QuickDraw: Detecting HIV in Whole Blood using an Integrated Paper-based Consumable that Enables Direct Amplification of Purified RNA from Paper

Alexander L. Evans[1]*, Alexandra K. Sogn[2]*, Andrea C. Mora[2], Moses N. Arthur[1], Justin Leach[3], Sebastian Bosch[3], Shruthika Araselvan[1], Jeffrey W. Beard PhD[1,4], Stephen Dewhurst PhD[3], Charles R. Mace PhD[2,]** Benjamin L. Miller PhD[1,4,5,]**

[1]Department of Biomedical Engineering, University of Rochester, Rochester, NY 14627

[2]Department of Chemistry, Tufts University, Medford, MA 02155

[3]Department of Microbiology and Immunology, University of Rochester, Rochester, NY 14642

[4]Department of Dermatology, University of Rochester, Rochester, NY 14642

[5]Institute of Optics, University of Rochester, Rochester, NY 14627

** Corresponding authors: Benjamin_miller@urmc.rochester.edu, Charles.mace@tufts.edu

Table of Contents

Figure S1 – QuickDraw Layer Stack

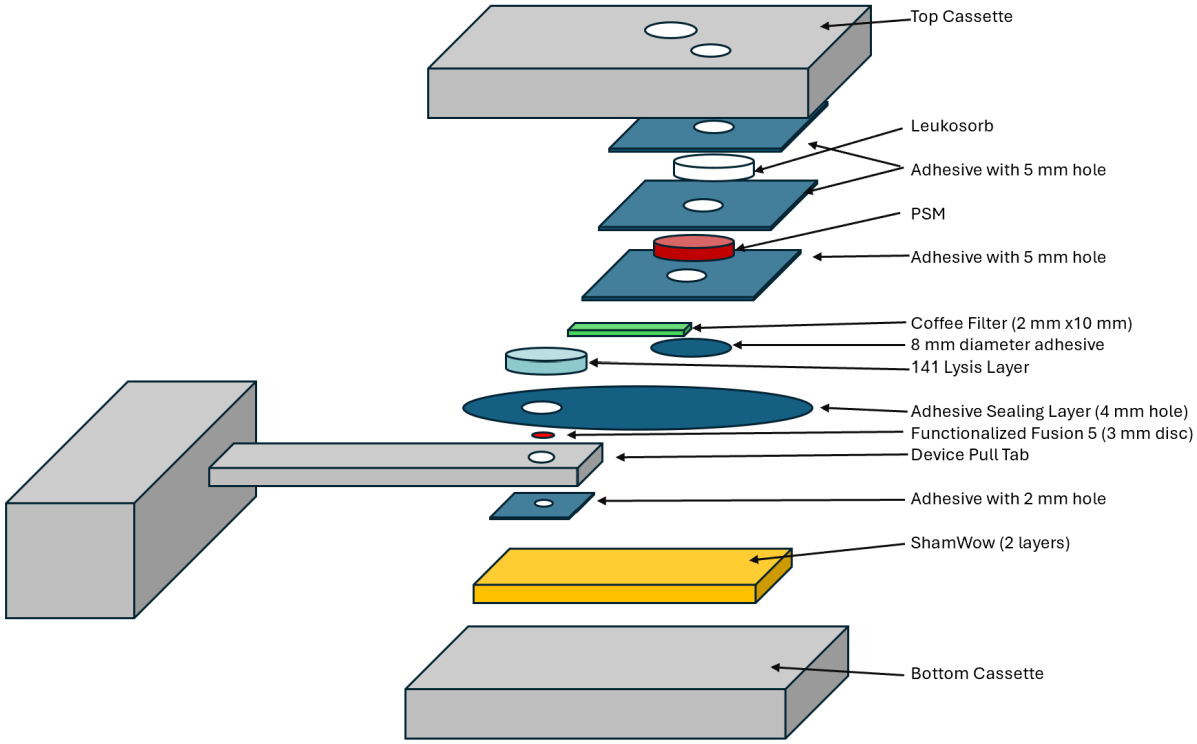


Figure S1: Detailed assembly of paper-based layers of the QuickDraw. The top cassette has a layer of adhesive placed on the bottom (interior) surface. Leukosorb is placed on that layer of adhesive and then covered with another layer of adhesive. PSM is then placed on the second layer of adhesive and covered with a third layer of adhesive. A channel made of A141 is placed on the adhesive, and the right side is covered with another piece of adhesive. The 141 layer is stuck to the adhesive sealing layer and then stuck to the rest of the top cassette. A small piece of adhesive is stuck to the bottom of the pull tab and then the FF5 is placed in the hole of the pull tab. Finally, ShamWow is placed in the bottom cassette and the 3 pieces are then stuck together using circle pillars from the top cassette into hexagonal holes in the bottom cassette.

