Supplementary Information for

Portable Sample Processing for Molecular Assays: Application to Zika Virus Diagnostics

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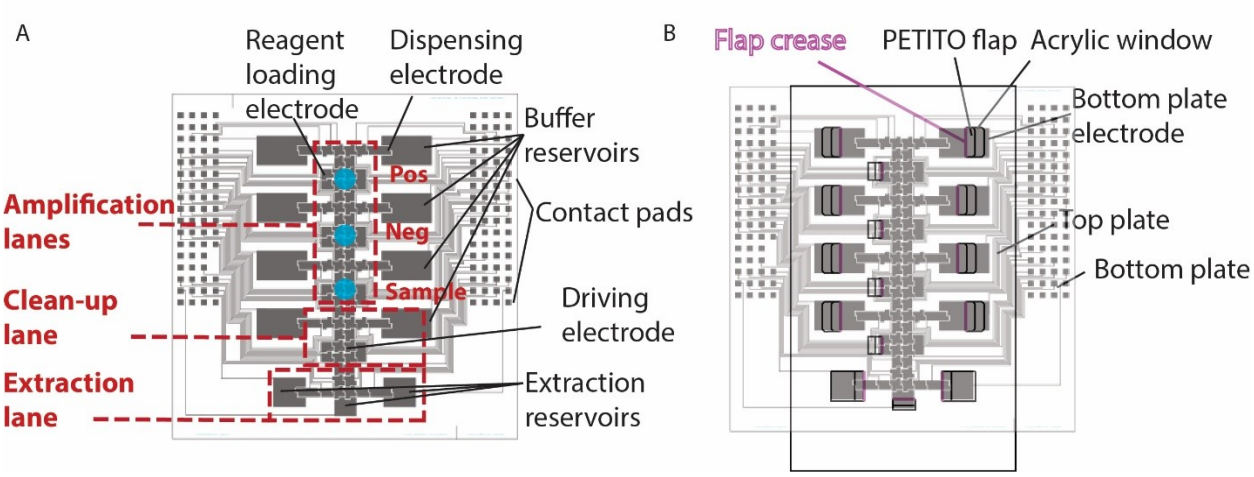

Figure S1: DMF cartridge structure. A) Schematic illustrating the DMF cartridge and process-flow layout, with lanes indicated in red dashed outlines. Samples are loaded in the extraction lane (bottom of figure), where the beads are separated from the liquid. The beads progress to the clean-up lane for washing (middle), and eluted RNA proceeds to the amplification lanes (top of figure), with spaces designated for extraction-amplification (EA) positive control, EA negative control, and sample. The layout of the bottom plate electrodes is also depicted, with driving, reagent loading, dispensing, and extraction reservoir electrodes indicated. B) Diagram illustrating the top plate of the cartridge, which features an acrylic layer on top of a PET-ITO layer. There are 15 windows through the top plate. The wider windows in the acrylic layer are outlined in black and pink. The narrower windows in the PET-ITO layer are outlined in black. Each pink line indicates the position of a folded "crease" in the PET-ITO. The "flap" that extends from the crease is bendable, such that fluid can be injected underneath it using a pipette during loading steps.

