

Supporting Information

A LEGO-Inspired Multipiece Chip for Portable Nucleic Acid Detection

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S1. Experimental Section

Ethical statement. This study was conducted in accordance with the ethical principles of the Declaration of Helsinki and in compliance with relevant national laws and regulations governing biomedical research involving human subjects in the People's Republic of China. All procedures were performed in accordance with the institutional guidelines of West China Second Hospital, Sichuan University. The study protocol was reviewed and approved by the Medical Ethics Committee of West China Second Hospital, Sichuan University (Approval No. 2022YFS0079). Written informed consent was obtained from all participants prior to enrollment and sample collection.

Materials. TwistAmp® Basic kit for recombinase polymerase amplification (RPA) was purchased from TwistDx Limited (UK), QIAamp DNeasy® Blood & Tissue Kit was purchased from Qiagen China, RNase-free double-distilled water and 1X Tris-HCl buffer (pH 8.0) were purchased from Sangon Biotech (China), polyethylene glycol (MW 4000), TWEEN20, magnesium acetate, and magnesium chloride were purchased from Titan Tech (China), isopropanol was purchased from Kelong Chemical (China). All DNA oligonucleotides were purchased from Sangon Biotech with HPLC purification.

LIM-Chip design and fabrication. The LIM-Chip was designed using Fusion360 software and fabricated via 3D printing technology. Detailed design drawings of the chips are shown in Figure S1. The male and female connectors (Figure 1a) and the liquid collector outer shell (Figure 1e) were 3D-printed using PLA plastic filament with a Pro3 Plus FDM 3D printer (Raise3D, China). The inner cartridge for reagent storage (Figure 1b) and the collector core (Figure 1e) were fabricated using transparent resin with a Form 3 SLA 3D printer (FormLabs, USA).

Protocol for PEG coating. The internal channels and cavities of the chip were filled with a 3% (w/v) aqueous solution of PEG (average molecular weight: 4000), incubated at room temperature for 30 minutes. The solution was then drained using a syringe, and the chip was baked in a DZF-6050 oven (Hengke, China) at 70 °C for 30 minutes, followed by cooling to room temperature.

Design and fabrication of the portable device. The LIM-Chip-Compatible Portable Integrated Instrument consists of a heating module, a liquid handling module, and a fluorescence imaging module. The thermal regulation module consists of two custom-machined 6-well aluminum heating blocks optimized for standard PCR tubes. Heating is provided by silicone rubber heating elements, regulated by a closed-loop feedback system utilizing an Arduino Nano microcontroller. The liquid handling module comprises three lead screw syringe pumps controlled by an Arduino Nano; each lead

screw syringe pump consists of a 1 mL plastic syringe, an SG90 servo motor as the power source, a 60 mm long M5 screw as the lead screw, and a 3D-printed housing for assembling the components. The fluorescence imaging module is an optical path system consisting of two blue LED lamps (equipped with 470 nm band-pass filters at the front) as excitation light sources and a 520 nm filter as the emission filter. The above three systems are powered by a 12000 mAh 24 V lithium battery. All components and the circuit system are encapsulated and integrated in an instrument housing designed with Fusion360 software and manufactured using a Pro3 Plus FDM 3D printer (Raise3D, China). The circuit design of the instrument is shown in Figure S8, and the overall design is illustrated in Figure 3.

LIM-Chip-based CLARISSA Assay Reagent preparation and chip assembly were performed on a GT20401 multi-temperature zone workbench (Mona, China). A standard LIM-Chip utilizes 6 base units, 1 liquid collector, and 1 PCR tube: Load one RPA lyophilized pellet (from the TwistAmp Basic kit) into the dry powder chip, then add 1 μL of 280 mM MgOAc to the PCR tube. Mix 15 μL of rehydration buffer (supplied with the kit), 2.5 μL of 10 μM forward/reverse primers, and 4 μL of deionized water, then load the mixture into a standard chip; load 20 μL of 40 nM helper strands into a standard chip, 42 μL of 20 nM gDz into a standard chip, and 8 μL of 400 nM fluorescent probe strands into a microchip. All chips were fitted with outer shells and O-ring seals, then sealed with parafilm at the channel openings and stored at 4°C until use.

The chip assembly order is shown in Figure 2b, and all DNA solution buffers (except for RPA) consist of 10 mM Tris-HCl (containing 1 mM MgCl₂): Add 5 μL of target DNA to the PCR tube, then assemble the liquid collector; transfer the RPA rehydration solution into the dry powder storage chip, and after 10 seconds of incubation for pellet rehydration, transfer the solution to the PCR tube via the liquid collector before placing the chip-connected PCR tube on a 37°C heating block for 20 minutes. Next, transfer the assembly DNA solution (from the chip) to the PCR tube via the liquid collector, heat at 95°C for 2 minutes, then immediately transfer to a 37°C heating block for another 2 minutes, followed by transferring the gDz and probe strands to the PCR tube via the liquid collector and incubating at 37°C for 40 minutes. After the reaction, insert the chip into the chip tray of the portable instrument, load it into the instrument, and capture images using a HUAWEI Mate 50 Pro smartphone (China) (parameters: ISO = 1000, aperture = 1.4, exposure time = 1.2 s). For data acquisition, convert the image to an 8-bit grayscale file using ImageJ software, outline the area corresponding to the PCR tube, and record the average grayscale value of all pixels in this area as the detection signal intensity.

