Compact Point-of-Care Device for Self-Administered HIV Viral Load Tests from Whole Blood

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Disposable plasma separation card

The design of this disposable plasma separation card was executed using AutoCAD software. Each layer of the card was meticulously patterned using a CO_2 laser cutting machine (Universal Laser Systems). Following this, all the patterned layers were aligned with exceptional precision and laminated with double-sided adhesive tape. This procedure ensured a consistent and robust assembly of the plasma separation card apparatus. By combining innovative design with precise construction techniques, we were able to manufacture an effective and reliable module, paving the way for convenient, one-time-use plasma separation.

Syringe-based RNA extraction module

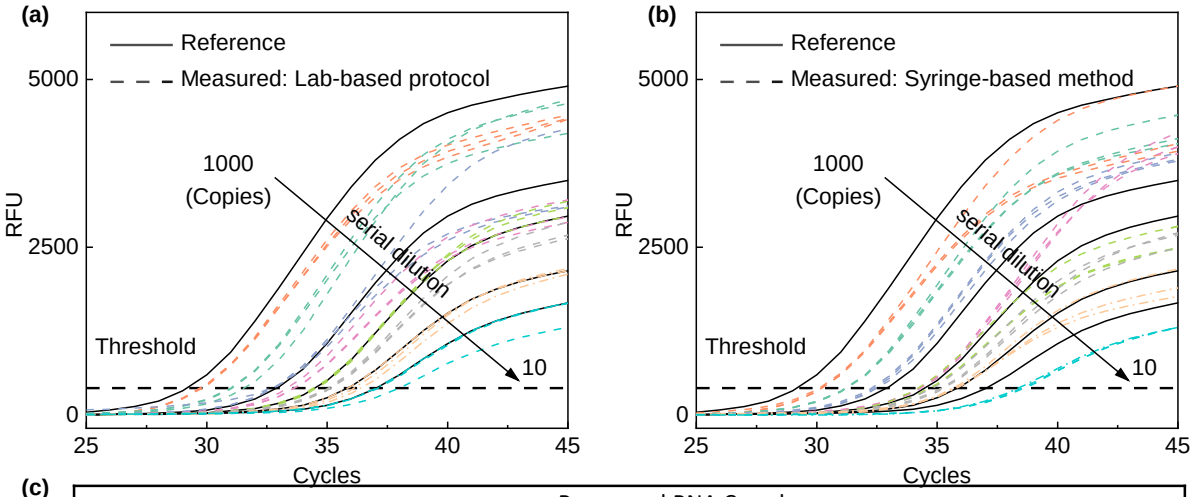
The design of a syringe-based RNA extraction module can be separated into three parts. Firstly, a silica membrane filter was designed to bind to RNA effectively at a high salt concentration. The QIAamp Viral RNA Kits Total RNA (52904) were modified by cutting and retrofitting the silica column. Quick-Turn Tube Couplings were connected to the modified column, and a sealing adhesive was used to secure and seal the connections, effectively transforming it into a silica membrane filter. Secondly, flexible Tygon PVC tubes (procured from McMaster) were employed to store the washing and elution buffers. Each segment of the reagent was separated by a custom-made manual valve. Each tube was equipped with a lure lock at its end to facilitate a quick and secure connection to a syringe. This feature accelerated the overall extraction process and minimized the risk of leaks or contamination. Finally, two syringes were used to generate a negative pressure, enabling the reagent in the flexible tubes to pass through the silica membrane filter. One syringe was dedicated to collecting the washing buffer, while the other was assigned to collect the eluted RNA. These syringes were procured from VWR.

Analyzer design and fabrication

The entire architecture of the analyzer was conceived and developed using PTC Creo software, while the containment unit for the device underwent fabrication via three-dimensional printing, utilizing METHOD X on a MakerBot system. The Printed Circuit Board (PCB) was intricately designed with Autodesk Eagle software, with manufacturing carried out by OSH Park. Laboratory