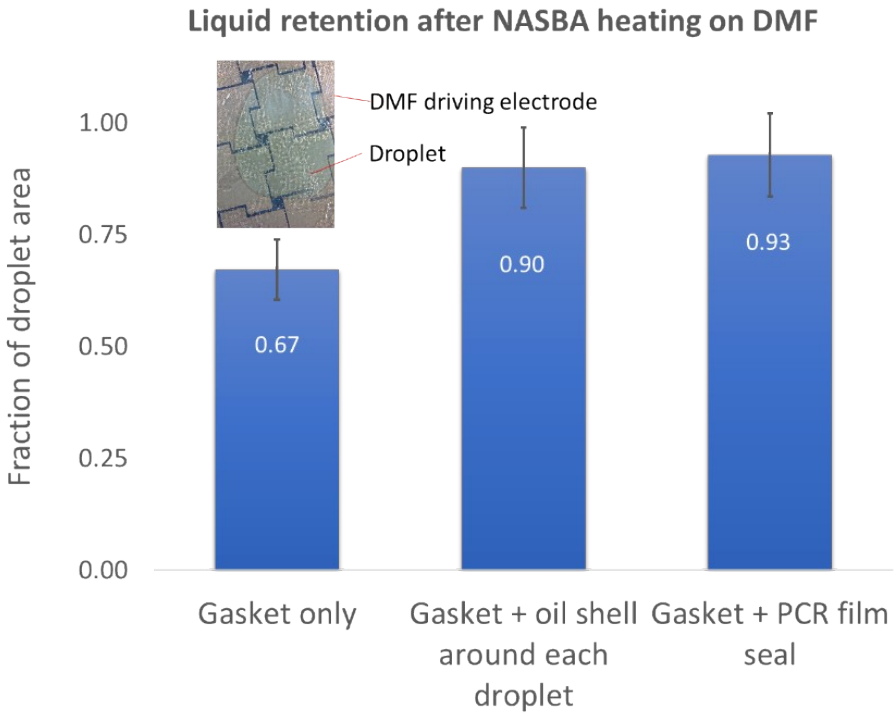

Figure S2: Plot of relative droplet area after heating to 45°C for 20 min for three different evaporation mitigation strategies. Apparent droplet areas (in pixels, inset) were estimated from images collected at a fixed height above the cartridge. The conditions tested included droplets of 4 µL DPBS (0.1% w/v Tetronics 90R4) in a cartridge formed with a gasket (left), engulfed in oil shells (1 µL per droplet, Vapor Lock™, Qiagen) in a cartridge formed with a gasket (middle), and in a cartridge formed with a gasket + PCR film seal (right). Error bars are ± 1 std. dev. for n=3 droplets evaluated per condition.

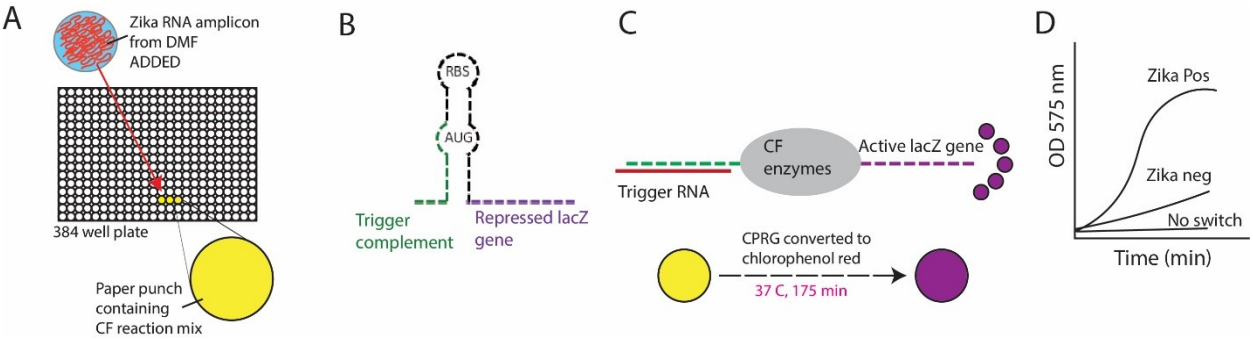

Figure S3: Schematic of the cell-free protein expression assay: A) Zika RNA amplicon (or "trigger", red) is added to a well plate containing in vitro protein synthesis reagents ("CF reaction mix"), the DNA toehold switch, a paper punch, and the beta galactosidase substrate chlorophenol red-β-D-galactoside (CPRG, which confers a yellow colour to the reagent mix). B) The DNA toehold switch contains a sequence complementary to the Zika trigger (green dashed line), ligated to sequences necessary for protein expression (including the ribosome binding site, RBS, and start codon, AUG) in a hairpin loop, ligated to a lacZ reporter gene (purple dashed line). C) When exposed to the cell-free protein expression enzymes at 37°C, the DNA switch is converted to an RNA switch, and binding of the Zika trigger causes the loop to open, triggering translation of Beta galactosidase. This enzyme catalyzes the conversion of CPRG (yellow) to chlorophenol red (purple). D) the reaction is quantified via absorbance measurements at 575 nm, where the optical density (OD) corresponds to the amount of Zika RNA amplicon in a Zika-trigger-positive sample (Pos). The toehold switch can be leaky, such that even in a Zika-trigger-negative sample (neg) some red colour develops with time as consequence of spontaneous switch opening. A no/low-signal "blank" can be found in a well not containing the toehold switch ("no switch") – see figure S4 for details about different kinds of blanks.