Standard CLARISSA Assay. The volume and concentration of reagents used in the microplate reader assay are identical to those in the chip-based assay. After the reagents complete the reaction in the test tube (incubated in an X50 thermal cycler, Eppendorf, USA), 90 μ L of the reaction mixture is transferred to a microplate. The microplate is then placed into the Cytation 5 microplate reader (BioTek, USA), with the excitation and emission wavelengths set to 485 nm and 525 nm, respectively, and the endpoint fluorescence intensity is measured.

Clinical validation of LIM-Chip-based CLARISSA assay. For 2 mL of cervical exfoliated cell swab samples: First, the samples are centrifuged at 8000 rpm for 3 minutes to enrich the samples and collect cells. Subsequently, nucleic acid extraction is performed using the QIAamp DNeasy® Blood & Tissue Kit, following the recommended protocol provided by the reagent manufacturer. The concentration of extracted DNA was verified using a Take3 micro-volume detection plate (Agilent, USA). The extracted DNA is stored in a -20 °C refrigerator until use. The DNA detection process for clinical samples is consistent with the process for detecting synthetic targets described earlier.

S2. Supporting Figures

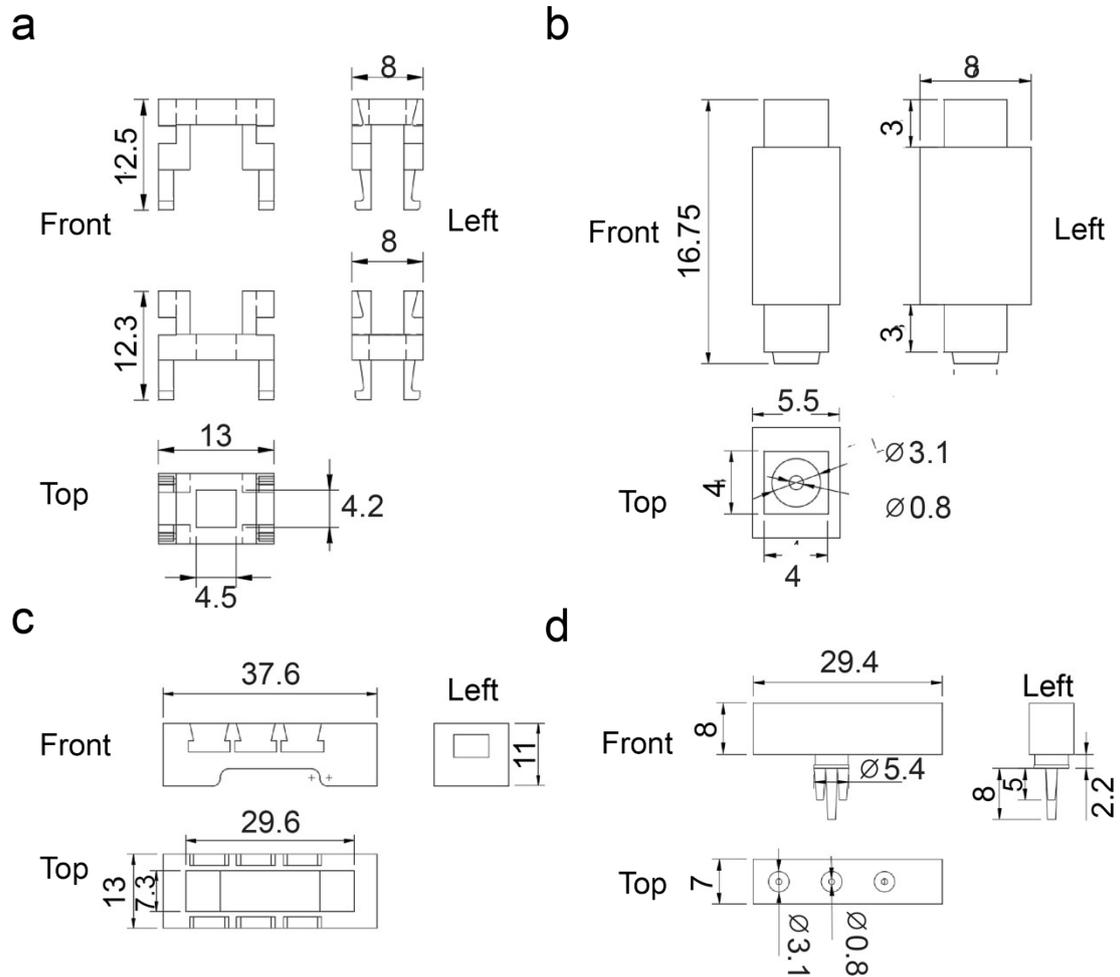


Figure S1. Design details of LIM-Chip. (a) Male and female connectors. **(b)** Standard inner cartridge. **(c)** Outer shell of liquid collector. **(d)** Inner channel design of the liquid collector. Units in drawing are millimeters.