personnel manually soldered the electronic constituents and Microcontroller Unit (MCU) onto the PCB. For heating regulation, a resistive-heating element (PWR263S-20-2R00J, Digi-Key) was adjoined to the reverse side of a specially tailored aluminum heating block through the application of thermal compound (AATA-5G, Artic Alumina). In the core of the heating plate, a thermistor (95C0606, Digi-Key) was carefully positioned for the continuous surveillance of temperature. To sustain the requisite temperature during Nucleic Acid Testing (NAT), negative thermal feedback control was executed by deploying an N-channel power Metal-Oxide-Semiconductor Field-Effect Transistor (MOSFET) (63J7707, Digi-Key). Our optical components comprised an adjustable Res Cermet Trimmer (3296W, Digi-Key) for LED intensity control, an IC Color Sensor (AS7341, Digi-Key) for optical detection, and a blue SMD LED (1497-1138-1, Digi-Key) for fluorescence excitation. Power stabilization for the optical components was provided by the capacitors, and a voltage regulator supplied the necessary power. An I_2C Multiplexer was used as the I_2C expander for the sensor. The control graphical user interface (GUI) software was developed using the MIT App Inventor. This GUI facilitates easy control and communication with the device via Bluetooth, offering a user-friendly control interface for the instrument.

Supplementary Figures



(c)

Recovered RNA Cq value										
RNA (Copies)	Lab-based protocol					Syringe-based method				
	Run 1	Run 2	Run 3	Avg.	Std.	Run 1	Run 2	Run 3	Avg.	Std.
20	38.29	37.38	37.30	37.66	0.45	37.84	37.75	37.60	37.73	0.10
40	36.35	36.66	36.11	36.37	0.22	36.67	36.45	36.66	36.59	0.10
60	35.26	35.37	35.34	35.32	0.05	36.01	36.09	35.56	35.89	0.23
80	34.72	34.66	34.81	34.73	0.06	35.46	34.97	35.48	35.31	0.23
100	34.38	34.40	34.08	34.29	0.15	34.49	34.56	34.50	34.51	0.03
200	33.03	33.02	33.01	33.02	0.01	33.02	33.23	33.11	33.12	0.08
400	32.26	31.73	31.68	31.89	0.26	31.89	31.94	31.97	31.94	0.03
1000	30.09	30.11	30.16	30.12	0.03	30.32	30.04	30.16	30.17	0.12

(d)

Recovered RNA copy number (Copies)										
RNA (Copies)	Lab-based protocol					Syringe-based method				
	Run 1	Run 2	Run 3	Avg.	Std.	Run 1	Run 2	Run 3	Avg.	Std.
20	5.29	9.39	9.89	8.19	2.06	7.05	7.46	8.19	7.57	0.47
40	17.95	14.78	20.84	17.86	2.47	14.66	16.88	14.72	15.42	1.03
60	35.53	33.09	33.89	34.17	1.01	22.21	21.11	29.40	24.24	3.68
80	49.78	51.78	47.24	49.60	1.86	31.35	42.50	30.92	34.92	5.36
100	61.56	60.93	74.72	65.74	6.36	57.63	55.17	57.42	56.74	1.11
200	143.79	145.06	146.12	144.99	0.95	144.85	127.18	137.28	136.44	7.24
400	233.25	325.84	335.62	298.24	46.13	293.98	284.96	280.69	286.54	5.54
1000	914.84	901.52	873.66	896.67	17.16	788.07	944.60	871.73	868.13	63.95

Figure S1. Characterization of the syringe-based RNA extraction module. **(a)** Data from real-time HIV-1 RT-qPCR amplification is presented. The solid line represents the reference reaction with known copy numbers, while the dashed line signifies RNA extracted using the lab-based protocol. Each concentration was tested in triplicate. **(b)** Similarly, data from real-time HIV-1 RT-qPCR amplification is shown. The solid line depicts the reference reaction with known copy numbers, and the dashed line illustrates RNA extracted using the syringe-based method. Each concentration was also tested in triplicate. **(c)** The CT value corresponds to the extraction from plasma samples containing HIV-1 RNA with varying copy numbers using two distinct methods. Mean and variance values are also reported. **(d)** Recovered RNA copy numbers were determined from the extraction of plasma samples containing different copy numbers of HIV-1 RNA using two different methods. Mean and variance values are likewise provided.

