection of Pathogens

Dongyang Cai^{a,c}, Meng Xiao^b, Peng Xu^a, Yingchun Xu^{b,*}, Wenbin Du^{a*}

AUTHOR INFORMATION

^a State Key Laboratory of Microbial Resources, Institute of Microbiology, Chinese Academy of Sciences, 100101 Beijing, China.

^b Department of Clinical Laboratory, Peking Union Medical College Hospital, 100730 Beijing, China.

^c Department of Chemistry, Renmin University of China, 100872 Beijing, China.

Corresponding Authors:

*E-mail: W. D.: wenbin@im.ac.cn; Y. X.: xycpumch@139.com.

Supporting Information

Chemicals and Materials. Bovine serum albumin (BSA) was purchased from Sigma-Aldrich. SsoFast EvaGreen Supermix (2X) was purchased from Bio-Rad Laboratories (Beijing, China). Mineral oil (DNase, RNase and Protease free), Dichlorodimethylsilane and Dimethoxydimethylsilane were purchased from TCI (Shanghai, China). Soda-lime glass plates coated with photoresist and chromium were ordered from Telic Company (Valencia, CA). ITO glass was purchased from CSG Company (Shenzhen, China). SU8 2005 was purchased from MicroChem Corp. (Newton, MA). The PCR mixture for preloading the microwells was prepared by mixing 25 μ L of 2× PCR master mixture (SsoFast EvaGreen SuperMix, Bio-rad), 5 μ L of 10 mg/mL BSA (Sigma) solution, 15 μ L of RNase free water and 2.5 μ L of each primer (0.5 μ M) for different species of pathogens. All primers for PCR amplification were ordered from Sangon biotech (Shanghai, China). Primer sequences were listed in Table S1.

A set of clinical strains isolated from patients' positive blood cultures from Department of Clinical Laboratory, Peking Union Medical College Hospital (PUMCH, Beijing, China) during January 1, 2013 to June 1, 2013 were used for evaluation of broad-range utility of the methods. The bacterial and fungal names and their serial numbers are:

- 1. Escherichia coli (E. coli): 13B00401, 13XB00121, 13B00357, 13B00431
- 2. Staphylococcus aureus (S. aureus): 13B01049, 13B02176, 13B02240, 13XB00584
- 3. Klebsiella pneumoniae (K. pneumoniae): 13B00139, 13B00154, 13B00601, 13XB00476
- 4. Pseudomonas aeruginosa (P. aeruginosa): 13B00286, 13B00597, 13XB00005, 13XB00144
- 5. Enterobacter aerogenes (E. aerogenes): 13B00472, 13B00809, 13B02461, 13B15683
- 6. Acinetobacter baumannii (A. baumannii): 13B00352, 13B01168, 13B02045, 13XB00144
- 7. Enterococcus faecium (E. faecium): 13B00474, 13B01142, 13B01509, 13B01945
- 8. Enterococcus faecalis (E. faecalis): 13B00631, 13B00767, 13XB00729, 13XB00730
- 9. Candida glabrata (C. glabrata): 13h04036
- 10. Candida albicans (C.albicans): 13z017401
- 11. Candida krusei (C. krusei): ATCC 6258
- 12. Candida parapsilosis (C. parapsilosis): ATCC22019

Name of Pathogen	Primer sequence	Target gene	Amplicon size (bp)
<i>E. coli</i> (ATCC 8739)	GAA CAT CAA ACA TTA AA	LacZ ¹	151
	AAT CAG TCG AAG ATA		
	CGG ACA TCC ATG TGA TAT GG		
<i>E. coli O157:H7</i> (ATCC		rfb O157 ²	259
35150)	TTG CCT ATG TAC AGC TAA		
	TCC		
P. aeruginosa (ATCC	GTC AGT GTT ACC TAA	23S rRNA ¹	377
9027)	GAA AGG ATC TTT GAA		
S. aureus (ATCC 6538p)	GGA GGA AGG TGG GGA TGA	16s rRNA ³	241
	CG		
	ATG GTG TGA CGG GCG GTG TG		
S. mutans	AGA TAT GAT TGC AAC AAT	dltA ⁴	412
	TGA A		
	CGC ATG ATT GAT TTG ATA AG		
C. tropicalis	CAA TCC TAC CGC CAG AGG	rRNA ITS ⁵	357
	TTA T		
	TGG CCA CTA GCA AAA TAA		
	GCG T		

Table S1. Names of pathogens and sequences of primer pairs tested by the SlipChip.

REFERENCE:

- L. E. Ferreira, K. Dalposso, B. B. Hackbarth, A. R. Gonçalves, G. A. Westphal, P. H. C. D. França and M. D. S. L. Pinho, *Rev. Bras. Ter. Intensiva*, 2011, 23, 36-40.
- I. M. Moussa, M. H. Ashgan, H. A. Alwathnani, K. F. Mohamed and A. A. Al-Doss, *Afr. J. Biotechnol.*, 2010, 9, 4356-4363.
- 3 N. Samadi, M. Alvandi, M. R. Fazeli, E. Azizi, H. Mehrgan and M. Naseri, J. Biol. Sci., 2007, 7, 359-363.
- 4 M. Palka-Santini, B. E. Cleven, L. Eichinger, M. Kronke and O. Krut, *BMC Microbiol.*, 2009, 9, 1.
- 5 G. Luo and T. G. Mitchell, J. Clin. Microbiol., 2002, 40, 2860-2865.



Figure S1. a) A photograph of the device with zoom-in view; the microchannels and microwells were loaded with dye solutions of different colors; b) Schematic illustration showed that the system was consisted of the top glass plate, bottom plate with 5 µm SU-8 coating and ITO microelectrodes; c) Interdigitated ITO microelectrodes before and after fabricating three capture grooves. The detailed geometry was shown in the zoom-in schematic drawing.



Figure S2. a) Schematic illustration of bacterial DEP capture by grooves with a ladder-like layout in five units; b) A fluorescence microphotograph of three grooves in unit 2 with captured RFP-tagged *E. coli*. Scale bar=100 μ m; The average fluorescence intensity profile perpendicular to the flow direction was shown on the right; c) The capture ratio of five units with equal groove width of 53.3 μ m; d) Capture ratio of the first unit with groove widths in the range of 20-40 μ m.