Figure S2 – Lysis Reagent Study

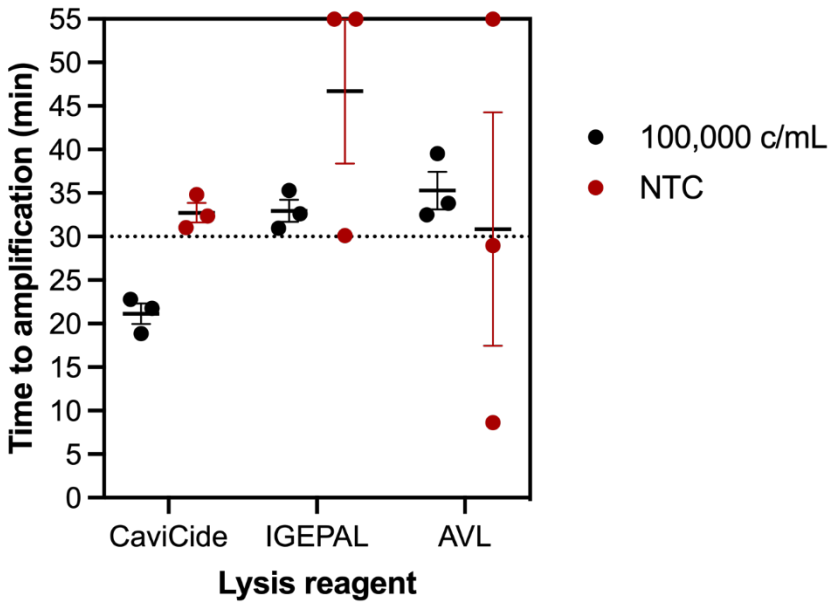


Figure S2. Results of a lysis buffer study. We tested three different lysis buffers on live HIV in human plasma: CaviCide, a common surface disinfectant, IGEPAL, a surfactant that is commonly used to solubilize membrane proteins, and AVL buffer from Qiagen, a commercial viral lysis buffer. For lysing live HIV, we found that CaviCide worked best, amplifying 100,000 RNA copies/mL in under 30 minutes, the cutoff for this assay.

Paper lysis protocol: Viral samples were diluted to 300,000 copies/mL before spiking into pooled normal human plasma. Viral samples in plasma were dried on the 141 filters for 2 hours, then treated with 60 µL molecular-grade water for 30 minutes, followed by 200 µL lysis buffer (with carrier RNA) for 15 minutes. Samples were centrifuged, washed (1X Zymo RNA shield and 0.01% Tween-20), eluted per Qiagen protocol, and analyzed by RT-qPCR. Controls included HIV+ and HIV– plasma, and buffer-only controls. PCR replicates (N=3) were performed for each condition.