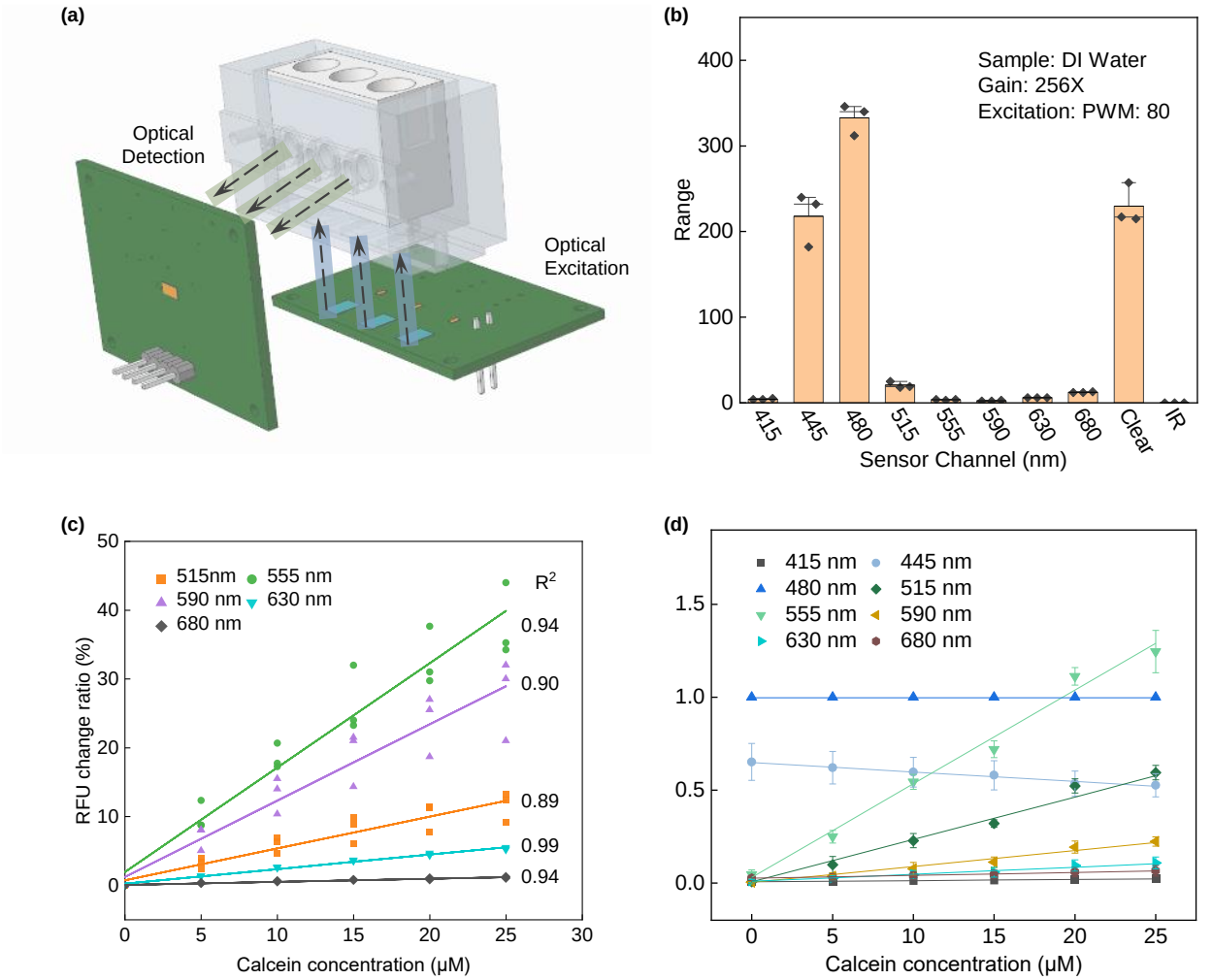


Figure S2. (a) Schematic of the Optical Module. On the excitation side, an LED light source (λ = 488 nm) illuminates the LAMP reaction chamber. On the detection side, light emitted from the LAMP reaction chamber to the optical senso. The excitation LED light is oriented perpendicularly to the optical sensor to minimize diffracted excitation light entry, thereby enhancing the signal-to-noise ratio. **(b)** Response of each channel in the optical module's sensor to water. The test was conducted at a gain of 256X, an integration time of 154 ms, and an 80% PWM of the excitation LED control. **(c)** Characterization of the optical sensor with Calcein. Each channel of the optical sensor exhibited a linear response to Calcein concentrations ranging from 0 to 25 μM. The test was carried out at a gain of 256X, an integration time of 154 ms, and an 80% PWM of the excitation LED control. **(d)** The optical module's profile when each channel's data from the color sensor is normalized with the channel (480nm) that corresponds to the LED light source (λ = 488 nm).

