Supporting Information

Reading Out Single-Molecule Digital RNA and DNA Isothermal Amplification in Nanoliter Volumes with Unmodified Camera Phones

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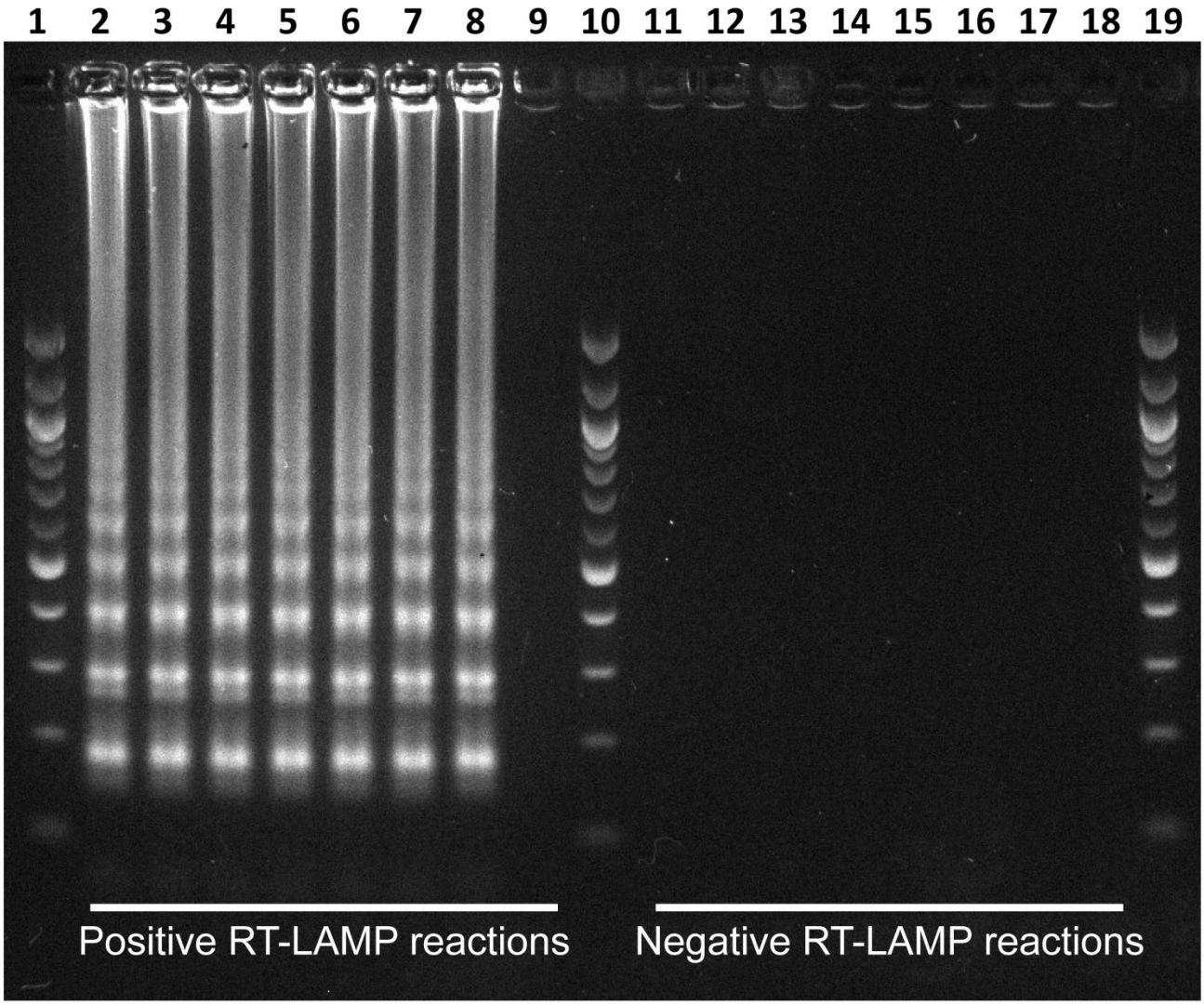


Figure S1. DNA gel electrophoresis for RT-LAMP product. Lanes 1, 10 and 19 are 100 bp DNA ladders. Lanes 2–9 are positive (HCV RNA) RT-LAMP reactions at two-fold increased EBT solution concentration (from 0.011 to 1.4 mM). Lanes 11–18 are negative RT-LAMP reactions at two-fold increased EBT solution concentration (from 0.011 to 1.4 mM). Lane 9 shows an inhibited RT-LAMP reaction in the presence of 1.4 mM EBT solution.