Figure S3 – FF5 RNA Capture Efficiency

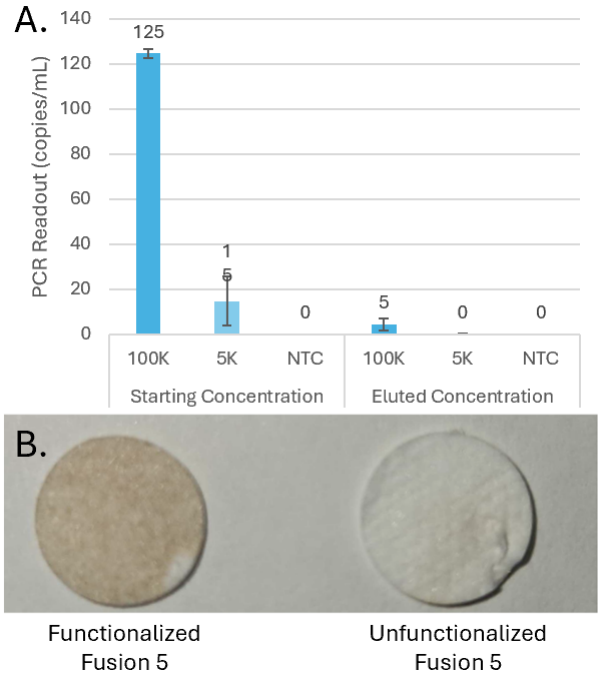


Figure S3: Paper-based ψHIV RNA capture experiments. FF5 was activated with pH4 buffer and then two different conditions of RNA (5,000 copies/mL and 100,000 copies/mL) was washed through the FF5. After capturing RNA on the FF5, PCR was performed on the eluate to show how much ψHIV RNA had been captured. A) PCR data for the capture of ψHIV RNA as seen in figure 2. B) Functionalization of F5 with chitosan confirmed via ninhydrin stain. Ninhydrin reacts with primary and secondary amines, forming a brown-purple color. As shown, FF5 turned the expected purple-brown color, while unfunctionalized F5 remained white.

FF5 Capture Protocol

Dilute RNA from PCR confirmed viral RNA stock sample to 100,000 copies/mL and 5,000 copies/mL. Assemble SpinX Columns (Corning, Corning, NY, USA) by placing an 8 mm disc of FF5 in the bottom. Add 100 µL of pH4 buffer and spin at 4000xg for 1 minute. Add 100 µL of each RNA sample and spin at 4,000 xg for 1 minute. Collect the eluate and check capture efficiency with RT-PCR.

Functionalization Test

A ninhydrin test was used to detect amino groups and confirm chitosan functionalization on FF5. A 0.3% (w/v) ninhydrin solution in 1-butanol containing 3% (v/v) glacial acetic acid solution was made and mixed until the ninhydrin had dissolved. Fusion 5 samples (functionalized or unfunctionalized) were briefly immersed in the ninhydrin solution and heated on a hot plate using tweezers for approximately 5 s, taking care to avoid burning the paper substrate.

Chitosan-functionalized FF5 turned purple upon reaction with ninhydrin, while unfunctionalized samples remained white.

Figure S4 – Schematic of Lysis and Capture of ψHIV samples

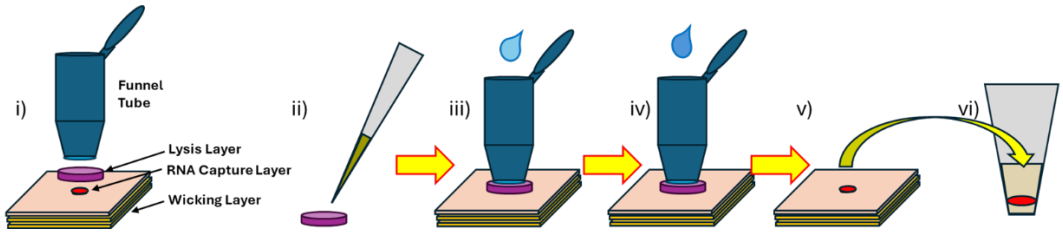


Figure S4: Cartoon schematic of experimental workflow for combined paper-based lysis and RNA capture using ψHIV. The funnel tube is just a 1.7 mL Eppendorf tube with the bottom cut off to a inner diameter of about 6 mm. Later iterations did not include the funnel tube, as just dropping wash buffers onto the lysis layer was enough to flush the RNA to the capture pad. i) The Plasma Lysis Layer (treated with Qiagen AVL), RNA Capture Layer, and wicking layers of the HIV QuickDraw were stacked together to compose a portion of the complete device. ii) 30 µL of pooled normal human plasma containing 100 to 100,000 ψHIV copies/mL was added to the Plasma Lysis Layer. iii) 0.5 mL of pH4 buffer was added and wicked through all layers via the wicking layer. iv) 0.5 mL of molecular grade water was filtered through the layers. v) The lysis layer was removed with sterilized forceps, the capture layer extracted with sterile forceps, and vi) placed in a PCR plate with LAMP reagents.