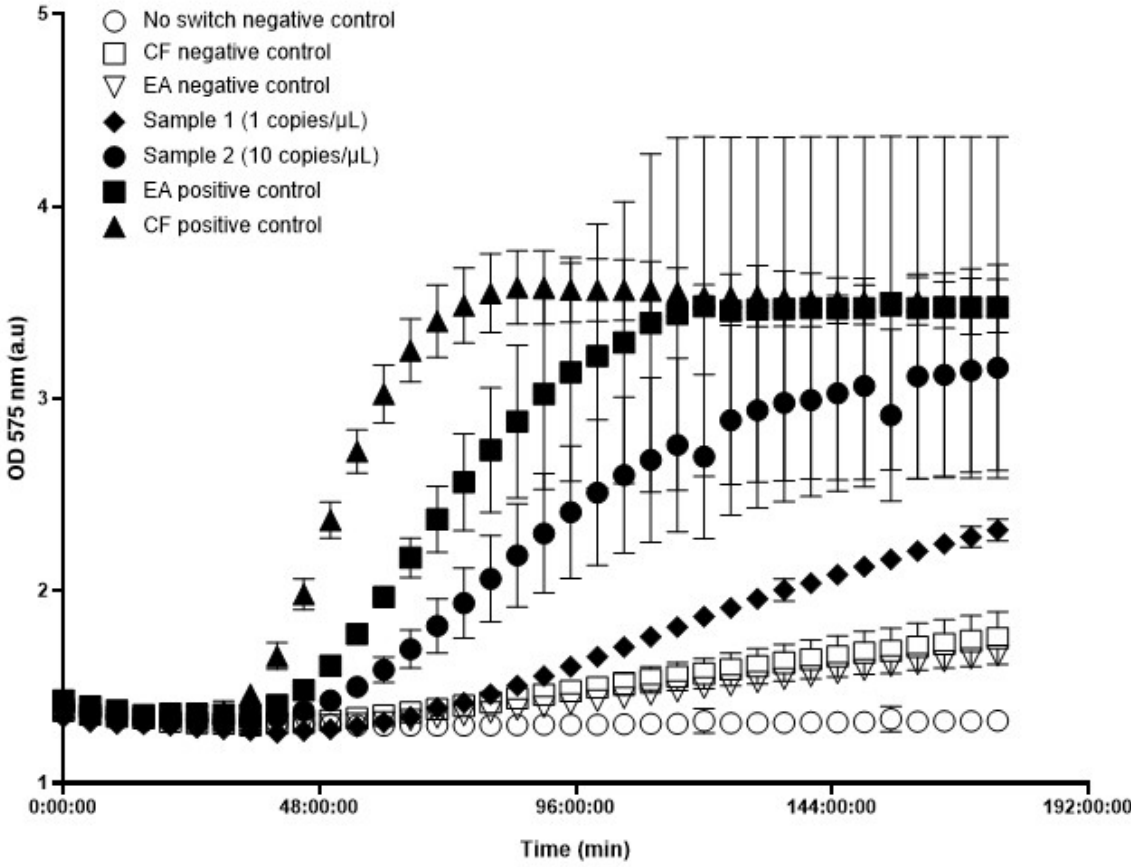

Figure S4: Representative OD 575 nm measurements as a function of time (min) for a cell-free protein expression assay for zika virus RNA. Eight data series are shown, where error bars represent ± 1 std. dev. for n=6 measurements per condition (see methods section for details). The "no switch negative control" (open circles) contains CF reagents but no toehold switch or sample. The CF negative control (open squares) contains CF reagents and toehold switch but no sample. The EA negative control (open upside-down triangles) contains CF reagents, toehold switch, and extracted/amplified serum with no Zika virus. Sample 1 (filled diamonds) contains CF reagents, toehold switch, and extracted/amplified serum spiked with 1 copies/µL Zika trigger RNA. Sample 2 (filled circles) contains CF reagents, toehold