SUPPLEMENTAL INFORMATION

A versatile PDMS/paper hybrid microfluidic platform for rapid and sensitive infectious diseases diagnosis

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EXPERIMENTAL SECTION

LAMP mixture

The Loopamp reaction mixture contained 20 mM Tris-HCl (pH 8.8), 10 mM KCl, 8 mM $MgSO_4$, 10 mM $(NH_4)_2SO_4$, 0.1% Tween 20, 0.8 M Betaine, 0.5 mM $MnCl_2$, 1.4 mM dNTPs, 8U Bst Polymerase, 1.6 μM each of the inner primer (FIP/BIP), 0.2 μM each of the outer primer (F3/B3), 0.4 μM each of the loop primer (LF/LB).

Artificial cerebrospinal fluid (ACSF) samples

Artificial cerebrospinal fluid (ACSF) was prepared according to previously published protocol.[1] The ACSF solution contained 119 mM NaCl, 26.2 mM $NaHCO_3$, 2.5 mM KCl, 1 mM NaH_2PO_4, 1.3 mM $MgCl_2$, 10 mM glucose. It was bubbled with 5% CO_2/95% O_2 for 10–15 min, before $CaCl_2$ was added to the solution to reach a final concentration of 2.5 mM $CaCl_2$. The prepared ACSF solution was filtered with a 0.20 μm filter apparatus, and stored at 4 °C before use.

Table S-1 *ctrA* LAMP primer sequences

Primer	Sequences (5'-3')	No. of bases
ctrA_FIP	CAAACACACCACGCGCATCAGATCTGAAGCCATTGGCCGTA	41
ctrA_BIP	TGTTCCGCTATACGCCATTGGTACTGCCATAACCTTGAGCAA	42
ctrA_F3	AGC(C/T)AGAGGCTTATCGCTT	19
ctrA_B3	ATACCGTTGGAATCTCTGCC	20
ctrA_FL	CGATCTTGCAAACCGCCC	18
ctrA_BL	GCAGAACGTCAGGATAAATGGA	22

Microfluidic platform fabrication

PDMS films were prepared following standard soft lithography procedures.[2] Briefly, the liquid PDMS base and the curing agent were mixed at a weight ratio of 10:1. Then the PDMS precursor mixture was poured onto a petri dish, degassed in a vacuum desiccator for ~30 minutes, and incubated at 60 °C overnight. Unlike the commonly used PDMS moulding, micro-channels were directly created on top of the PDMS film via ablation using the laser cutter. Inlet reservoir in the top PDMS layer, outlet reservoirs and LAMP zones in the middle PDMS layer were excised using biopsy punches. After 30 seconds exposure in an oxidizing air Plasma Cleaner (Ithaca, NY), PDMS films and the glass slide were face-to-face sandwiched to bond irreversibly. After biochip assembly, the specific primers for *N. meningitidis* and PC DNA were pre-loaded into the LAMP zones with paper inside and dried in a vacuum desiccator. Thus, the biochip became ready for use.

Microorganism culture

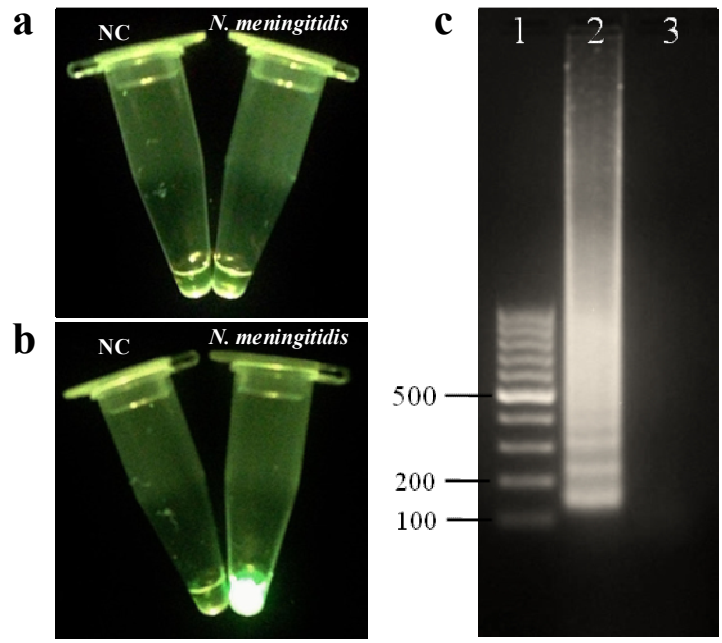
The *Nerisseria meningitidis* (*N. meningitidis*, ATCC 13098), *Streptococcus pneumoniae* (*S. pneumoniae*, ATCC 49619) and *Haemophilus influenzae* (*H. influenzae*, ATCC 33533) were obtained from American Type Culture Collection (ATCC, Rockville, MD). *N. meningitidis* and *H. influenza* were grown on chocolate II agar supplemented with haemoglobin and IsoVitalex plates (BD, Sparks, MD), and incubated at 37 °C for 48 h in an aerobic environment with 5% CO_2. *S. pneumoniae* was grown in TSA II agar plates supplemented with 5% sheep blood (BD, Sparks, MD) and incubated at 37 °C for 48 h in an aerobic environment with 5% CO_2.

