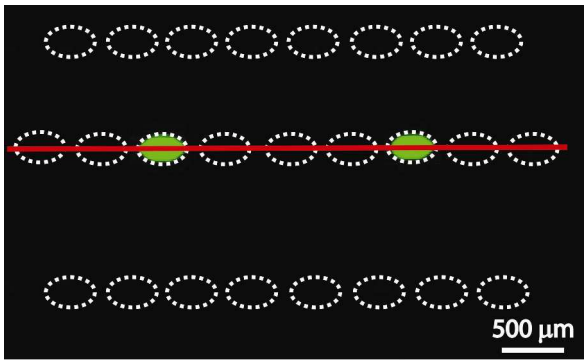
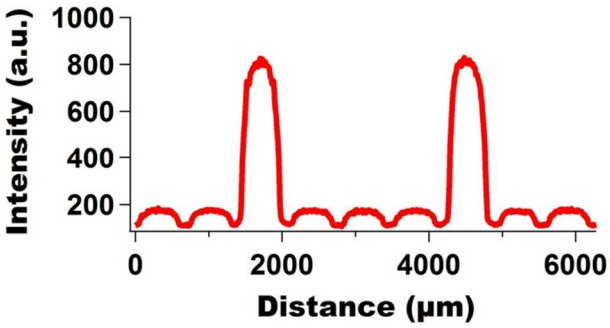
Mechanistic evaluation of the pros and cons of digital RT-LAMP for HIV-1 viral load quantification on a microfluidic SlipChip and improved efficiency via a two-step digital protocol

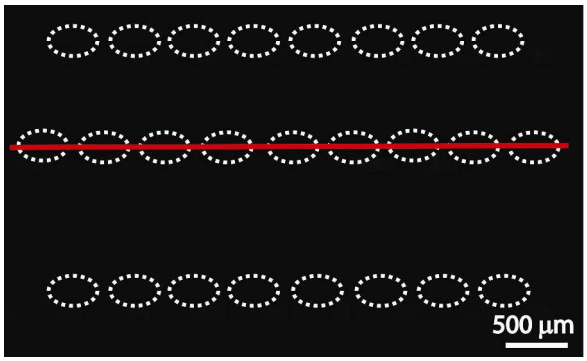
Bing Sun[†], Feng Shen[‡], Stephanie E. McCalla[†], Jason E. Kreutz[§], Mikhail A. Karymov[†], and Rustem F. Ismagilov[†]*

[†]Division of Chemistry and Chemical Engineering, California Institute of Technology, 1200 East California Boulevard, Pasadena, California 91125, United States

Figure S1. Line-scan fluorescence measurement of glass device using microscope (background was not subtracted). Intensity measured in arbitrary units (a.u.).







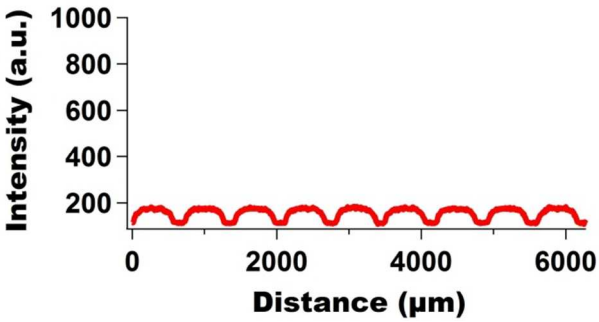
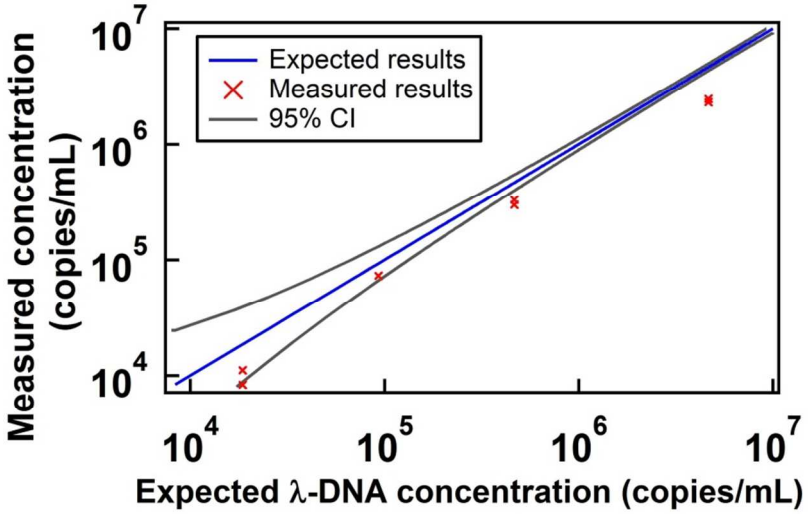


Figure S2 Quantification results of λ-phage DNA concentration (copies/mL) on SlipChip using dLAMP plotted against concentration measured by Qubit fluorometer (n=2). Error bars represent standard deviation.