Figure S5 – Amplification of ψHIV samples with and without LAMP pH Modification

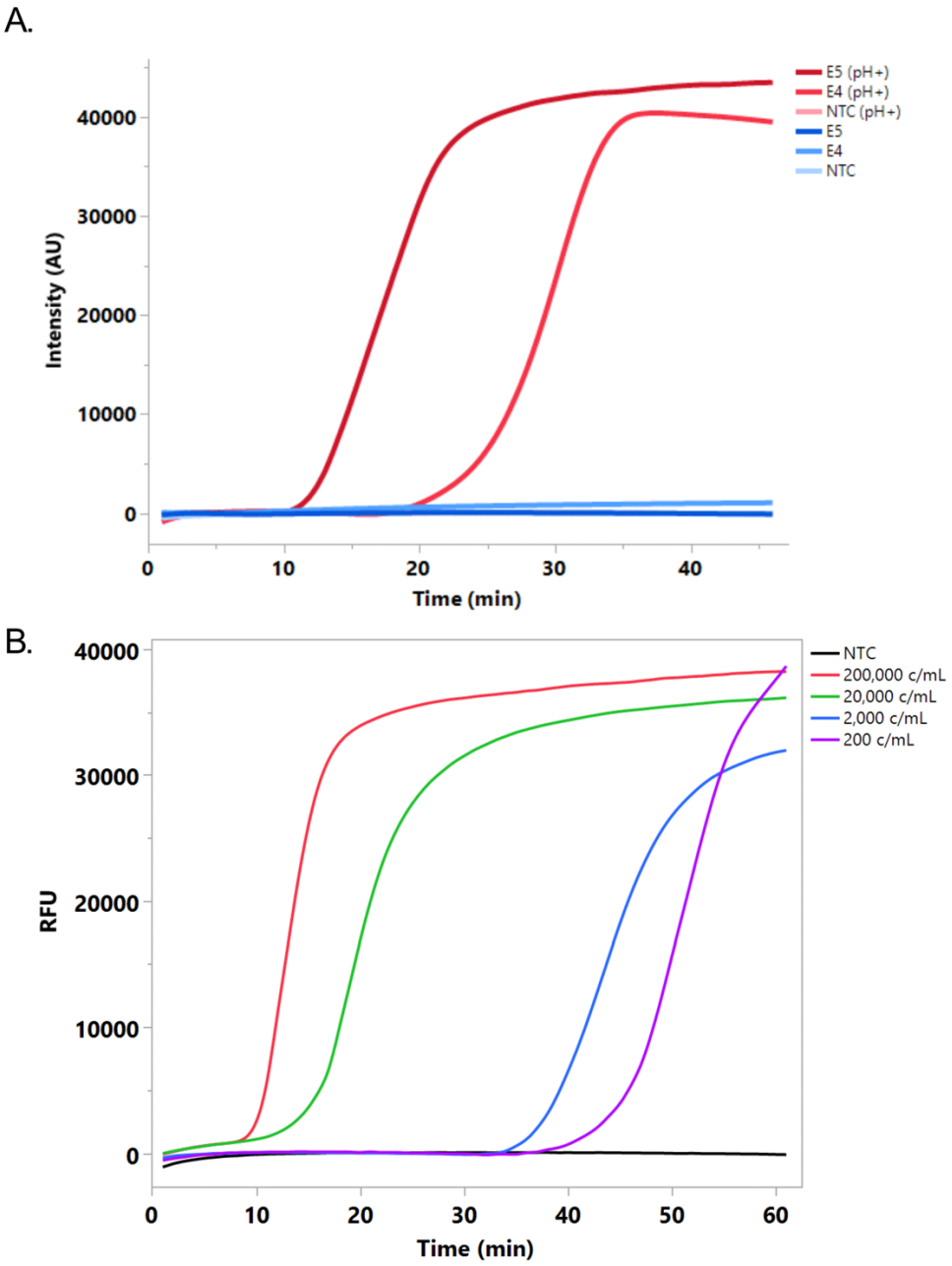


Figure S5. a) Comparison between modified LAMP buffer and unmodified LAMP buffer. The unmodified LAMP buffer did not allow for amplification of the viral RNA, likely due to the fact that the RNA was fully captured on the FF5 and the enzymes were unable to adequately the sequence. By adding the pH 8 TE buffer into the LAMP mix, the chitosan was partially de-protonated, allowing for some of the captured RNA to become free, and thereby permitting amplification to proceed. b) Using the modified LAMP buffer (Table S2), we were able to detect viral RNA from a plasma sample down to 200 copies/mL (RFU = relative fluorescence units).

