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>
<thead>
<tr>
<th>Bacterial Strain</th>
<th>Forward Primer</th>
<th>Reverse Primer</th>
<th>Probe</th>
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<tbody>
<tr>
<td>Escherichia coli O157:H7</td>
<td>GTTAACTTTACCATTTGCAAAGTGATATGTA</td>
<td>GAAATATACTTTATAACGCATCGACCATTGATT</td>
<td>CCTTCAGAGTAGCGCCAAGATCTGTG-T(FAM)-T-dSpacer-AGT(BHQ-1)-GCCTGTCGCTAC</td>
</tr>
<tr>
<td>Listeria monocytogenes</td>
<td>CGCCTGCAAGTCCTAAGACGCCTCAATCGAAA</td>
<td>CTGCATCTCCGTGGTATAGTAACTAATACATTGTTTA</td>
<td>CGAAAAAGAAAACACGGGATGAAAATCGATAAG(FAM)[THF][BHQ-1]ATACAA GGATTGGA</td>
</tr>
<tr>
<td>Salmonella enterica</td>
<td>CGTCTACGTAATGATTCTCTATTGATTAT</td>
<td>CATCAATCAAATAAGACCGTAAATTGTCGATGGCGAGGGCCTGGACGATACAGCA-T(FAM)-CGAT-T(BHQ-1)-TTGATTAATGAGAAT</td>
<td>GCGATGGCGAGGGGCTGGACGATTACAGCA-T(FAM)-CGAT-T(BHQ-1)-TTGATTAATGAGAT</td>
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**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](image)

**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