Sequences of primers and HIV viral RNA used in the paper

Primers used for RT-LAMP amplification were selected from a previous publication[1] with several single point mutations:

GAGAACCAAGGGGAAGTGA (p24_Loop_B), TTTAACATTTGCATGGCTGCTTGAT (p24_Loop_F), TATTGCACCAGGCCAGATGATTTTGTACTAGTAGTTCCTGCTATG (p24_BIP), CAGCTTCCTCATTGATGGTCTCTTTTAACACCATGCTAAACACAGT (p24_FIP), ATTATCAGAAGGAGCCACC (p24_F3), CATCCTATTTGTTCCTGAAGG (p24_B3).

LTR primers used for RT-PCR amplification were the same as in a previous publication[2].

Sequencing results for HIV viral RNA at p24 gene region

Acrometrix_sample

ATTATCAGAAGGAGCCACCCCACAAGATTTAAACACCATGCTAAACACAGTGGGGGGACATC
AAGCAGCCATGCAAATGTTAAAAGAGACCATCAATGAGGAAGCTGCAGAATGGGATAGAGT
GCATCCAGTGCATGCAGGGCCTATTGCACCAGGCCAGATGAGAGAACCAAGGGGAAGTGAC
ATAGCAGGAACTACTAGTACCCTTCAGGAACAAATAGGATG

Patient_sample_#1

ATTATCAGAAGGAGCCACCCCACAAGATTTAAACACCATGCTAAATACAGTGGGGGGACATC
AAGCAGCTATGCAAATGTTAAAAGAGACCATCAATGAGGAAGCTGCAGAATGGGATAGAGT
GCATCCAGTGCATGCAGGGCCTATTGCACCAGGCCAGATGAGAGAACCAAGGGGAAGTGAC
ATAGCAGGAACCACTAGTACCCTTCAGGAACAGATAGGGTG

Patient_sample_#2

ATTATCGGAAGGAGCCACCCCACAAGATTTAAACACCATGCTAAACACAGTGGGGGGACATC
AAGCAGCCATGCAAATGTTAAAAGAGACCATCAATGAAGAAGCTGCAGAATGGGATAGAGT
ACATCCAGTGCATGCAGGGCCTATTGCACCAGGCCAGATGAGAGAACCAAGGGGAAGTGAC
ATAGCAGGAACTACTAGTACCCTTCAGGAACAAATAGGATG

Patient_sample_#3

ATTATCAGAAGGAGCCACCCCACAAGATTTAAACACCATGCTAAACACAGTGGGGGGACATC
AAGCAGCTATGCAAATGTTAAAAGAGACCATCAATGATGAAGCTGCAGAATGGGATAGATTA
CATCCAGTGCATGCAGGGCCTGTTGCACCAGGTCAGATGAGAGAACCAAGGGGAAGTGACAT
AGCAGGAACTACTAGTACCCTTCAGGAACAAATAGGATG

Patient_sample_#4

ATTATCAGAAGGAGCCACCCCACAAGATTTAAACACCATGCTAAACACAGTGGGGGGTCATC
AAGCAGCCATGCAAATGTTAAAAGAGACTATCAATGAGGAGGCTGCAGAATGGGATAGATT
ACATCCAGTGCAGGCAGGACCAGTTGCACCAGGTCAGATGAGAGAACCAAGGGGAAGTGAC
ATAGCAGGAACTACTAGTACCCTTCAGGAACAAATAGGATG