Figure S6 – Real-Time LAMP Amplification of ψHIV samples

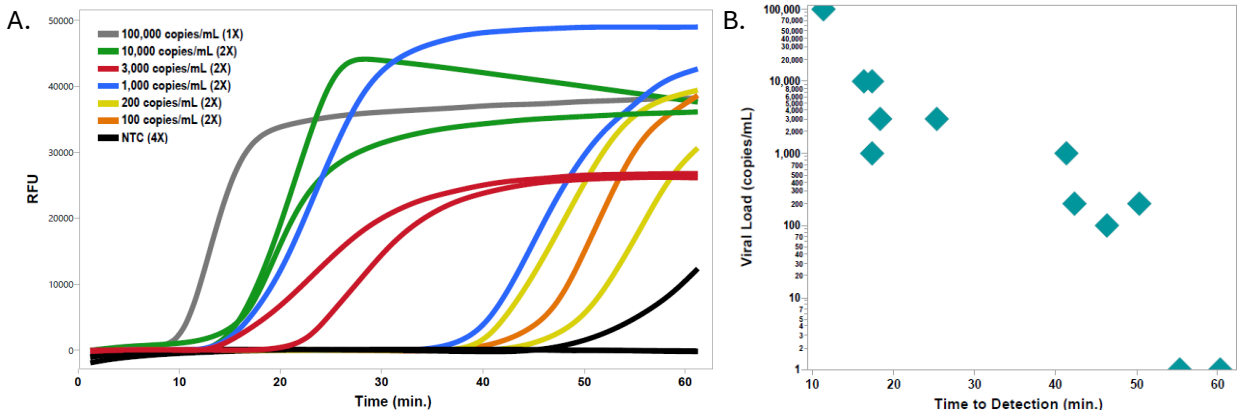
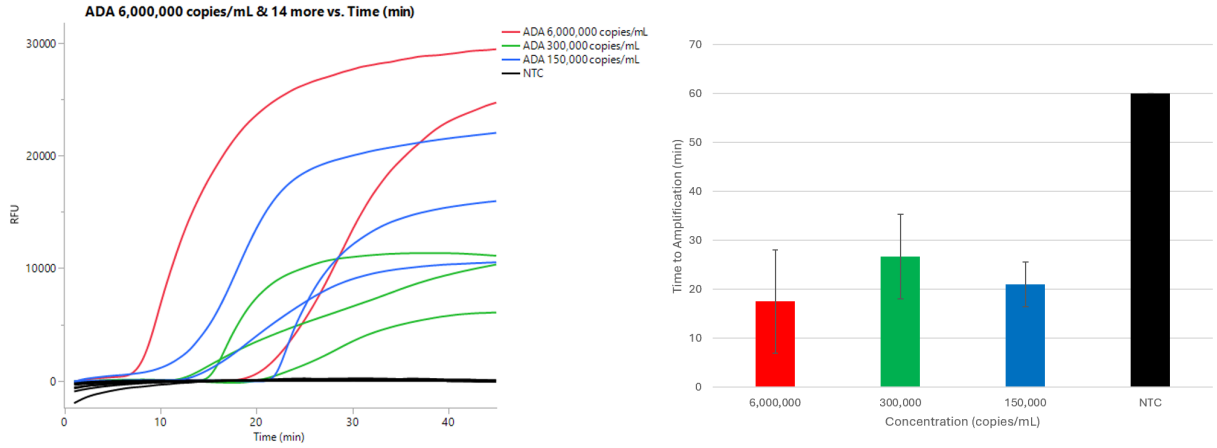


Figure S6. Real-time RT-LAMP amplification results and time to detection of experiments run using the adjusted LAMP mix. The no target controls (NTC) were processed the same as the other samples using HIV-negative plasma. The RT-LAMP reaction was conducted, and fluorescence intensity measured using a CFX Opus Real-Time PCR (BioRad). To allow RT-LAMP to occur, the thermocycler was set to 65 °C for 1 minute and a SYBR Green fluorescence reading was taken. This was repeated every minute for 60 minutes to find the optimal cutoff time. Time-to-detection was determined as the time to reach > 5,000 RFU. The light to dark shaded background shows binned viral loads by time-to-detection.