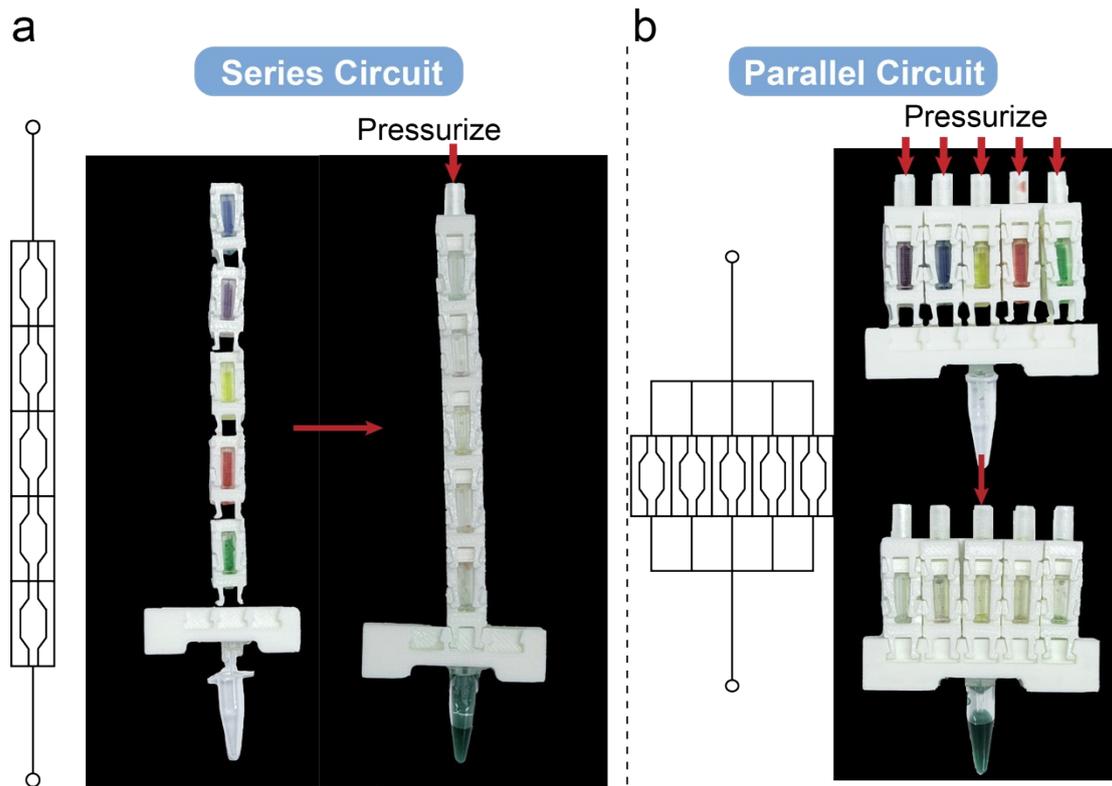


Figure S2. Serial and parallel assembly of the LIM-Chip. (a) Serial assembly of 6 base units and 1 liquid collector for sequential mixing of 6 solutions. (b) Parallel assembly of 6 base units on 1 liquid collector for the simultaneous mixing of 6 solutions.

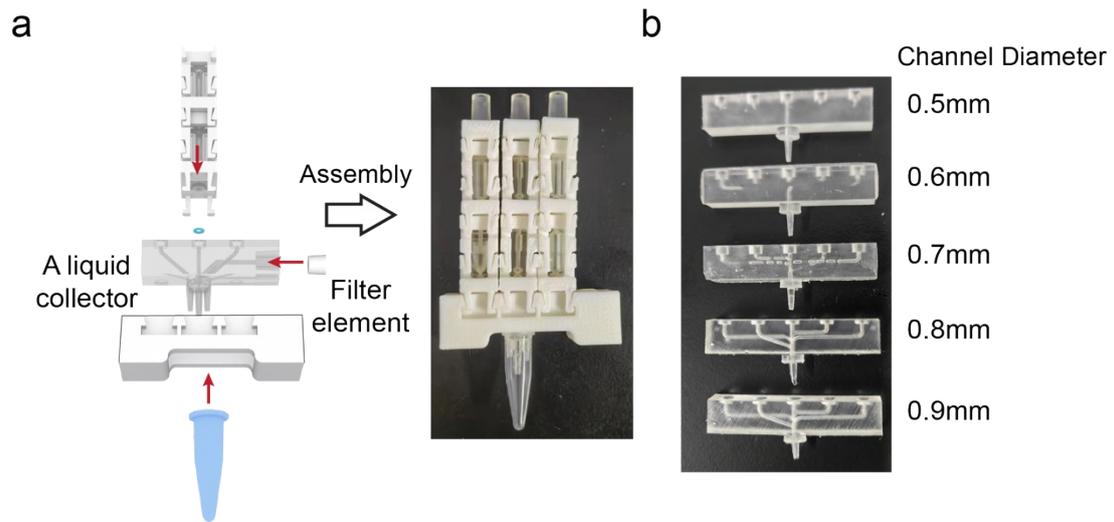


Figure S3. Combined serial and parallel assembly of the LIM-Chip. (a) Schematic illustration of the assembly among base units, the liquid collector, and collection tube. (b) Liquid collectors with varying inner channel diameters.