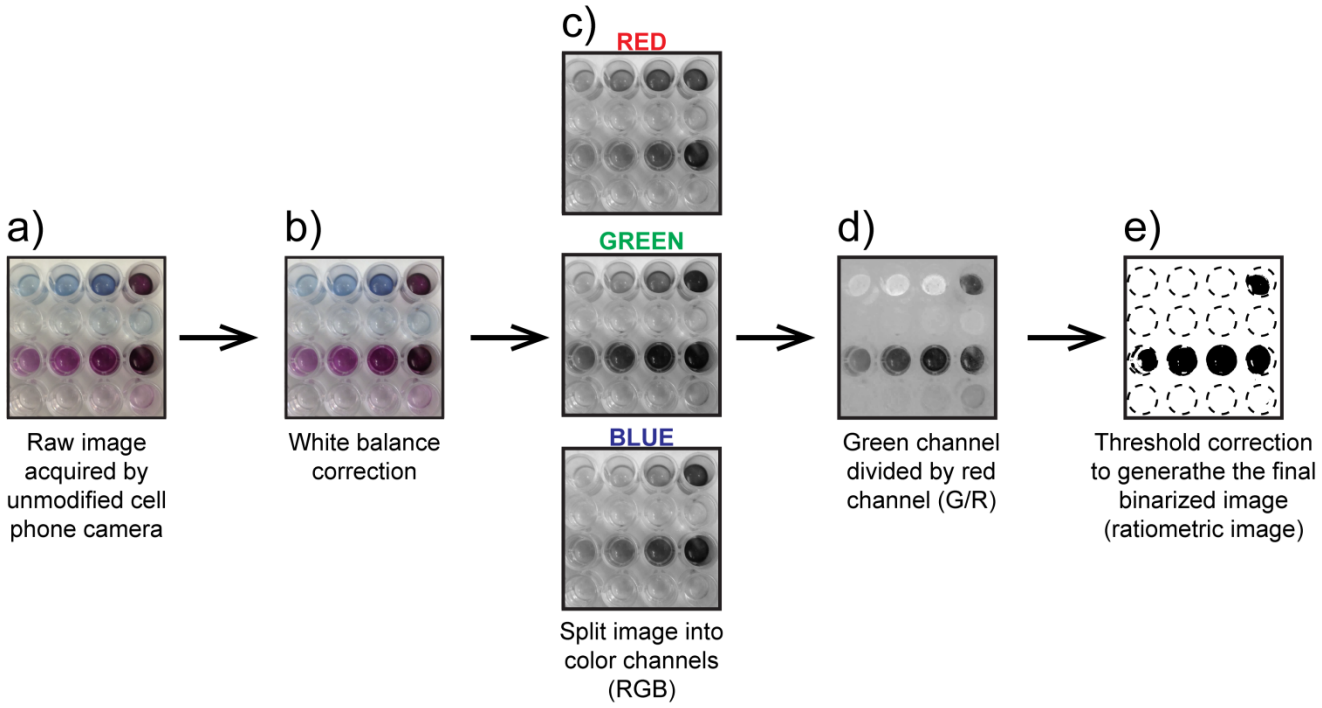


Figure S2. Each step of the G/R process algorithm. This experiment was performed with HCV RNA as a clinically relevant target and the raw image was acquired with an Apple iPhone 4S under fluorescent light. The top row of each panel (eight wells) shows a positive RT-LAMP reaction containing EBT solution at two-fold increasing concentrations from 10.9 µM to 1.4 mM (from left to right). The bottom row of each panel (eight wells) shows negative RT-LAMP reactions containing EBT solution at two-fold increasing concentrations from 10.9 µM to 1.4 mM. a). A raw image acquired by a cell phone camera. b) The same image after white balance correction. c) Red, green and blue color channels separated. d) Resulting image after green channel is divided by red channel. e) The binary image after a threshold correction. Positive reactions (originally blue) are white and negative reactions (originally purple) are black. Image processing was performed with Image J (ver. 1.49).