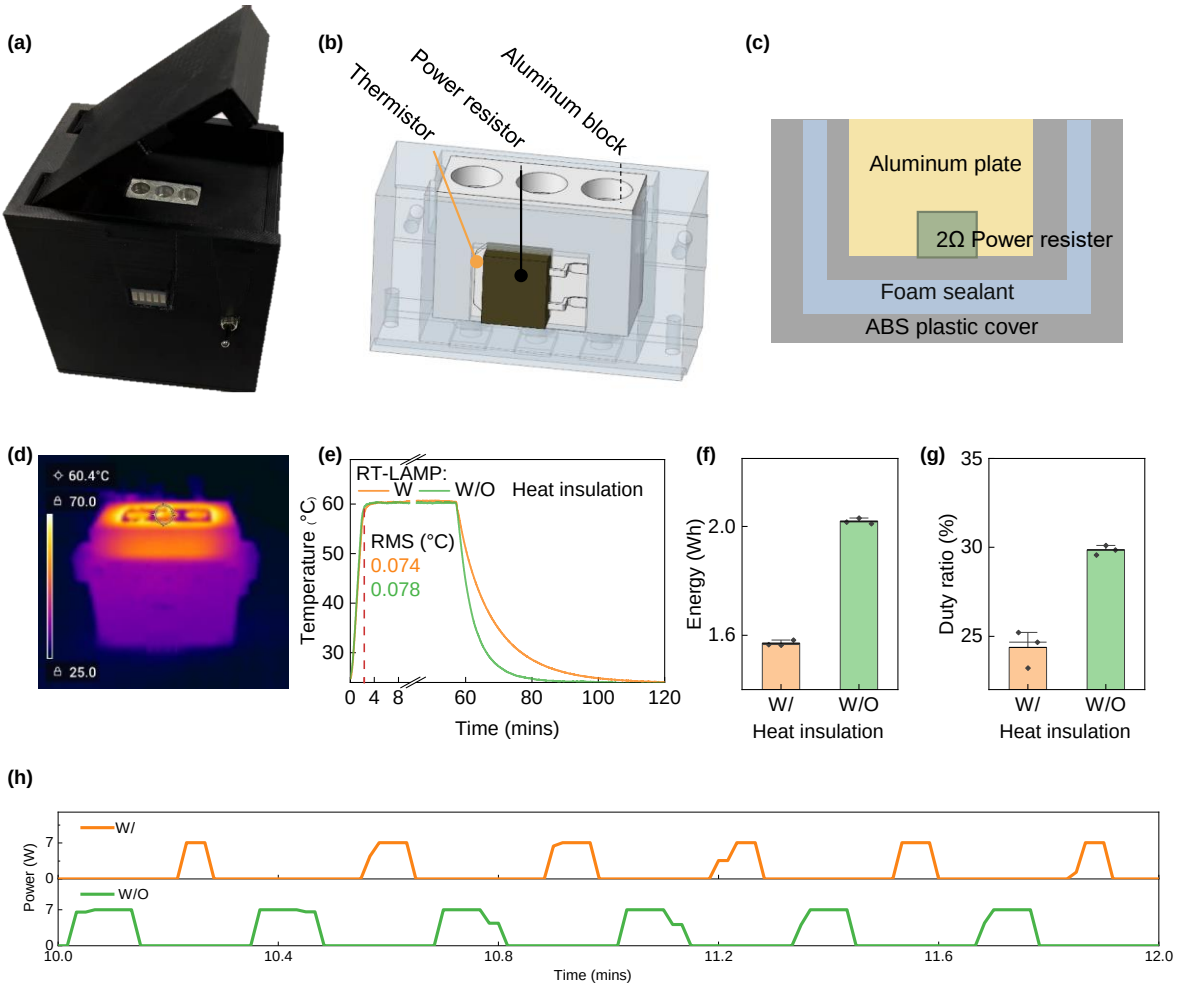


Figure S3. (a) Photographic depiction of the analyzer, illustrating the process of installing the aluminum block into the heating insulation case and the analyzer. **(b)** The thermal module comprises a power resistor (as a heating source), a thermistor (as a temperature feedback sensor), a CNC aluminum plate, and a housing for heat insulation to conserve energy. **(c)** The inner and outer sides of the thermal insulation module are made of 3D-printed ABS material, with foam sealant filling the middle layer to boost thermal insulation performance. **(d)** Thermal image of the heating module. (The core temperature of the AI plate is 60 ℃, and the exterior temperature of the insulation layer is around 30 ℃, thus minimizing heat loss.) **(e)** Temperature change curve for RT-LAMP, including the heating up, incubation, and natural cooling stages. The time required for the reaction solution to heat up remains consistent, regardless of the presence of the thermal insulation module. **(f)** Utilizing thermal insulation modules can decrease energy consumption during the heating and incubation phases by approximately 22%. **(g)** With the thermal insulation module, the duty cycle of the heating resistor during the heating and incubation stages is reduced by 16%. **(h)** The on/off operational profile of heating resistors during incubation for 2 minutes after the temperature remains stable at 60 ℃.