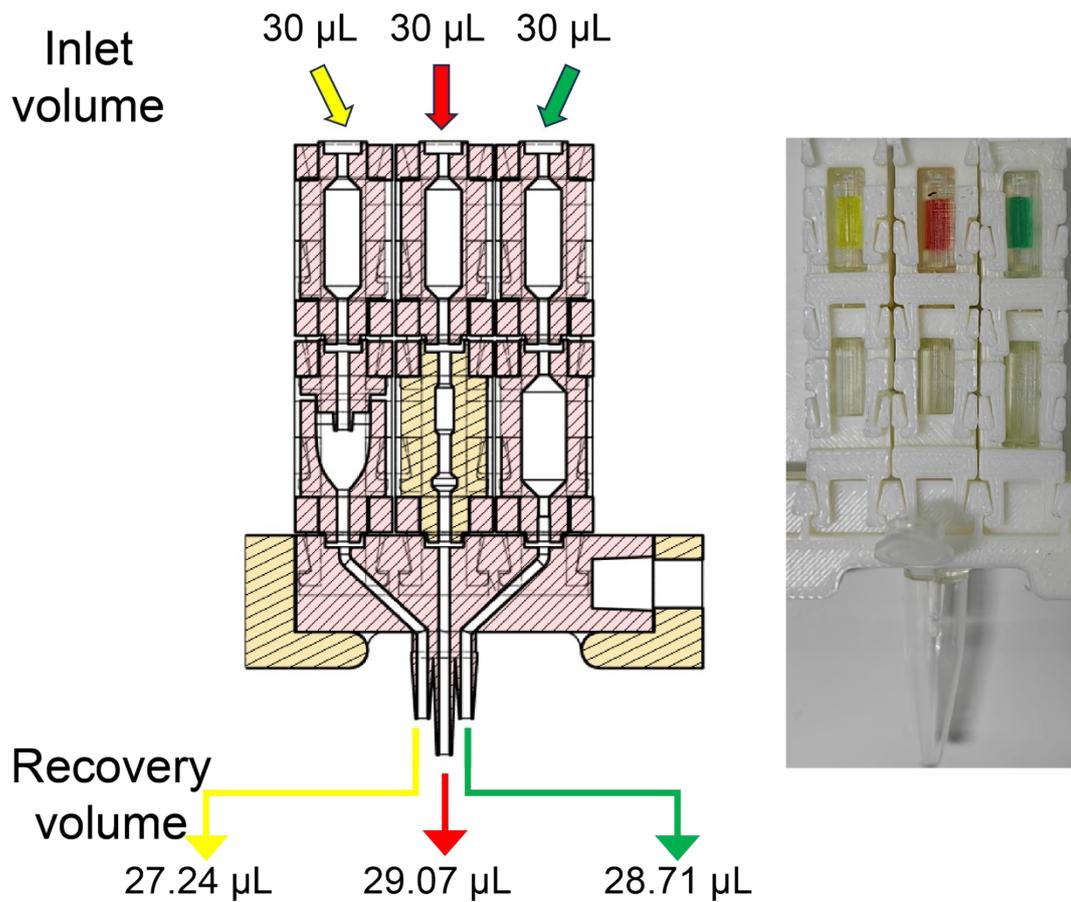


Figure S4. Characterization of the recovery of LIM-Chip using colored solutions.

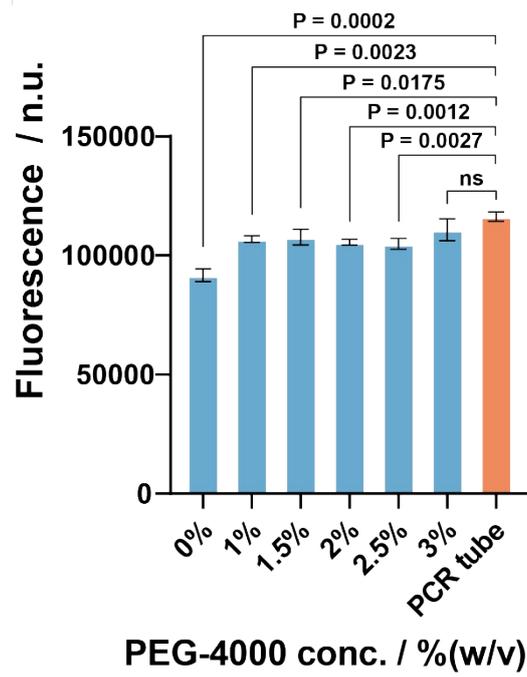


Figure S5. Optimization of the concentrations of PEG-4000 for coating the LIM-Chip channels.