ψHIV in Plasma Lysis and Amplification Protocol

Assemble lysis and capture layer stacks as seen in Figure S4. In a biosafety cabinet, dilute ψHIV viral stock with a PCR confirmed concentration into pooled normal human plasma at concentrations of 100,000 copies/mL down to 100 copies/mL. For the no target control (NTC) use plasma. Add 30 µL of each concentration to the lysis layer. After a 3 minute lysis time, add on each lysis layer to a capture layer stack. Add 500 µL of pH4 buffer. After the pH 4 buffer is fully wicked through, add 500 µL of MGW. After the MGW has been wicked through to the ShamWow, remove the lysis layer with sterilized forceps, grab the RNA capture pad, and place it into the PCR tube with the modified LAMP mix. To allow LAMP to occur, the thermocycler was set to 65 °C for 1 minute, a fluorescence reading was taken. This process was repeated for 60 minutes.

Figure S7 – ADA HIV Amplification



	6,000,000 copies/mL	300,000 copies/mL	150,000 copies/mL	NTC
1	10	19	16	60
2	25	36	25	60
3		25	22	60
Average	17.5	26.66667	21	60
Standard Deviation	10.6066	8.621678	4.582576	0

Figure S7. QuickDraw testing results with ADA HIV. This experiment was conducted with live ADA HIV virus was doped into a whole blood sample. LAMP was run on the FF5 capture layer with the captured RNA immediately after ejection for 45 minutes at 65 °C. From these experiments we were able to show detection of 150,000 copies of viral RNA/mL. This estimate of viral load was confirmed by performing a control PCR reaction using commercially lysed and isolated RNA samples of known concentration.

Table S1 – LAMP Primers

Table S1. LAMP primers for detection of HIV-1 RNA, *gag* and *pol* regions.

LAMP Primer	Sequence
F3	AGTTCCCTTAGATAAAGACTT
B3	CCTACATACAAATCATCCATGT
FIP	GTGGAAGCACATTGTACTGATATCTTTTTGGAAGTATACTGCATTTACCAT
BIP	GGAAAGGATCACCAGCAATATTCCTCTGGATTTTGTTTTCTAAAAGGC
LF	GGTGTCTCATTGTTTATACTA
LB	GCATGACAAAAATCTTAGA

Table S2 – LAMP Mix Composition

Table S2: Making modified LAMP Mix for 1 LAMP reaction

Reagent	Volume per Reaction
WarmStart LAMP 2X Mix	12.5 uL
10X Primer Mix	2.5 uL
LAMP Dye	0.5 uL
TE buffer (pH=8.0)	4.75 uL
Molecular grade water	4.75 uL

Table S3 – LAMP Primer Example Dilution

Table S3: Example dilution of LAMP Primers to make 10X Primer mix for LAMP reactions

Primer Name	Initial Concentration for Dilution (µM)	Concentration of primer needed for 10X mix (µM)	Volume needed to prep. 10X mix (µL)
			add to 10X tube
F3	100	2	30
B3	100	2	30
FIP	100	16	240
BIP	100	16	240
LF	100	4	60
LB	100	4	60
		MGW	840
		Total	1500

Table S4 – HIV PCR Primers

Table S4. PCR primers for detection of HIV-1 RNA.

PCR Primer	Sequence
HIV Forward	TACTGACGCTCTCGCACC
HIV Reverse	TCTCGACGCAGGACTCG

Movie S1 – QuickDraw Video

Movie S1: QuickDraw process and features animation (Credit: Erik Patak, Erik Patak Creative)

Movie S2 – Lab Demonstration of QuickDraw

Movie S2: Video showing the full QuickDraw Process. i) add blood sample, ii) add pH4 buffer, iii) add molecular grade water, iv) pull RNA capture layer out from beneath the lysis layer, v) extract

RNA into LAMP tube, vi) add LAMP mix, vii) Perform LAMP in a thermocycler, viii) add color indicator dye (yellow-green if positive, orange if negative).