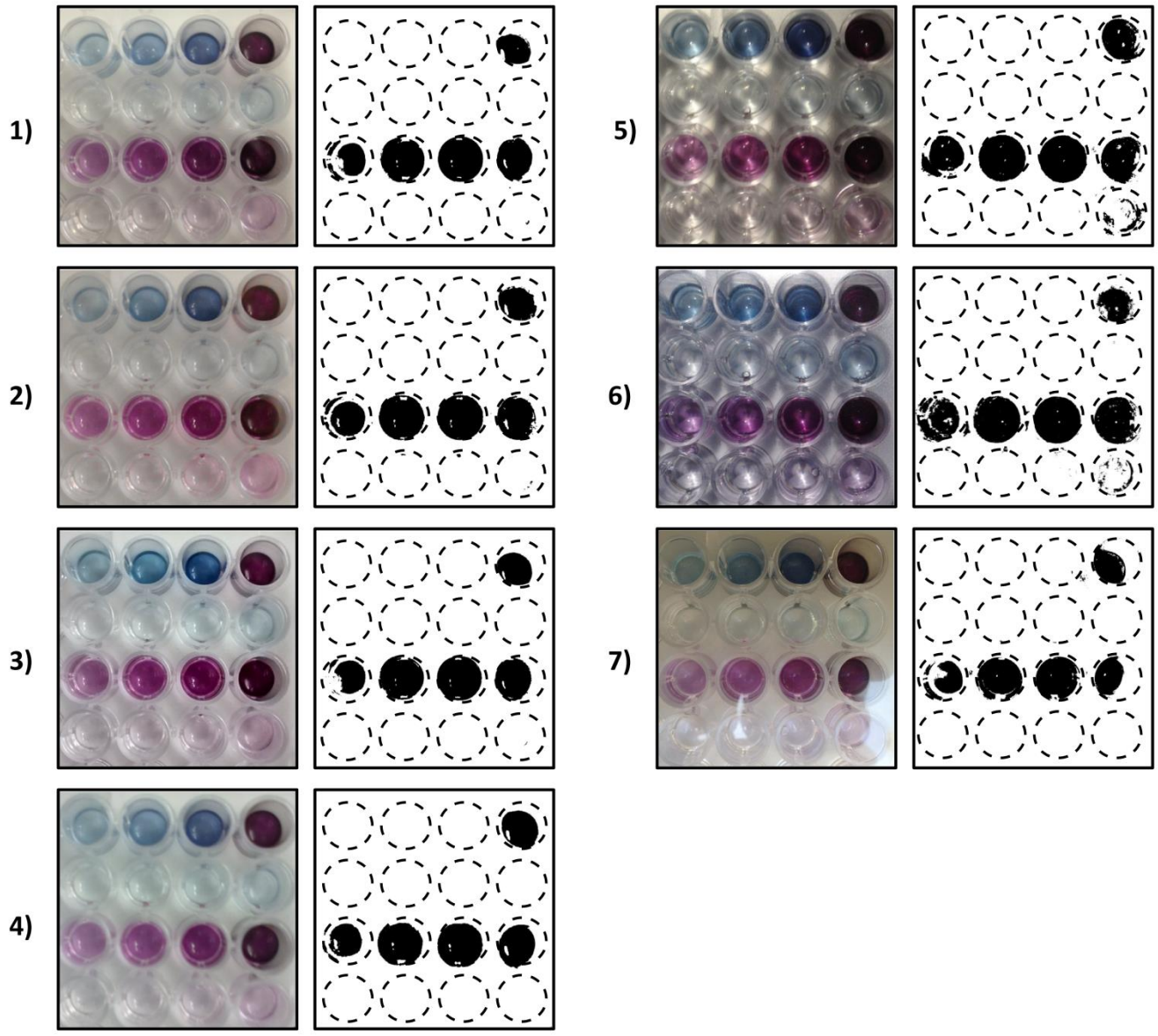


Figure S3. Original and G/R-processed images acquired with unmodified cell phone cameras. Original color images show negative (bottom two rows) and positive (top two rows) RT-LAMP reactions. From left to right, EBT concentration is increased in two-fold increments between 10.9 μM to .088mM (bottom row) and .175mM to 1.4mM (second row from the bottom). Positives contained HCV RNA and the same EBT concentration pattern was repeated. Negative wells are purple and positive wells are blue. Ratiometric G/R-processed images show the binary result in which the negative wells become black and the positive wells become white. (1–4) Images collected with four common cell phones under fluorescent light: (1) Apple iPhone 4S, (2) HTC inspire 4G, (3) Motorola Moto G and (4) Nokia 808 PureView. (5–7) Images collected with Apple iPhone 4S under different light conditions: (5) incandescent light, (6) direct sunlight and (7) indirect sunlight. Image processing was performed with ImageJ (ver. 1.49).

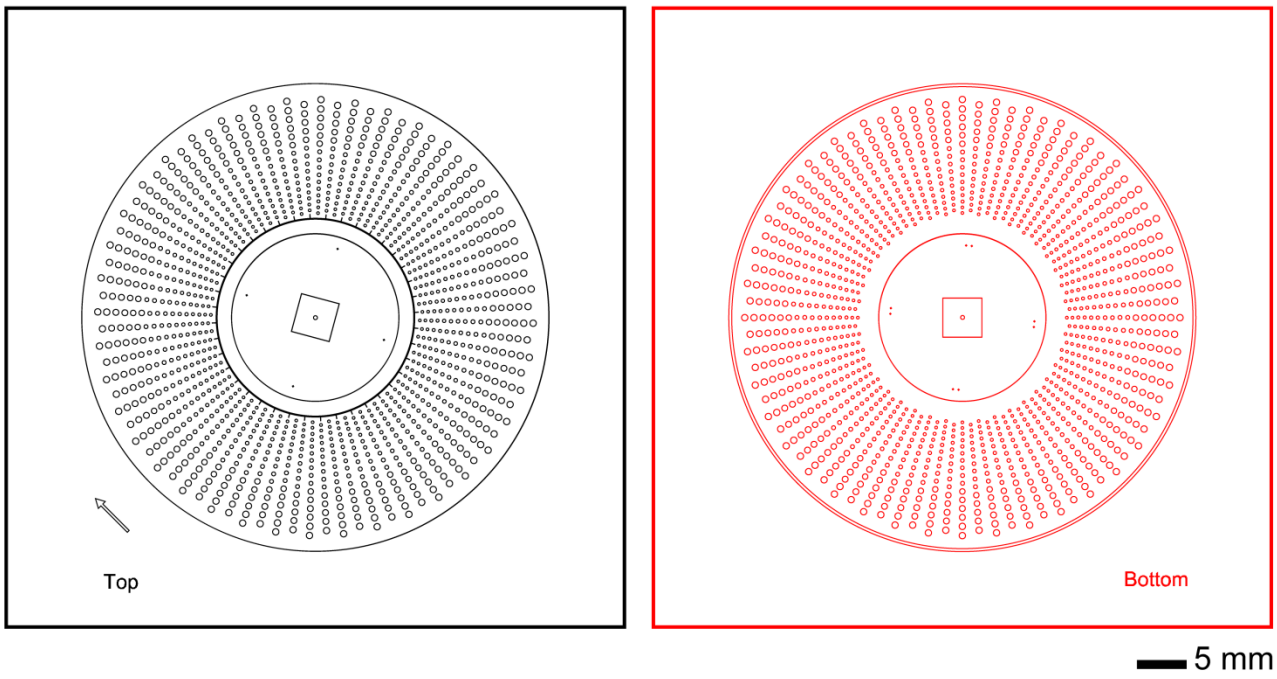


Figure S4. Schematic of the top (left) and bottom (right) plates of the multivolume rotational SlipChip device used in the one-step digital LAMP experiments before being assembled. The top plate shows the direction of the rotational 4.5° slip.