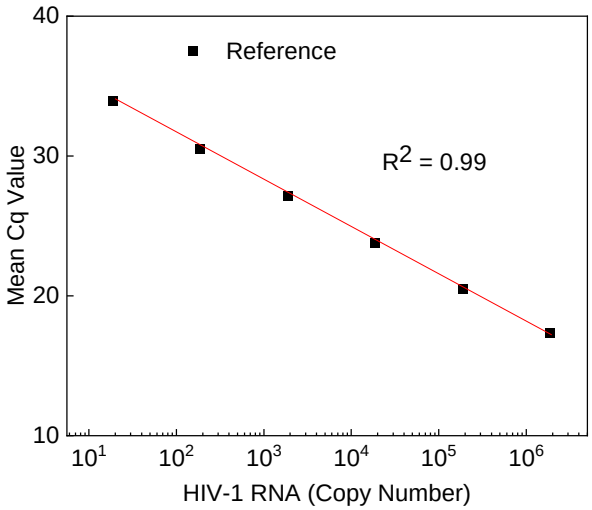


Figure S4. The Cq values obtained from the RT-PCR versus the target RNA copies. Cq values showed a logarithmic relationship with the sample concentration and verified our assay.

Supplementary Tables

Table S1. RT-LAMP reaction setup

Component	Volume (µL)/test	Final Concentration
Lyo-Ready RT-LAMP master mix	6.25	4 X
F3	0.05	0.2 µM
B3	0.05	0.2 µM
FIP	0.4	1.6 µM
BIP	0.4	1.6 µM
LF	0.2	0.8 µM
LB	10.2	0.8 µM
$MgSO_4$	1	4 mM
Calcein	0.63	25 µM
$MnCl_2$	1.88	75 µM
RNA sample	1	1 µL/rxn
PCR Grade Water	12.95	-

Table S2. RT-LAMP primer set targeting HIV-1

Primer	Sequence (5' → 3')
F3	AGTTCCCTTAGATAAAGACTT
B3	CCTACATACAAATCATCCATGT
FIP	GTGGAAGCACATTGTACTGATATCTTTTTGGAAGTATACTGCATTTACCAT
BIP	GGAAAGGATCACCAGCAATATTCCTCTGGATTTTGTTTTCTAAAAGGC
Loop F	GGTGTCTCATTGTTTATACTA
Loop B	GCATGACAAAAATCTTAGA

Table S3. Summary of the hit rate

HIV-1 RNA (copy)	1	2	4	8	16	32	64	128	256	512	1024	10^4	10^5
No. positive/ no. of replicates	0/6	1/6	3/6	4/6	6/6	6/6	6/6	6/6	6/6	6/6	6/6	6/6	6/6
Hit rate (%)	0	16.7	50	66.7	100	100	100	100	100	100	100	100	100

All six reactions with four or more copies of RNAs can be amplified successfully. The copy number sensitivity of the HIV-1 lyophilized RT-LAMP was determined to be 16 copies.

Table S4. RT-qPCR primer set targeting HIV-1

Primer	Sequence (5' → 3')
Forward	CATGTTTTCAGCATTATCAGAAGGA
Reverse	TGCTTGATGTCCCCCCACT
Probe	FAM-CCACCCCACAAGATTTAAACACCATGCT

