Electronic Supplementary Material (ESI) for Lab on a Chip.

A "Sample-in- multiplex-digital-answer-out" chip for fast detection of pathogens

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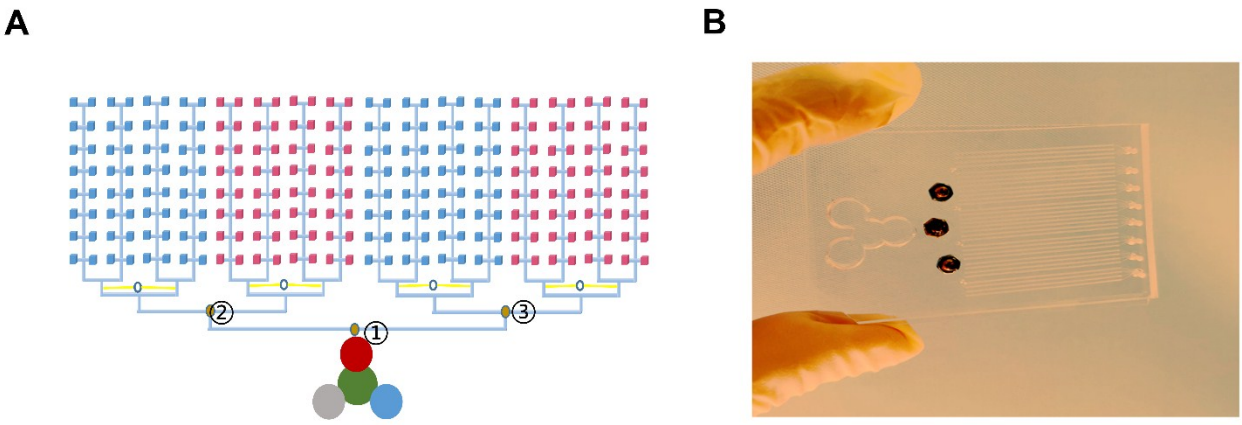

Figure S1 Design of the integrated microfluidic chip.

A Schematic diagram of the chip's planar structure. 1, 2, 3 represent the position of the 3 screws. One of the detection areas (blue) was used as a negative control and the other three areas (red) were the detection areas of the three food-borne pathogens.

B: Photograph of integrated multi-detection chip

Table S1 The Primer and probe sequence using in this manuscript

Bacterial Strain	Forward Primer	Reverse Primer	Probe
Escherichia coli O157:H7[1]	GTTAACTTTACCATTTGCAAAGGTATATGTAC	GAAATATACTTATAACGCATCGACCAATGATT	CCTTCAGAGTAGCGCCAAGATCTGTCG-T(FAM)-TG-dSpacer-AGT(BHQ-1)-GCCTGTCGCTAC
Listeria monocytogenes[2]	CGCCTGCAAGTCCTAAGACGCCAATCGAAAAGAAAC	CTGCATCTCCGTGGTATACTAATACATTGTTTTTA	CGAAAAGAAACACGCGGATGAAATCGATAAG[FAM][THF][BHQ-1]ATACAA GGATTGGA
Salmonella enterica[1]	CGTCTACGTAGTCAGTTCTTTATTGATTAT	CATCAAATCAAAATAGACCGTAAATTGTTC	GCGATGGCGAGGGCCTGGACGATAACAGCA-T(FAM)-CGAT-T(BHQ-1)-TTGATTAATGAGAT

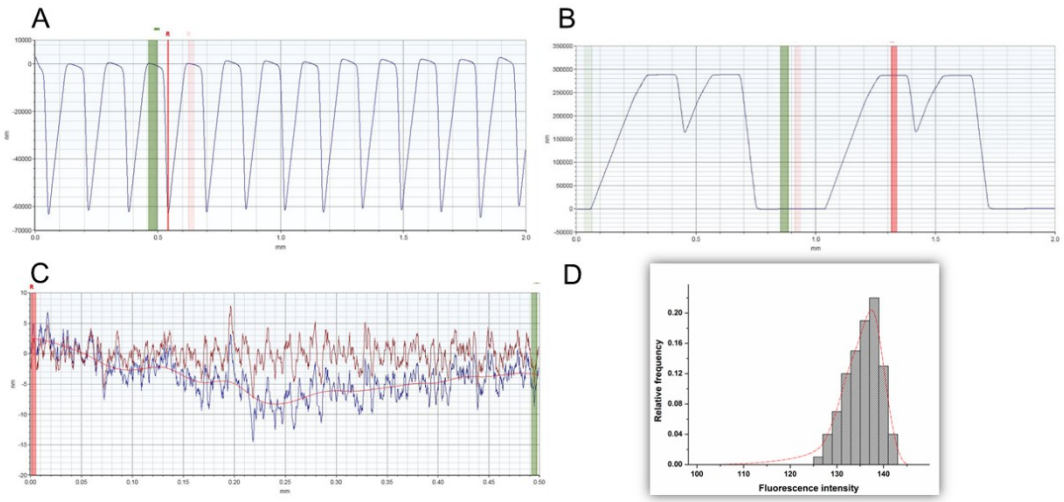

Figure S2 Height uniformity and fluorescence intensity uniformity analysis of the integrated multiplex digital RPA chip. A: The uniformity of the height of the same column. B: Uniformity of heights of different columns. C: Surface roughness of the chip. D: uniformity of brightness