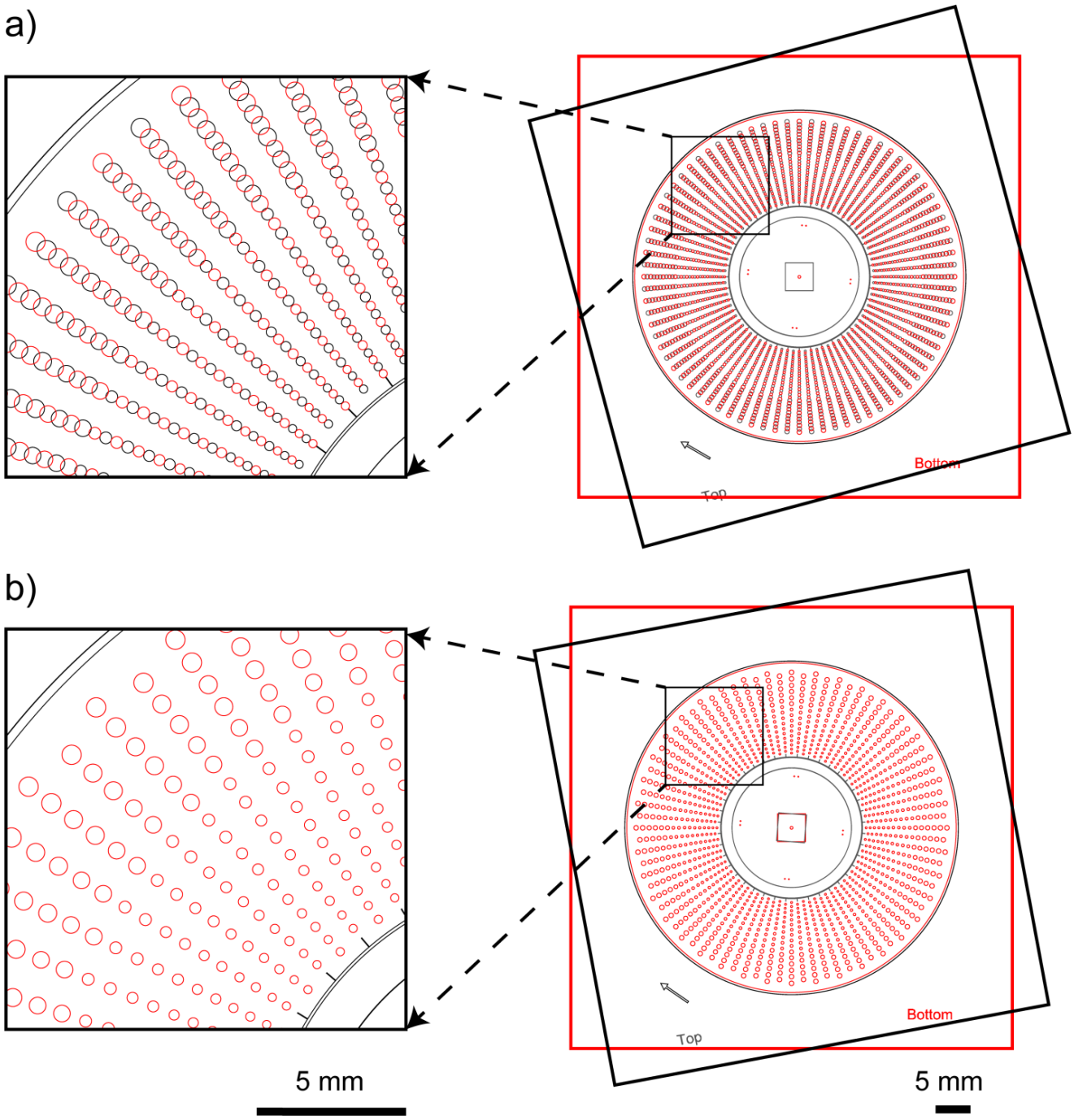


Figure S5. Schematic of the multivolume rotational SlipChip device used for one-step digital LAMP experiments after being assembled. Drawing shows the layout of top and bottom piece of the entire device on the right and a zoomed-in region (black box) on the left. a) Relative position of the two pieces when they are aligned to allow loading of solution through the channel, and b) the relative position of the two pieces when they are slipped (top slide rotated 4.5°) to separate droplets from one another and form compartments. Features shown are before isotropic glass etching.

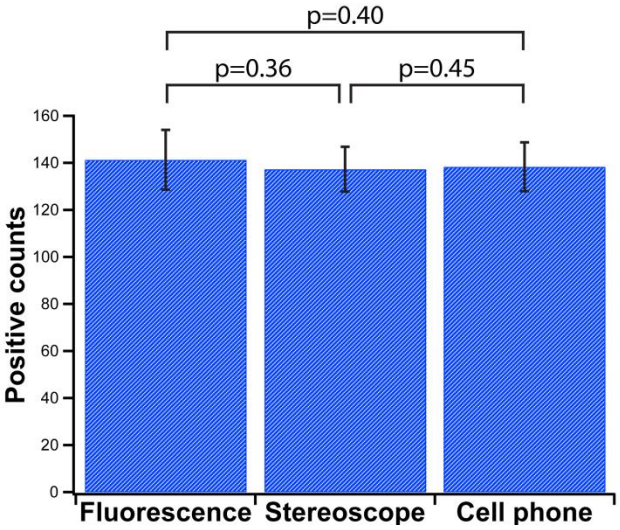


Figure S6. Positive counts obtained from single-molecule digital LAMP reactions performed with lambda DNA on a one-step SlipChip device imaged by a house-built real-time fluorescence microscope, a Leica MZ FI III stereoscope, and an unmodified cell phone camera (Apple iPhone 4S) under fluorescent light. One-step visual readout was performed on SlipChip devices composed of 800 wells of 27 nL volumes. LAMP amplification mix contained 0.7 mM eriochrome black T dye solution, SYTO® 9 Stain and phage lambda DNA. Automated counting was performed by self-developed Labview software for fluorescent images and freeware Fiji image processing for bright field G/R processed images. Data are mean positive counts and error bars are S.D. (N = 3). Student's t-tests were used for statistical comparisons, showing no significant differences among counts obtained by the three imaging methods (*P* values > 0.05).