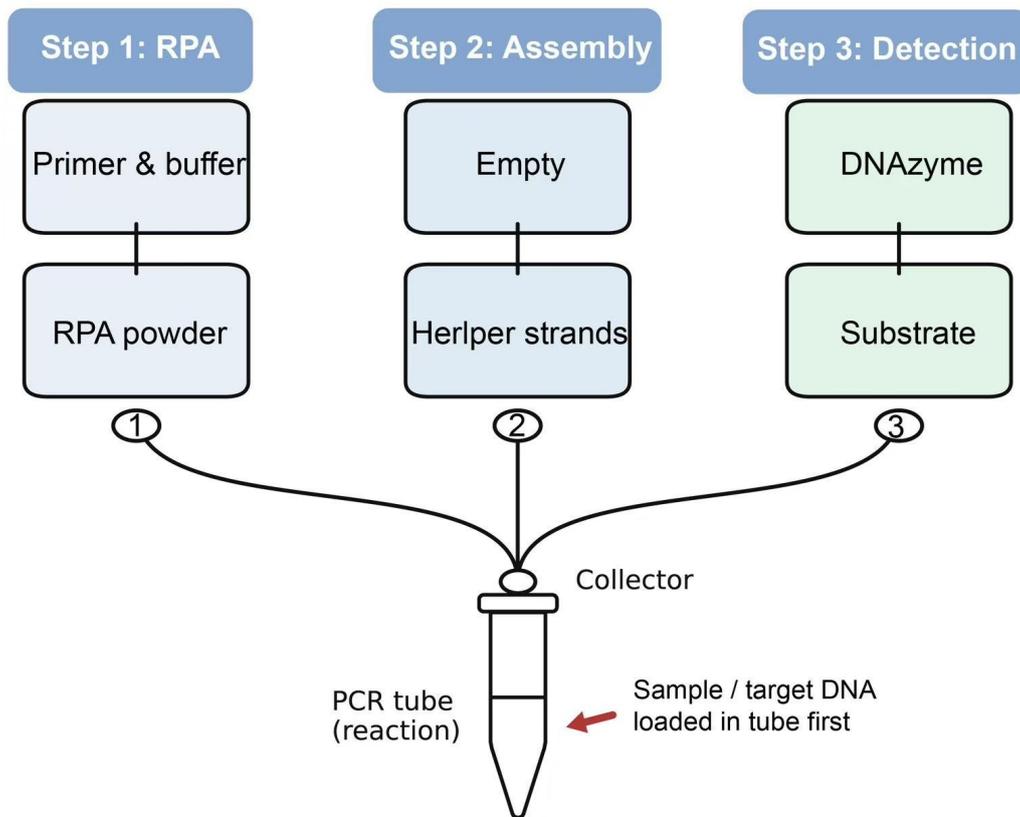


Figure S6. Schematic illustration of the sequential reagent delivery workflow for a single-sample CLARISSA assay on the LIM-Chip.

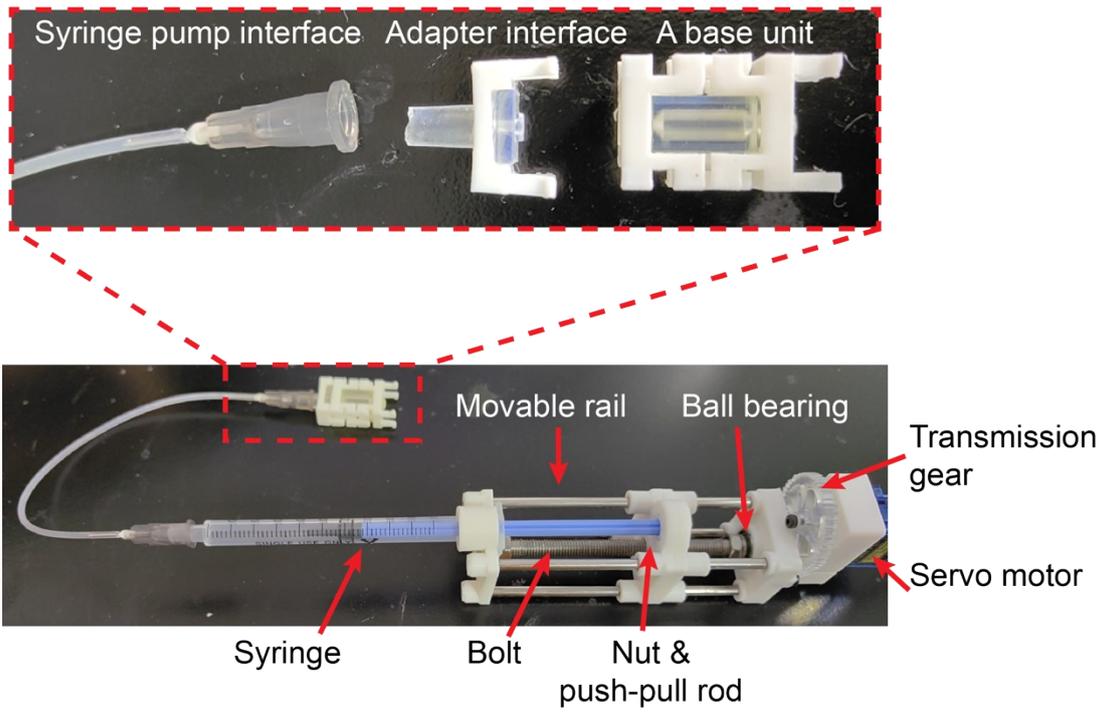


Figure S7. Design and image of the automated syringe pump design for the portable device.