DNA preparation

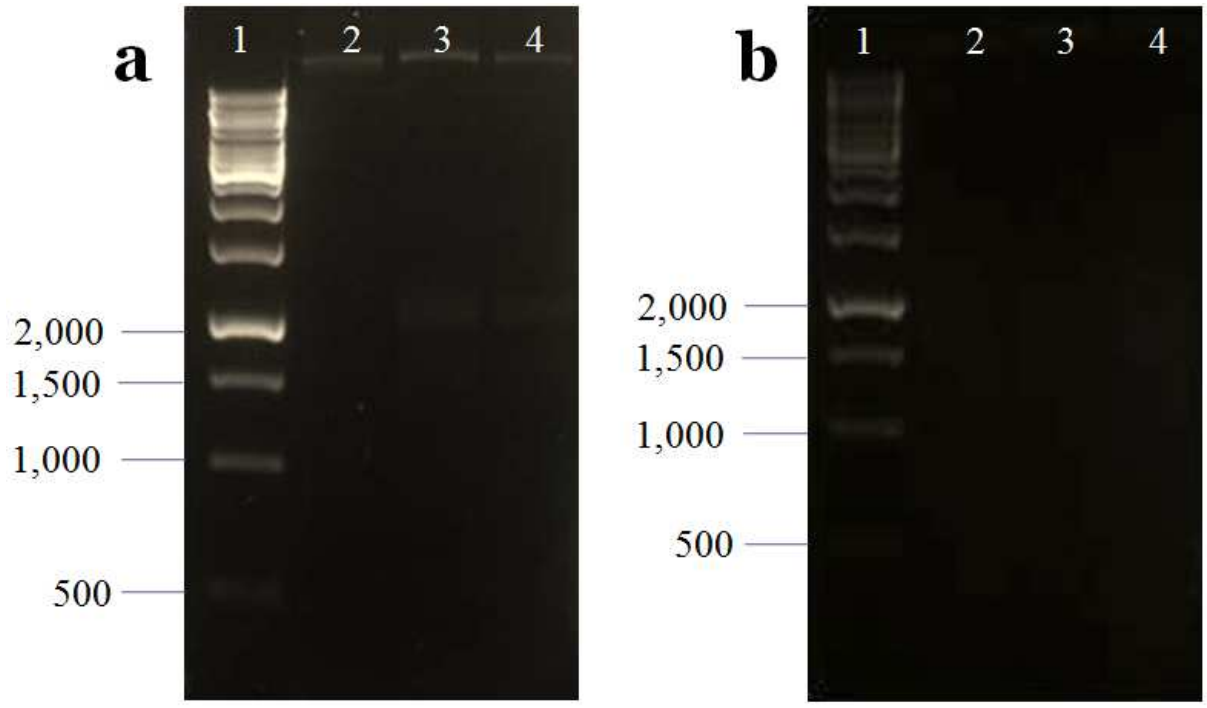
DNA was extracted by using Qiagen DNA Mini kit following a slightly modified protocol from the manufacturer. Briefly, harvested bacterial cells in 5 ml sterile saline (maximum 2×10^9 cells, adjusted to 0.5 turbidity McFarland standard) from a bacterial culture plate were centrifuged at 5000 × *g* (or 7500 rpm) for 10 min. After discarding the supernatant, the bacterial pellet was re-suspended in a mixture solution containing 180 μL lysis buffer ATL and 20 μL proteinase K (600 mAU/mL), and was incubated at 56 °C for 1 h in a water bath. The manufacturer's protocol was followed for the remaining DNA preparation procedures.

Post-chip analysis

LAMP products were collected from each outlet of the microfluidic device for further confirmatory tests using conventional gel electrophoresis (Sub-Cell GT, Bio-Rad, CA) and DNA quantitative analysis using Nanodrop (Nanodrop 1000, Thermo Scientific, MA). During gel electrophoresis, amplified products were resolved by applying 90 V for 1 hour in 1.5% agarose gel.



Supplementary Figure S-1 Confirmatory analysis of on-chip LAMP products. Collected reaction mixtures in PCR tubes were placed under portable UV light before (a) and after (b) LAMP reactions. Neither tube showed notable fluorescence before LAMP reaction. After the LAMP reaction, the collected *N. meningitidis* LAMP products showed bright green fluorescence under portable UV light. (c) Gel electrophoresis of on-chip LAMP products. Lanes 1-3: 100 bp ladder, *N. meningitidis* products, NC. Ladder-pattern DNA bands were observed in *N. meningitidis* products, whereas no DNA bands were observed in NC.



Supplementary Figure S-2 Detection of *N. meningitidis* microorganisms in ACSF by using (a) lysis buffer ATL from Qiagen and (b) MagNA pure bacteria lysis buffer from Roche Applied Science. Lanes 1-4 of the gel electrophoresis images: 1 kb marker, Sample 1, Sample 2, NC. No ladder-pattern multiple bands were observed in lane 2 and lane 3, indicating the two-step direct detection of *N. meningitidis* microorganisms was unsuccessful by using the two lysis buffer.

References

(1) *Cold Spring Harbor Protocols* **2011**, *2011*, pdb.rec065730.
(2) Y. Xia and G.M. Whitesides, A. *Rev. Mater. Sci.* **1998**, *1998*, 31.