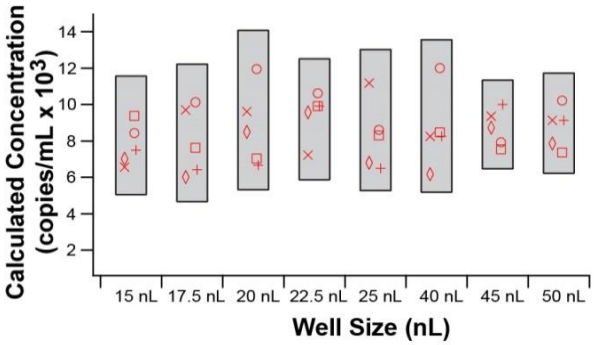


Figure S7. Five multivolume experiments were performed, and the concentration of each volume was calculated based on the methods of Kreutz *et al*.[57] Gray boxes denote the 95% confidence interval for the set of experiments at each volume. Concentrations calculated at each volume are consistent, and there is no bias based on the volume in which the reaction is performed.

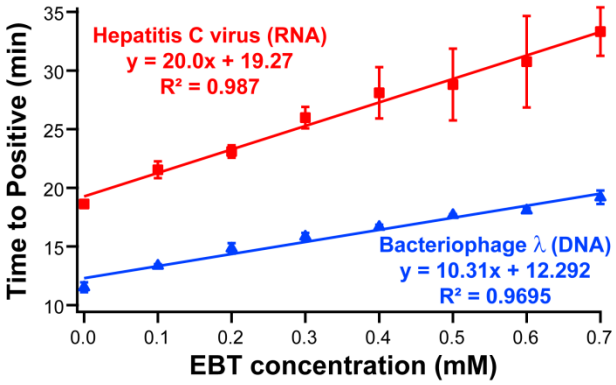


Figure S8. Performance of bulk LAMP reactions at increasing concentrations of the amplification indicator dye eriochrome black T (EBT). All reactions performed in 10 μL volumes with concentrations of EBT solution ranging from 0.0 to 0.7 mM, SYTO® 9 Stain and either 1,000 copies of HCV RNA (red) or 1,000 copies of phage lambda DNA (blue). All reactions were run in triplicate.

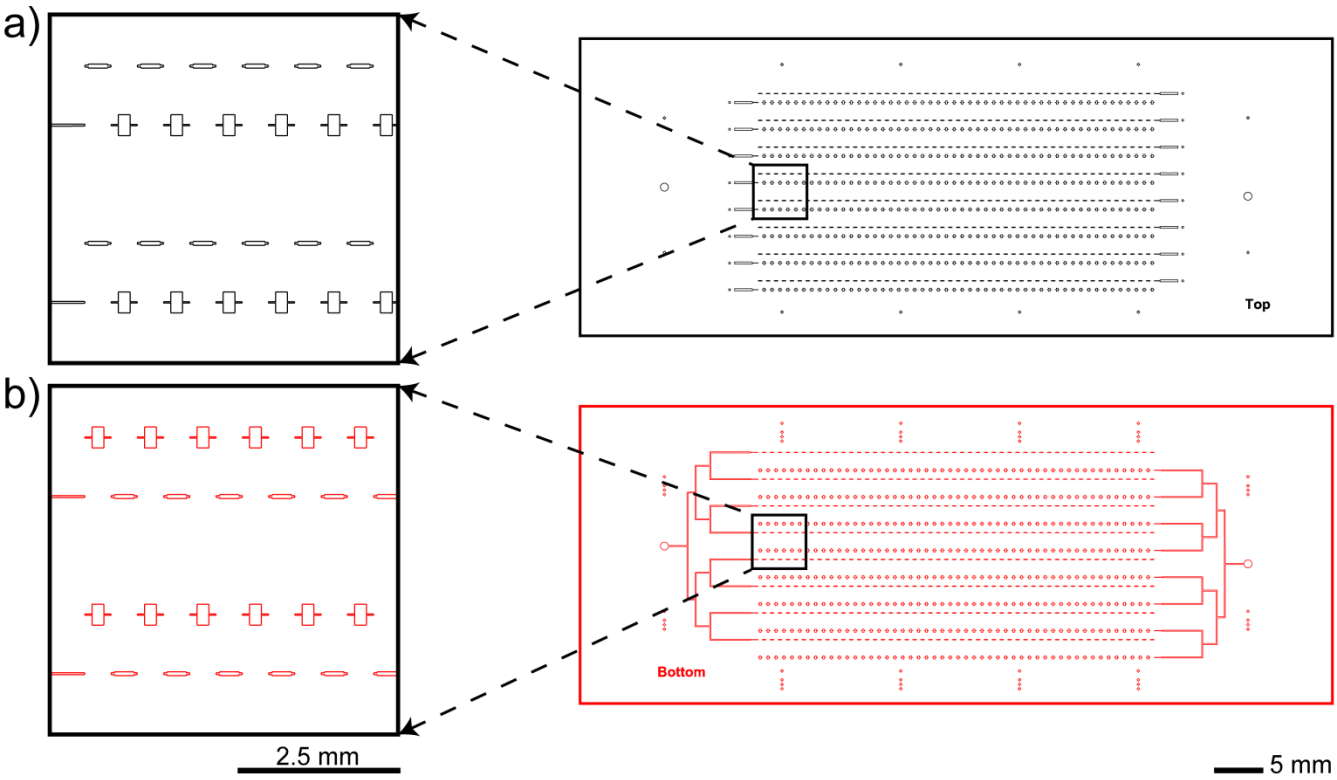


Figure S9. Schematic of the two-step SlipChip device before assembly. Drawings show the top (a) and bottom (b) device plates with a selected region (black box) magnified on the left to show locations of the 5 nL and 9.5 nL wells. Features are shown before isotropic glass etching. The design of the two-step SlipChip device was based on previously published SlipChip designs.[13]