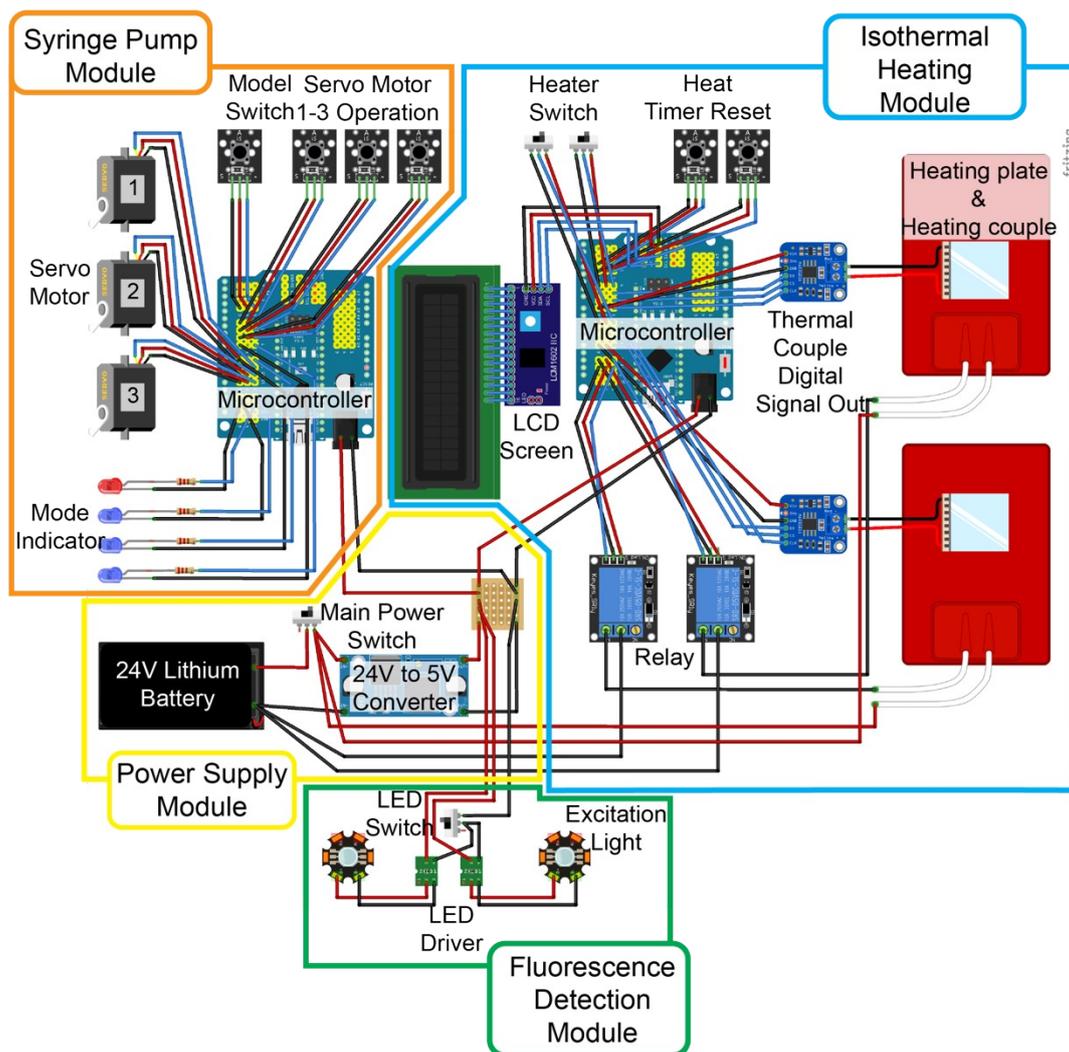


Figure S8. Electrical circuit design of the portable device for LIM-Chip operation.