Supplementary Table S5. Bill of materials of the analyzer

System	Description	Part#	Function	Unit Cost ($)	Unit Qty.	Ext. Cost ($)
Electronics	Arduino Nano	7630049200173	Microcontroller	6.00	1	6.00
Electronics	Adafruit Bluefruit BLE	2633	Bluetooth Low	14.00	1	14.00
Electronics	Capacitor 10µF	P10425CT	Power stabilizing	0.147	1	0.15
Electronics	Power Jack/Connector	PRT-00119	Power Connector	1.25	1	1.25
Electronics	36-pin male header	392	Headpins	4.95	0.1	0.50
Thermal	Aluminum CNC block	CP-0.91-0.91	Heating Stage	5.75	0.25	1.44
Thermal	2Ω Power Resistor	MP725-2.00-FCT	Heater	7.01	1	7.01
Thermal	SMD Resistor, 1kΩ	311-1.00KHRCT	Tem. control	0.0072	1	0.01
Thermal	Thermistor	235-1057	Tem. sensing	3.5	1	3.50
Thermal	SMD Resistor, 10kΩ	RNCP0603FTD10K0CT	Tem. control	0.1	1	0.10
Thermal	Power MOSFET	DMN1019USN-7DICT	Switch for heater	0.354	1	0.35
Optics	Res Cermet Trimmer 500K ohm	3296W-1-501RLF	Adjust LED intensity	0.6	3	1.80
Optics	SMD Resistor, 1kΩ	RNCP0805FTD1K0CT	Optical module	0.0277	3	0.08
Optics	SMD Resistor, 10kΩ	RNCP0805FTD10K0CT	Optical module	0.0277	8	0.22
Optics	IC Color Sensor	AS7341	Optical detection	3.00	3	9.00
Optics	SMD LED, Blue	1497-1138-1	Fluorescence excitation	0.528	3	1.58
Optics	Capacitor 10µF	P10425CT	Power stabilizing	0.147	3	0.44
Optics	Capacitor 0.1µF	P01425CT	Power stabilizing	0.147	1	0.15
Optics	Voltage Regulator	AP7312-3.3/1.8	Power supplier	0.8	1	0.80
Optics	I_2C Multiplexer	TCA9548A	I_2C expender	1.10	1	1.10
Enclosure	ABS Filament (Black)	-	3D material	69.00	0.05	3.45
Enclosure	Screws (M2 cap screw)	91290A015	Assembly	0.14	10	1.40
			Total Cost			**$54.33**

Table S6. Cost per test

Reagent cost /test

Reagents	Vendor	Function	Stock Vol (mL)	Unit Cost ($)	Volume (μL)/rxn	Ext Cost/rxn
RT-LAMP Mixes	Meridian Life Science	LAMP master mix	5	198	6.25	0.2475
F3	IDT	LAMP master mix	4.8	9.4	0.15	0.0003
B3	IDT	LAMP master mix	4.3	8.46	0.15	0.0003
FIP	IDT	LAMP master mix	0.9	62.68	1.2	0.0836
BIP	IDT	LAMP master mix	0.9	64.56	1.2	0.0861
LF	IDT	LAMP master mix	4.6	9.4	0.6	0.0012
LB	IDT	LAMP master mix	4.3	75.34	0.6	0.0105
Calcein	Sigma-Aldrich	LAMP master mix	1.5	24.25	2.4	0.0388
$MnCl_2$	Sigma-Aldrich	LAMP master mix	3.2	107	2.1	0.0702
$MgSO_4$	NEB	LAMP master mix	6	20	1.2	0.004
Lysis Buffer	Invitrogen	Sample Prep.	25	6	200	0.02
Wash Buffer	Invitrogen	Sample Prep.	37.5	54	500	0.12
Elution Buffer	Invitrogen	Sample Prep.	11	35	30	0.1
Ethanol	Sigma-Aldrich	Sample Prep.	100	10	200	0.02
					Total	**$0.8025**

Cartridge cost /test

Material	Vendor	Function	Stock Vol (sq. m.)	Unit Cost ($)	Volume (sq.mm)/rxn	Ext Cost/rxn
1/32" Acrylic Sheet	ePlastics	PSM card	0.5	16.13	20	0.65
Vivid PSM-GR	Pall Corporation	PSM card	0.06	30.00	200	0.30
Tygon PVC Tubing	McMaster	RNA extraction	25 ft	11.50	1 ft	0.46
Quick-Turn Coupling	McMaster	RNA extraction		0.50	3	1.50
Silica column	Qiagen	RNA extraction		2.00	1	2.00
Syringe	VWR	RNA extraction		0.30	2	0.60
PCR tube		Fisher scientific		0.01	1	0.01
					Total	**$5.52**