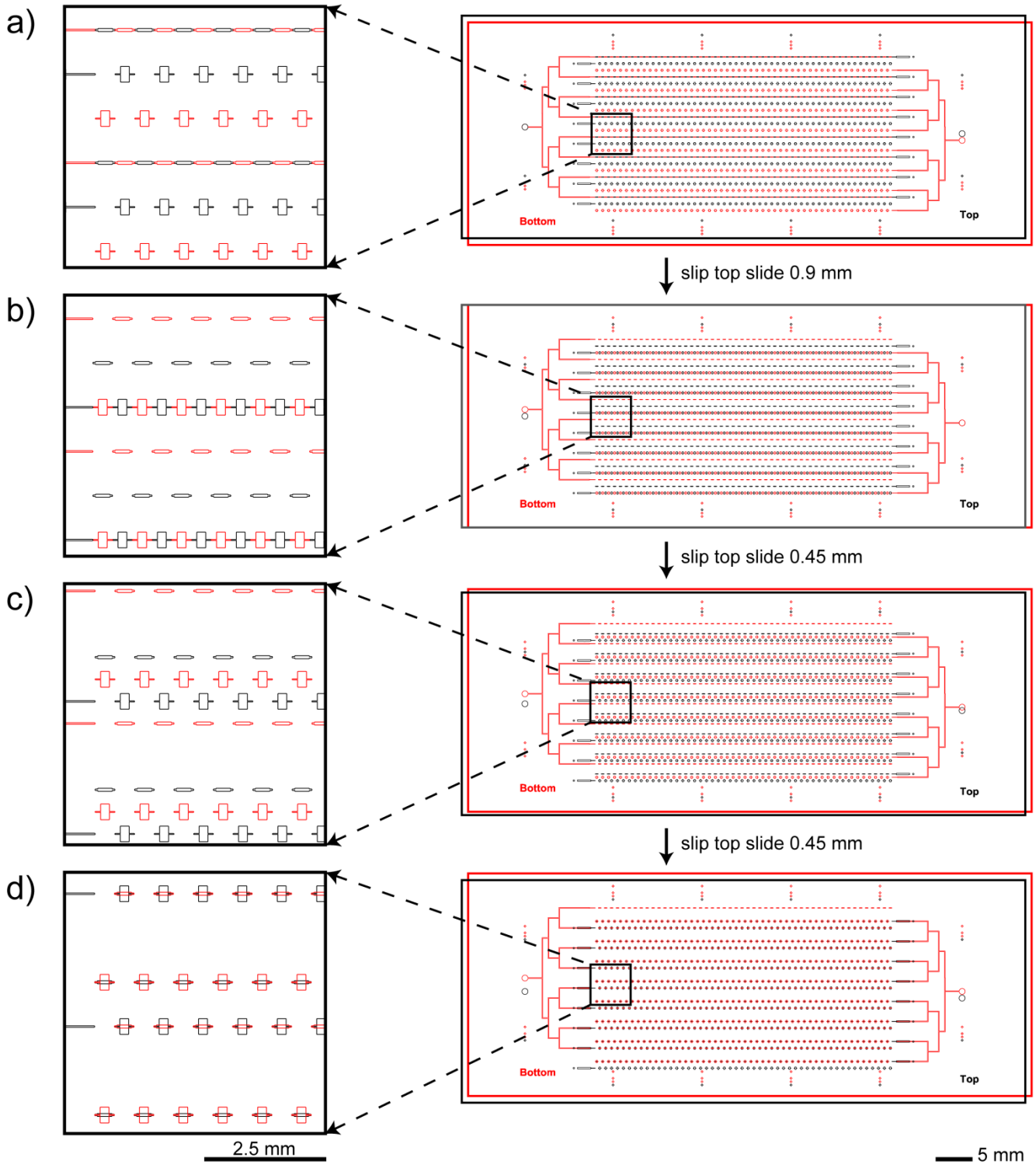


Figure S10. Schematic of the two-step SlipChip device after assembly and its operation. Drawings show the layout of the top and bottom plates on the right and a magnified region (black box) on the left. (a) Loading conformation for the first set of wells (5 nL each). (b) Loading conformation for the second set of wells (9.5 nL each). (c) Incubation conformation. (d) Final mixing conformation ready for imaging with a cell phone camera. Features are shown before isotropic glass etching.

Table S1 Sequence of primers used in RT-LAMP experiments for detection of hepatitis C RNA.[15]

primer	sequence (5'-3')
F3	CCTCCCGGGAGAGCCATAG
FIP	TCCAAGAAAGGACCCIGTCTTTTTCTGCGGAACCGGTGAGTAC
LF	TTICCGGIAATTCCGGT
B3	GCACTCGCAAGCACCITATC
BIP	TTGGGCGTGCCCCCGCIAGATTTTTCAGTACCACAAGGCCITTCGCIACC
LB	CTGCTAGCCGAGTAGIGTTG

Table S2 Sequence of primers used in LAMP experiments for detection of phage lambda DNA.[17]

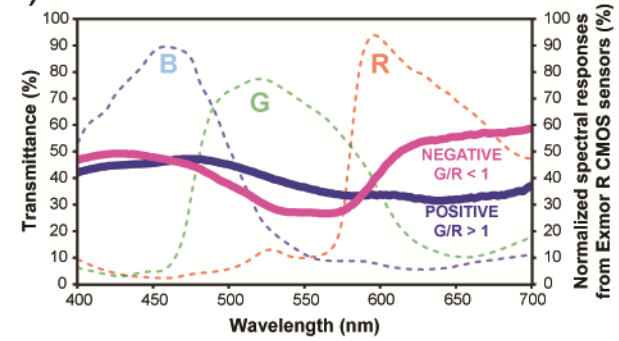
primer	sequence (5'-3')
F3	GAATGCCCGTTCTGCGAG
FIP	CAGCATCCCTTTCGGCATACCAGGTGGCAAGGGTAATGAGG
LF	GGCGGCAGAGTCATAAAGCA
B3	TTCAGTTCCTGTGCGTCG
BIP	GGAGGTTGAAGAACTGCGGCAGTCGATGGCGTTCGTACTC
LB	GGCAGATCTCCAGCCAGGAACTA

Table S3. Multivolume device designs for viral load quantification.