switch, and extracted/amplified serum spiked with 10 copies/µL Zika trigger RNA. The EA positive control (filled squares) contains CF reagents, toehold switch, and extracted/amplified serum spiked with 1,000 copies/µL Zika trigger RNA. The CF positive control (filled triangles) contains CF reagents, toehold switch and high concentration trigger RNA stock (10^{10} copies/µL) in lieu of amplified sample.

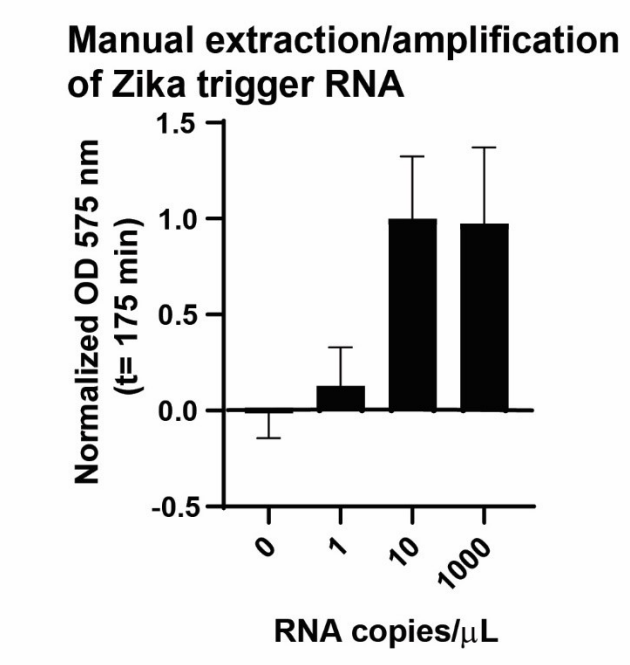

Figure S5: Optimized extraction conditions for detection limit using the Genesig Easy® kit. Plot showing normalized cell-free expression assay results implemented with the optimized ratio of Carrier RNA solution: Sample solution volume (0.25) using the manual workflow, which reduced detection limits to 1 copy/μL trigger RNA. Error bars represent 1 std. dev. from n = 3 replicates in each condition.