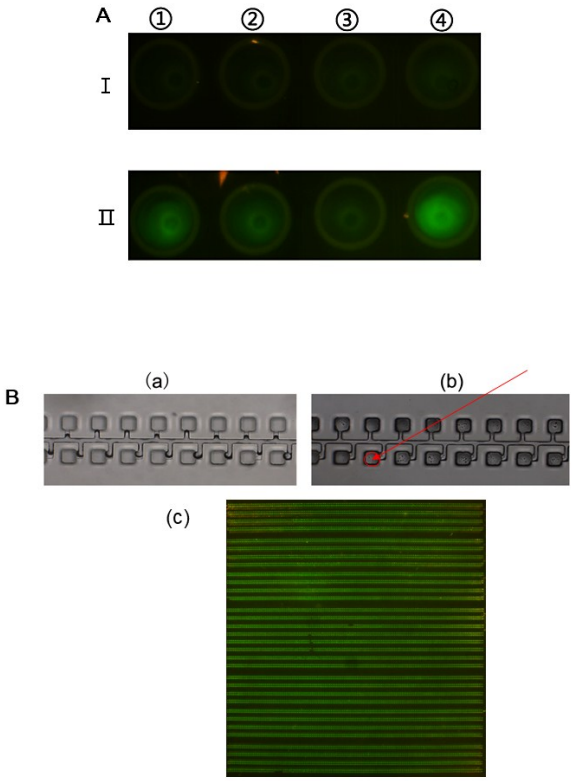

Figure S3 The feasibility of off-chip RPA reaction and the reliability of freeze-dried components

A：Off-chip reaction, showing that the primers and probes used can be used for RPA reactions. Line Ⅰ:Fluorescent picture inside the tube before reaction. Line Ⅱ:Fluorescent picture inside the tube after reaction ①:E. coli O157:H7;②:*S. enterica;*③:Control group; ④:*L. monocytogenes.* B： The state of the chip before and after lyophilization. Microscope picture of the microwells before lyophilization. Microscope picture of the chamber after lyophilization. Powdered ingredients can be observed. (c) A picture of the RPA reaction using a chip embedding the reaction component, indicating that all the chambers can perform the RPA reaction normally

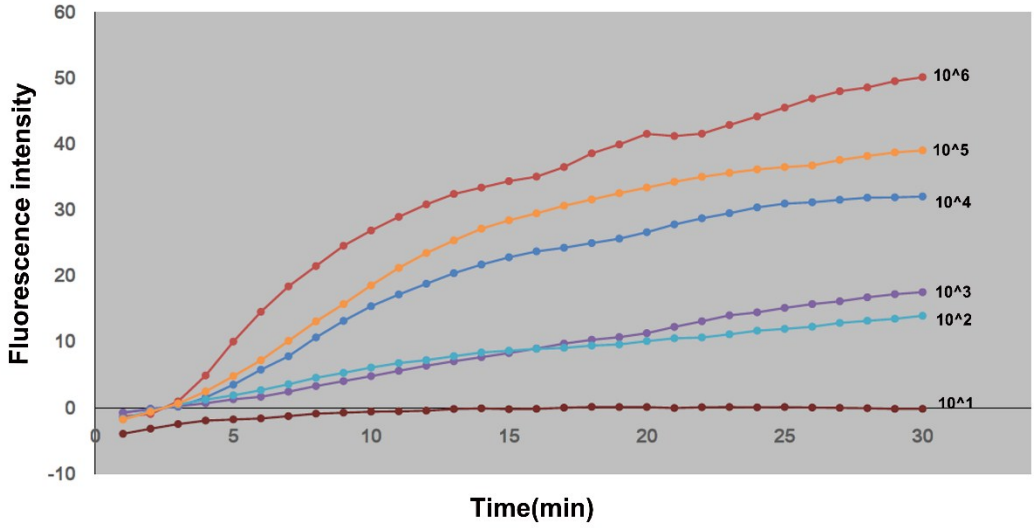

Figure S4 The real-time fluorescence curve of RPA reaction. The fluorescence signal was collected by ABI7900, and the results showed that the detection of gene copy number below 10 copies could not be accurately detected by real-time fluorescence quantitative method.

References

[1] Choi G, Jung J H, Park B H, et al. A centrifugal direct recombinase polymerase amplification (direct-RPA) microdevice for multiplex and real-time identification of food poisoning bacteria[J]. Lab on a Chip, 2016, 16(12): 2309-2316.

[2]Li Z, Liu Y, Wei Q, et al. Picoliter well array chip-based digital recombinase polymerase amplification for absolute quantification of nucleic acids[J]. PLoS One, 2016, 11(4): e0153359.