Volumetric step	Number of well volumes	Well volume range (nL)	Number of wells per device	LDL – ULQ (copies/mL)	DR (log)
2	6	5 – 160	2,700	500 – 1,000,000	3.3
2	6	5 – 160	2,700	50 – 1,000,000	4.3
5	3	5 – 125	1,350	500 – 1,000,000	3.3
5	3	5 – 125	1,350	50 – 1,000,000	4.3
25	2	5 – 125	900	500 – 1,000,000	3.3
25	2	5 – 125	940	50 – 1,000,000	4.3

The lower detection limit (LDL) is defined as the concentration which would have a 95% probability of generating at least one positive well. The upper limit of quantification (ULQ) is defined as the concentration where the probability of all wells being positive is 5%. DR: dynamic range. Calculations were performed according to the equations and algorithms found in *Kreutz JE, Munson T, Huynh T, Shen F, Du W, Ismagilov RF. "Theoretical design and analysis of multivolume digital assays with wide dynamic range validated experimentally with microfluidic digital PCR." Anal Chem. 2011 83(21):8158-68*.

a)

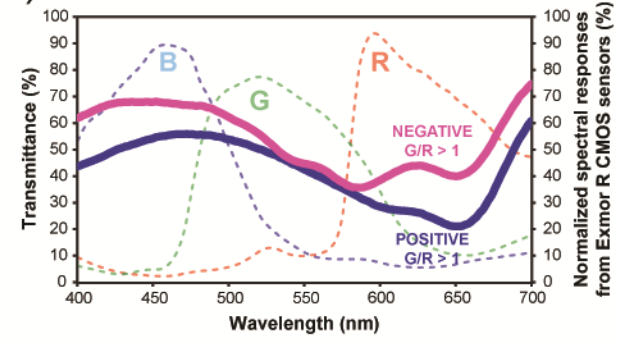


b)

Predicted ratiometric values and difference between positive and negative LAMP reactions in the presence of Eriochrome Black T indicator dye

Process	positive	negative	positive-negative (%)
Green/Red	1.06	0.87	0.19 (17.9)
Blue/Red	1.13	0.97	0.16 (14.2)
Green/Blue	0.94	0.90	0.04 (4.3)

c)

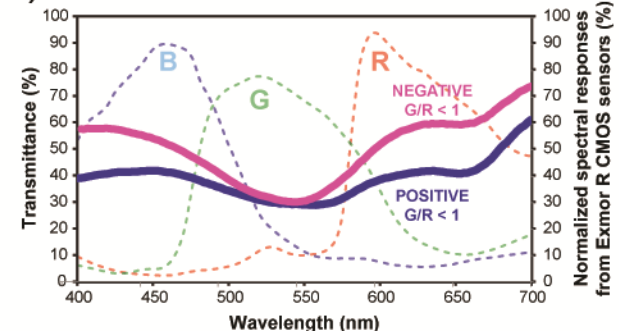


d)

Predicted ratiometric values and difference between positive and negative LAMP reactions in the presence of Hydroxynaphthol Blue indicator dye

Process	positive	negative	positive-negative (%)
Green/Red	1.17	1.06	0.11 (9.4)
Blue/Red	1.25	1.17	0.08 (6.4)
Green/Blue	0.94	0.90	0.04 (4.3)

e)



f)

Predicted ratiometric values and difference between positive and negative LAMP reactions in the presence of Calmagite indicator dye

Process	positive	negative	positive-negative (%)
Green/Red	0.92	0.86	0.06 (6.5)
Blue/Red	0.98	0.95	0.03 (3.1)
Green/Blue	0.94	0.91	0.03 (3.2)

Figure S11. (a, c and e) Measured spectral transmittance (%) in the range of visible light (400–700 nm) for positive (solid blue line) and negative (solid purple line) RT-LAMP reaction solutions, each containing 0.7 mM of eriochrome black T, hydroxynaphthol blue or calmagite as the amplification indicator dye. Dashed lines correspond to normalized spectral responses for red (R), green (G) and blue (B) channels of an Exmor R CMOS sensor, a common sensor in cell phone cameras. (b, d and f) Predicted ratiometric values for positive and negative LAMP amplification reactions processed for each ratiometric combination, Green/Red, Blue/Red and Green/Blue. Tables show absolute differences (positive – negative) and the relative difference (in %) between positive and negative ratiometric values are shown. All experiments were performed with HCV RNA as a template. Dye stock solutions were prepared as described in the Methods section "Preparation of EBT solution."