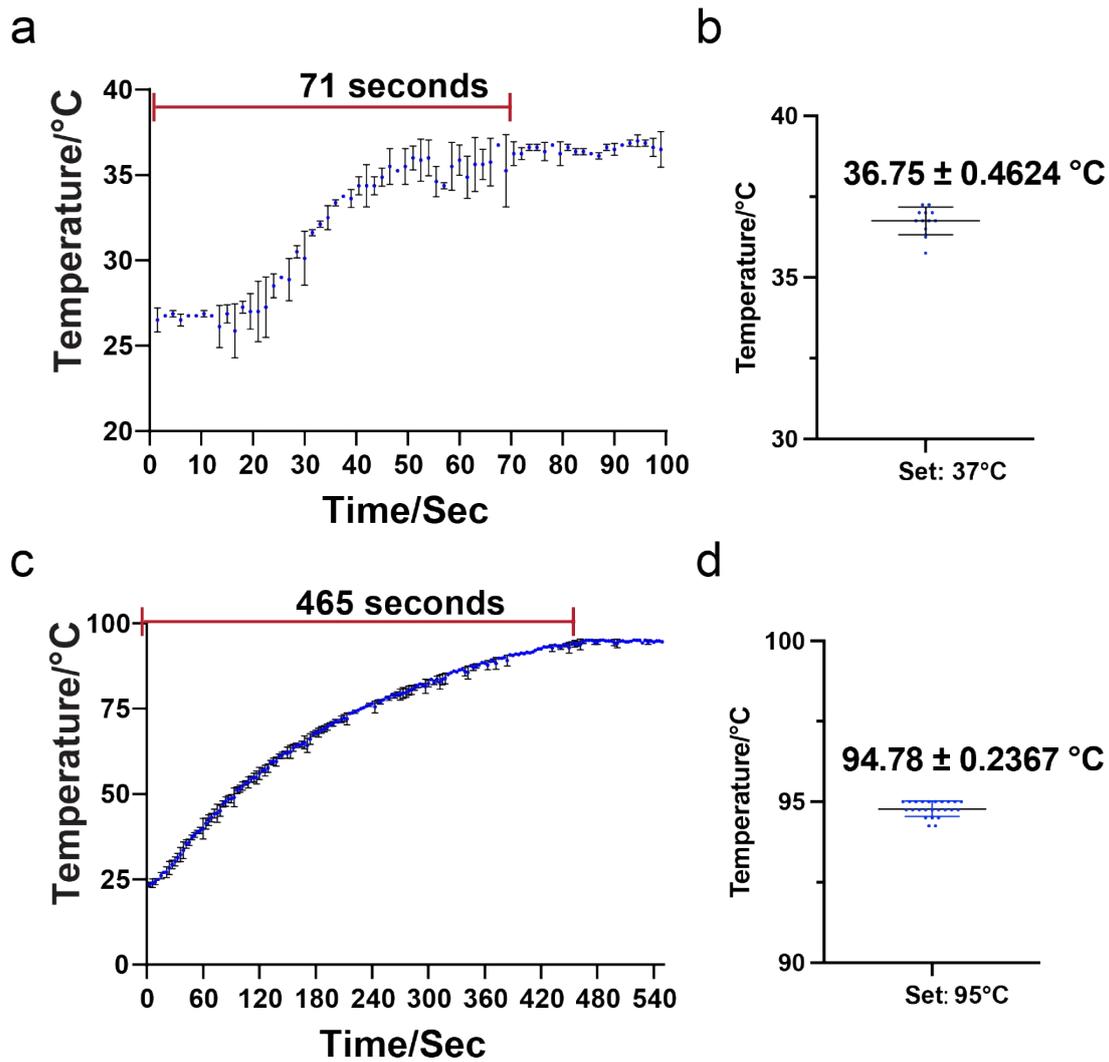


Figure S9. Characterization of the heating performance and temperature stability of a portable device at 37 °C (a, b) and 95 °C (c, d).

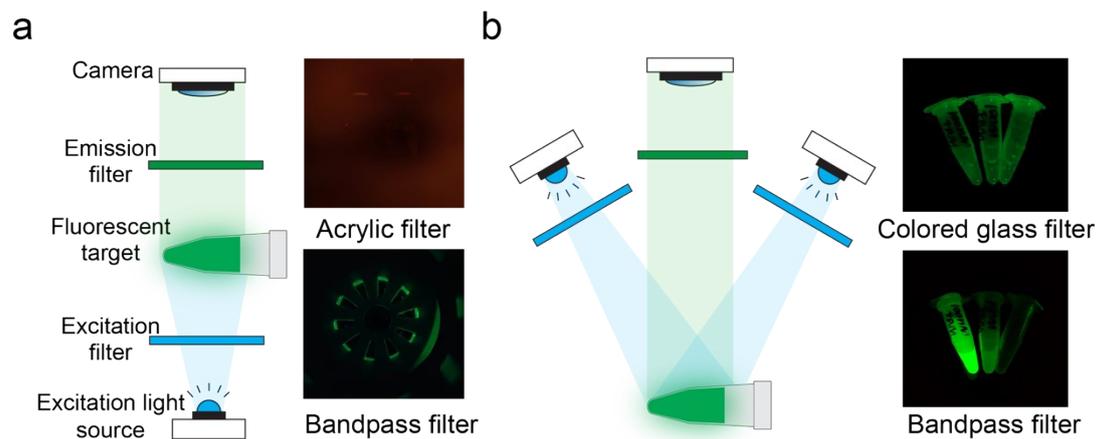


Figure S10. Optical design of the fluorescence imaging system of the portable device. (a) Schematic illustration and images of the optical system via a linear light path. **(b)** Schematic illustration and images of the optical system via an epi-fluorescence light path.