Chemicals and materials

All solvents and salts purchased from commercial sources were used as received unless otherwise stated. The LoopAmp® DNA amplification kit, the LoopAmp® RNA amplification kit, and the Calcein fluorescence indicator kit were purchased from SA Scientific (San Antonio, TX, USA). SsoFast EvaGreen Supermix (2X) was purchased from Bio-Rad Laboratories (Hercules, CA). Bovine serum albumin (BSA) and λ-DNA isolated from bacteriophage λ cl857 Sam 7 were purchased from Roche Diagnostics (Indianapolis, IN). Hybridase™ Thermostable RNase H was purchased from Epicentre Biotechnologies (Madison, WI). All primers were ordered from Integrated DNA Technologies (Coralville, IA). Mineral oil (D Nase, R Nase, and Protease free), tetradecane, and DEPC-treated nuclease-free water were purchased from Fisher Scientific (Hanover Park, IL). Dichlorodimethylsilane was purchased from Sigma-Aldrich (St. Louis, MO). AcroMetrix® HIV-1 Panel Copies/ml, EXPRESS One-Step SYBR® GreenER™ Universal, iPrepTM purification instrument, and iPrepTM PureLinkTM virus kit were purchased from Life Technologies (Grand Island, NY). PCR Mastercycler and in situ adapter were purchased from Eppendorf (Hamburg, Germany). Spectrum food color was purchased from August Thomsen Corp (Glen Cove, NY). Photomasks were designed in AutoCAD 2012 and ordered from CAD/Art Services, Inc. (Bandon, OR). Soda-lime glass plates coated with layers of chromium and photoresist were ordered from Telic Company (Valencia, CA). Polycarbonate SlipChip devices were designed in AutoCAD 2012 and purchased from microfluidic ChipShop GmbH (Jena, Germany).

Fabrication of SlipChips

The procedure of fabricating desired glass SlipChips using soda lime glass was based on previous work.[2,3] The two-step exposing-etching protocol was adapted to create wells of two different depths (5 μm for thermal expansion wells, 55 μm for all the other wells). After etching, the glass plates were thoroughly cleaned with piranha acid and DI water, and dried with nitrogen gas. The glass plates were then oxidized in a plasma cleaner for 10 minutes and immediately transferred into a desiccator for 1 hour of silanization. They were rinsed thoroughly with chloroform, acetone, and ethanol, and dried with nitrogen gas before use.

Plastic polycarbonate SlipChip devices were directly oxidized in a plasma cleaner for 15 minutes after they were received from microfluidic ChipShop GmbH, and then transferred into a desiccator for 90 minutes of silanization. They were soaked in tetradecane for 15 minutes at 65 °C and then rinsed thoroughly with ethanol, then dried with nitrogen gas before use. Plastic SlipChip devices were not reused.

Assembling and loading the SlipChips

The SlipChips were assembled under de-gassed oil (mineral oil: tetradecane 1:4 v/v). Both top and bottom plates were immersed into the oil phase and placed face to face. The two plates were aligned under a stereoscope (Leica, Germany) as shown in Figure 2 and fixed using binder clips. Two through-holes were drilled in the top plate to serve as fluid inlets. The reagent solution was loaded through the inlet by pipetting.

HIV viral RNA purification from AcroMetrix® HIV-1 Panel Copies/mL

400 μL plasma containing a modified HIV virus (5 million copies/mL, part of AcroMetrix® HIV-1 Panel Copies/mL) was loaded onto the iPrep™ PureLink® Virus cartridge. The cartridge was

placed in the iPrep™ purification instrument and the purification protocol was performed according to the manufacturer's instructions. The elution volume was 50 μL. The purified HIV viral RNA was diluted 10, 10^2, 10^3 fold in 1 mg/mL BSA solution, aliquoted and stored at -80 °C for further use. HIV viral RNA purified from patient plasma was also aliquoted and stored at -80 °C upon receipt.

Image acquisition and analysis

The bright-field image in Figure 2 and the fluorescence images in real-time dRT-LAMP experiments were acquired using a Leica MZ 12.5 Stereomicroscope. All other fluorescence images were acquired using a Leica DMI 6000 B epi-fluorescence microscope with a 5X / 0.15 NA objective and L5 filter at room temperature unless stated otherwise. All the images were analyzed using MetaMorph software (Molecular Devices, Sunnyvale, CA).

The data analysis method was adapted from a previous publication.[4]

References

(1) Curtis, K. A.; Rudolph, D. L.; Owen, S. M. *J. Virol. Methods* **2008**, *151*, 264.

(2) Shen, F.; Sun, B.; Kreutz, J. E.; Davydova, E. K.; Du, W.; Reddy, P. L.; Joseph, L. J.; Ismagilov, R. F. *J. Am. Chem. Soc.* **2011**, *133*, 17705.

(3) Du, W.; Li, L.; Nichols, K. P.; Ismagilov, R. F. *Lab Chip* **2009**, *9*, 2286.

(4) Kreutz, J. E.; Munson, T.; Huynh, T.; Shen, F.; Du, W.; Ismagilov, R. F. *Anal. Chem.* **2011**, *83*, 8158.