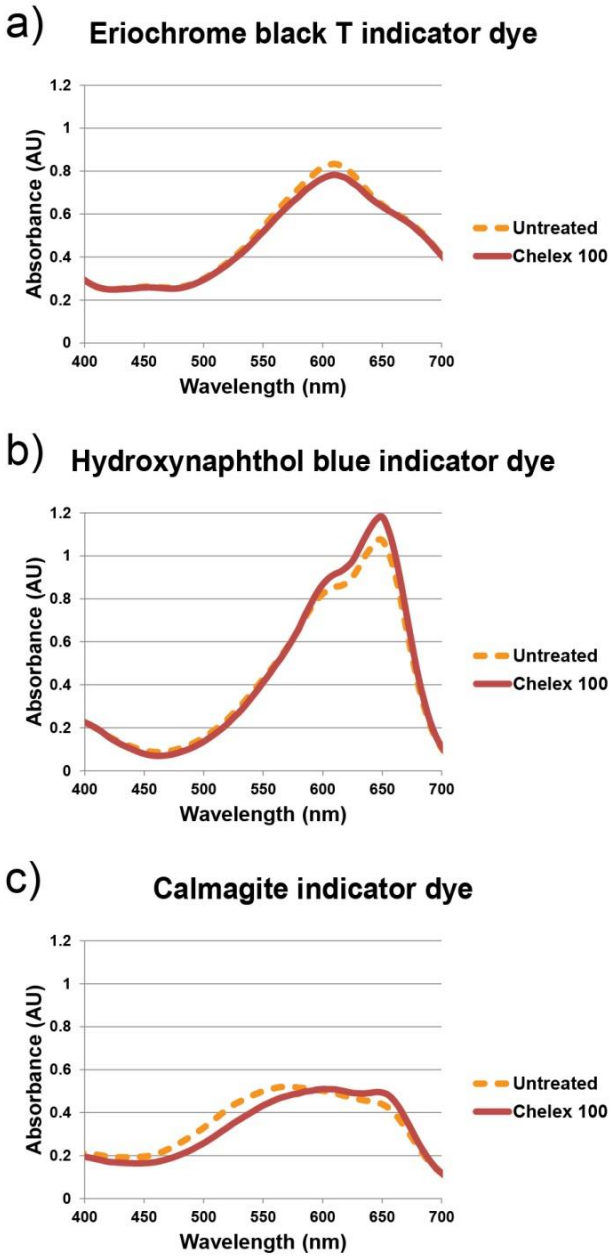


Figure S12. Comparison of the spectral absorbance (Absorbance Units) of untreated indicator dye stock solutions (dashed orange lines) and solutions treated with Chelex® 100 resin (solid red lines) for (a) eriochrome black T (EBT), (b) hydroxynaphthol blue (HNB) and (c) calmagite indicator dyes. The EBT, HNB and calmagite stock solutions were prepared by dissolving the dyes in 20 mM Tris-HCl buffer (pH 8.8) at 0.7 mM. The solutions were sonicated for 10 min and mixed on a rotator at room temperature for 1 h. The solutions were split into two equal volumes for the comparison; one volume was treated with Chelex® 100 ion exchange resin (5% w/v).

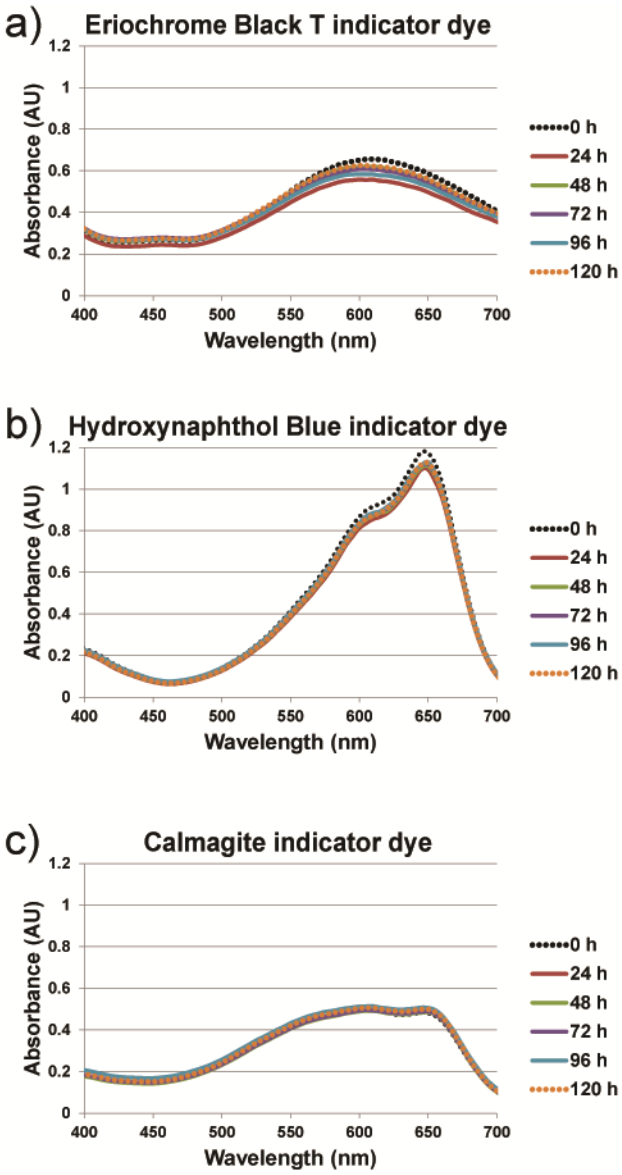


Figure S13. Storage stability of amplification indicator dyes by drying the stock solutions in the presence of stabilizer trehalose. Measured spectral absorbance (Absorbance Units) in the range of visible light (400–700 nm) for (a) eriochrome black T (EBT), (b) hydroxynaphthol blue (HNB) and (c) calmagite indicator dyes solutions. Time "0 h" is the spectral absorbance of the dye stock solution prior to drying in a dessiccator under vacuum. The following time points correspond to the length of time the dye stock solution was maintained in its dried state before being resuspended in distilled water. Full protocol is in Methods in the section "Storage stability of amplification indicator dyes by drying in the presence of stabilizer trehalose."