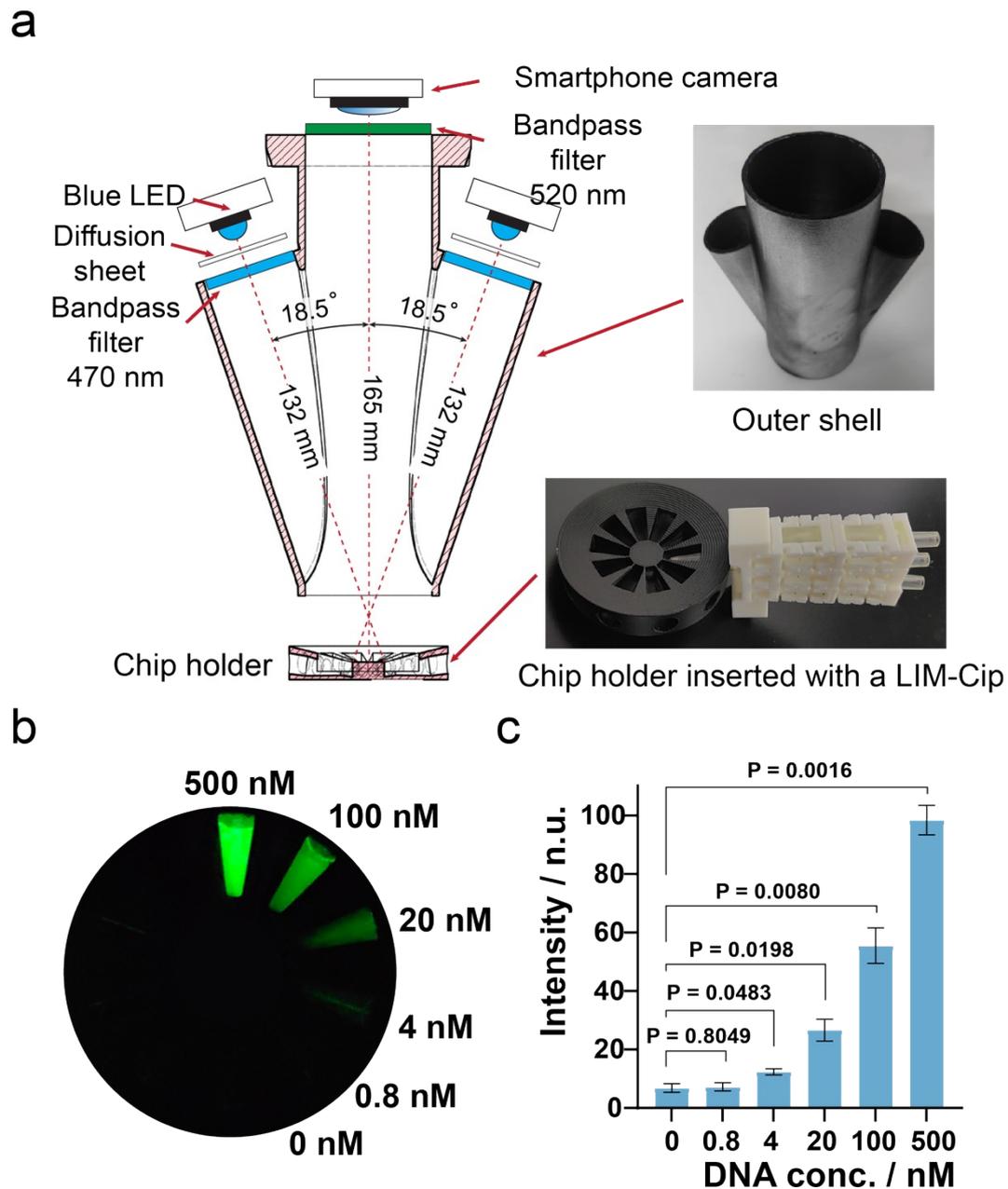


Figure S11. Design and fabrication of optical barrel for the epifluorescence light path system. (a) Schematic illustration and photos of the design of optical path system. (b) Fluorescence images as readouts of gDz-based detection of HPV16 DNA within the LIM-Chip. (c) Gray scale intensities plotted as a function of the DNA concentrations.

S3. Supporting Table

Table S1. Comparison of LIM-Chip with current microfluidics-based NATs for HPV and other pathogens.

Name	Amplification strategy	Reconfigurable	Reagent pre-storage	Target	LOD	Time	Sample Volume
Integrated microfluidic HPV16/18 RT-qPCR system ¹	qPCR	NO	YES	SARS-CoV-2, respiratory viruses	200 copies per mL	30 min	45 μ L
MEDIC-PCR ²	RT-qPCR	NO	YES	SARS-CoV-2	19 copies per μ L	15 min	1 μ L
All-in-one microfluidic qPCR chip ³	qPCR	YES	YES	Human Cytomegalo virus	1 copy per μ L	30 min	2 mL
MUSAL chip ⁴	LAMP	NO	YES	SARS-CoV-2, respiratory viruses	0.5 copies per μ L	30 min	2 mL
Self-contained microfluidic device ⁵	LAMP	NO	YES	SARS-CoV-2, respiratory viruses	100 copies per μ L	70 min	50 μ L
SP-dChip ⁶	LAMP	NO	NO	Zika Virus	100 copies per μ L	60-65 min	10 μ L
MiCaR ⁷	CRISPR-based	NO	YES	HPV and respiratory viruses	0.16 copies per μ L	40 min	150 μ L

Passive microfluidic RT-RPA/CRISPR chip ⁸	CRISPR-based	NO	YES	respiratory viruses	10 copies per μL	45 min	280–320 μL
SCADL ⁹	CRISPR-based	NO	NO	SARS-CoV-2	470 aM	60 min	100 μL
LIM-Chip (This work)	RPA	YES	YES	HPV16	10 aM	60 min	5 μL

Table S2. DNA oligonucleotides and modifications¹⁰

Name	Sequence (5'-3')	Purpose
HPV16-Dz	CTACTTCAGGCTAGCTACAACGAGAACTAC A TATAAATTTTTTATATGTAGTTTCTGAAGTA GA TATGGCA	gene-specific DNAzyme (gDz)
HPV16-probe	FAM-GTAGTTTCrGrUGAAGTA-BHQ2	Probe strand, 5'-end labeled with 6-FAM, 3'-end labeled with BHQ2
HPV16-FP	TTGTTGGGGTAACCAACTATTTGTTACTGTT	RPA Forward Primer
HPV16-RP	CCTCCCATGTCGTAGGTACTCCTTAAAG	RPA Reverse Primer
HPV16-Target	TTGTTGGGGTAACCAACTATTTGTTACTGTTG T TGATACTACACGCAGTACAAATATGTCATTAT G TGCTGCCATATCTACTTCAGAACTACATATA A AAATACTAACTTTAAGGAGTACCTACGACAT G	HPV16 synthetic target

	GGGAGG	
HPV16-h-1	TTTGTACTGCGTGTAGTATCAACA	HPV16 helper strands
HPV16-h-2	TATGTCATTATGTGCTGCCATAT	
HPV16-h-3	CTACTTCAGAACTACATATAAAAATACTAA	
HPV16-h-4	TCCCCATGTCGTAGGTACTCCTTAAAGTT	
HPV16-h-5	TGTTGATACTACACGCAGT	
HPV16-h-6	TAAGGAGTACCTACGACATGGGGA	
Target DNA	CTAAGAAGCGATCTACAAGAGTAGAAATTAA A AAGGTC-FAM	3'-end labeled with 6- FAM

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