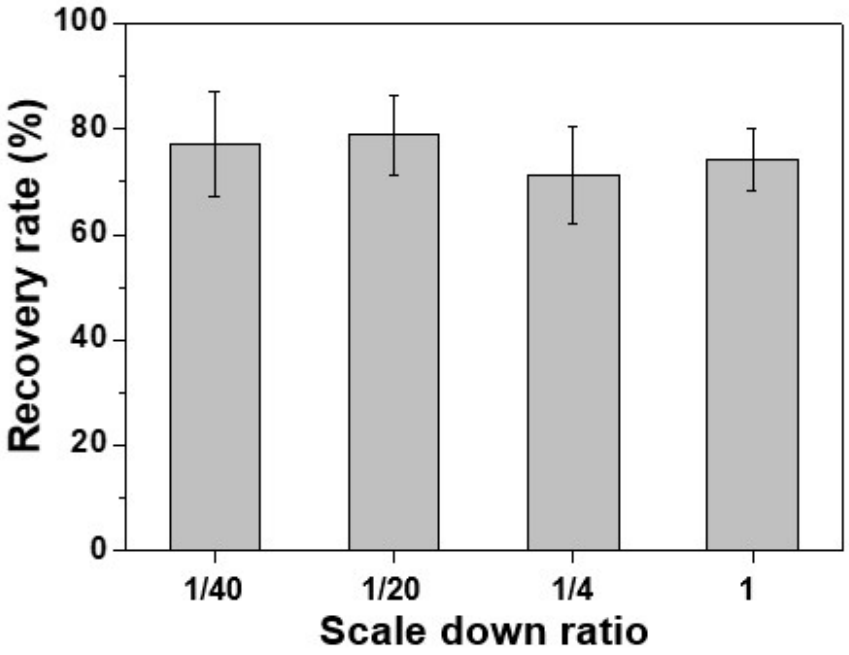

Figure S6: Optimization of sample volume for RNA extraction using the Genesig Easy® kit. Plot of RNA extraction efficiencies for repeated extractions of Zika trigger RNA at 10^5 copies/µL in serum carried out at different scale-down ratios (see Table S1 for details). Extraction efficiencies were determined by fluorometry after extraction and amplification, and error bars represent ±1 std. dev. from n = 3replicates in each condition.

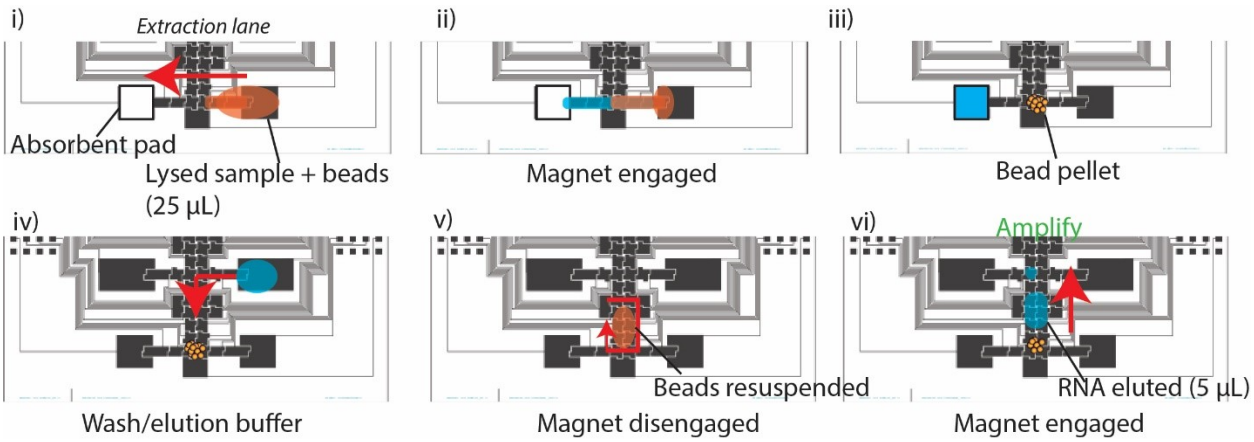

Figure S7: Schematic illustrating the P-CLIP extraction procedure. i) A mixture of sample, lysis reagents, and suspended beads (pink), is loaded into the device by activating a continuous pathway of electrodes leading into an absorbent pad (white), with the magnet engaged (ii), to pellet the beads (brown) such that the liquid phase only (blue) is wicked to the pad (iii). A droplet of wash buffer (blue) is dispensed from a reservoir with the magnet disengaged (iv) to resuspend, disperse, and wash the beads (v). Steps iv and v are repeated six times with different wash buffers, followed by elution in elution buffer (vi).

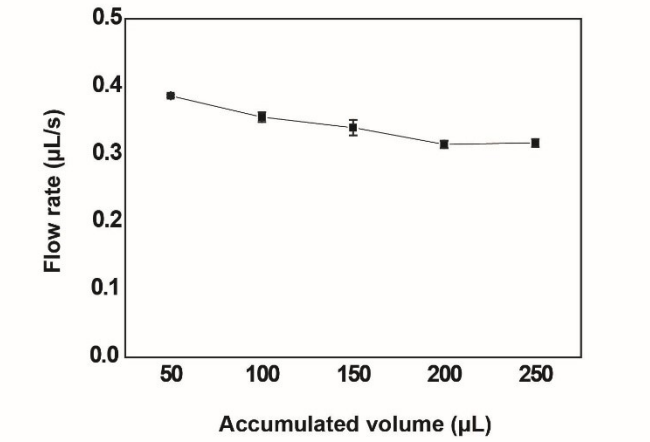

Figure S8: Plot of average flow rate as a function of sample volume measured during sample loading in P-CLIP experiments for solutions of sample and preprocessing reagents. Error bars represent ± 1 std. dev. from n = 5 replicates in each condition.

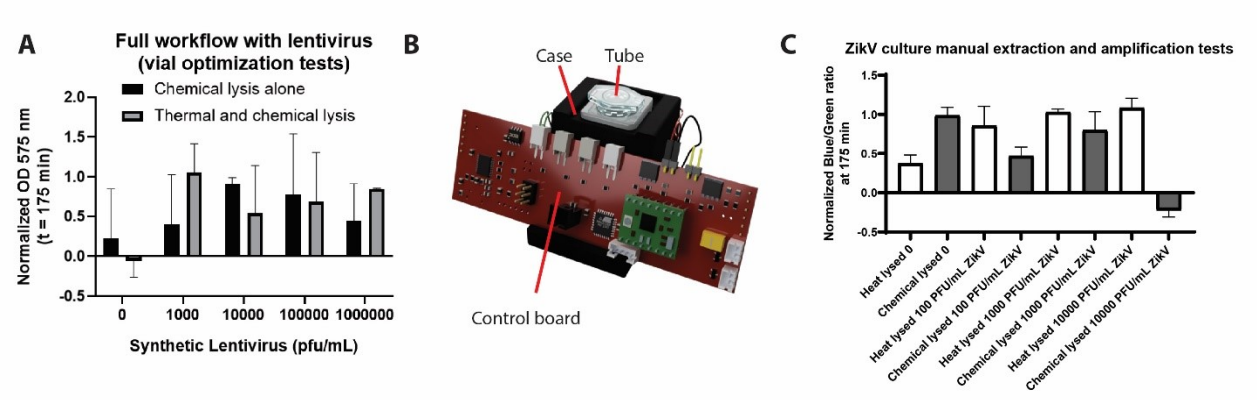

Figure S9: Workflow optimization of viral particle-containing samples.

A) Plot of normalized, end-point (t = 175 min) OD 575 nm readouts for the cell free expression assay for lentivirus samples pre-processed with (grey) or without (black) a 2 min 95° C lysis step via the P-E_{man}-A_{man}-C manual workflow. Samples were artificial lentivirus bearing Zika RNA at various plaque forming unit (PFU) densities. Error bars represent 1 std. dev. from n = 3 replicates in each condition. B) Rendered image of the ancillary tube-heater. A 3D printed case (black, PLA, Ultimaker) with dimensions 41x40x53 mm with an embedded heating element (not shown) holds a 600 µL Eppendorf centrifuge tube (gray), and is mounted to the control board (red), monitored by a laptop computer running a Jupyter Notebook script. C) Plot of normalized blue/green ratios for the cell free expression assay of Zika virus cultures at various densities in Recife, Brazil using the PLUM portable well-plate reader. Samples were pre-processed with (white) or without (grey) a 2 min 95° C lysis step using the ancillary heater shown in (B) followed by extraction and amplification via the the P-E_{man}-A_{man}-C manual workflow. Error bars represent 1 std. dev. from n = 3 replicates in each condition.

Table S1: Volumes of Genesig Easy® kit reagents tested at scale-down ratios of 1, 1/4, 1/20, and 1/40. As described in the text, the volumetric ratios of reagents to sample matched the manufacturer's instructions, except for carrier RNA, which was used at a ratio of 0.25. All volumes stated below are in units of µL.

	1/40	**1/20**	**1/4**	**1**
Sample	5.00	10.0	50.0	200
Lysis buffer	5.00	10.0	50.0	200
Proteinase K	0.50	1.00	5.00	20.0
Carrier RNA	1.25	2.50	12.5	50.0
Binding buffer	12.5	25.0	125	500
Wash buffer	12.5	25.0	125	500
80% ethanol	12.5	25.0	125	500
Elution	5.00	10.0	50.0	200

Table S2: Correlation between the number of Zika RNA PFUs, and RT-qPCR cycle threshold (Ct) values for the Zika virus preparation used in this work.

PFU	**RT-qPCR Ct value**
100,000	15.8
10,000	19.8
1,000	26.5
100	32.0
10	36.9