Rapid Simultaneous Self-Testing of HIV and HCV Viral Loads with Integrated RNA Extraction and Multiplex RT-PCR in Under 1 Hour

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Statistical analysis

The statistical analysis and regression modeling presented in this report have been conducted with the help of MATLAB R2020 software from Natick, MA. Additionally, almost all data visualization, including the development of the figures and plots, has been completed with the use of MATLAB as a primary tool. Microsoft PowerPoint has been used to adjust the layout of the materials and bring the final product into its current format. It should be noted that the mean has been used to represent every plot otherwise specified, and when provided, a plot shows three standard deviations. These measures have been collected from three similar tests and used as triplicates to avoid any uncertainty. A similar principle has been applied under all conditions to ensure the most precise statistical comparison. Overall, the visualization of the final results would not be possible without MATLAB, as this platform has been used not only for the initial data but also for every additional stage of the development, and therefore, the steps could be viewed as necessary parts of a single process.

Supplementary Tables

Table S1. Cost per test

Reagents	Vendor	Function	Stock Vol (mL)	Unit Cost ($)	Volume (μL)/rxn	Ext Cost/rxn
RT-LAMP Mixes	Meridian Life Science	LAMP master mix	5	198	6.25	0.2475
HIV-1 Forward	IDT	PCR master mix	0.856	12.05	0.4	0.00563
HIV-1 Reverse	IDT	PCR master mix	0.67	9.15	0.4	0.00546
HIV-1 Probe	IDT	PCR master mix	0.185	381	0.4	0.82378
HCV Forward	IDT	PCR master mix	0.697	9.64	0.4	0.00553
HCV Reverse	IDT	PCR master mix	0.87	10.60	0.4	0.00487
HCV Probe	IDT	PCR master mix	0.201	311	0.4	0.61891
RnaseP Forward	IDT	PCR master mix	0.763	9.15	0.4	0.0048
RnaseP Reverse	IDT	PCR master mix	0.68	9.64	0.4	0.00567
RnaseP Probe	IDT	PCR master mix	0.231	284	0.4	0.49177
Lysis Buffer	Invitrogen	Sample Prep.	25	6	200	0.02
Wash Buffer	Invitrogen	Sample Prep.	37.5	54	500	0.12
Elution Buffer	Invitrogen	Sample Prep.	11	35	30	0.1
Ethanol	Sigma-Aldrich	Sample Prep.	100	10	200	0.02
Material	**Vendor**	**Function**	**Stock Vol (sq. m.)**	**Unit Cost ($)**	**Volume (sq. mm)/rxn**	**Ext Cost/rxn**
Vivid PSM-GR	Pall Corporation	PSM card	0.06	30.00	200	0.30
Silica column	Qiagen	RNA extraction		2.00	1	2.00
PCR tube		Fisher scientific		0.01	1	0.01
					Total	**$4.28**

Supplementary Table S2. 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 LE SPI	2633	Bluetooth Low Energy	14.00	1	14.00
Electronics	Capacitor 10uF	P10425CT	Power stabilizing	0.147	1	0.15
Electronics	DC Barrel Power Jack/Connector	PRT-00119	Power Connector	1.25	1	1.25
Electronics	36-pin stripe 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	Temperature/EM control	0.0072	1	0.01
Thermal	Thermistor	235-1057	Temperature sensing	3.5	1	3.50
Thermal	SMD Resistor, 10kΩ	RNCP0603FTD10K0CT	Temperature control	0.1	1	0.10
Thermal	N Channel Power MOSFET	DMN1019USN-7DICT	Switch for heater and fan	0.354	2	0.70
Thermal	FAN AXIAL 40X15MM 12VDC	603-1006-ND	Fan for colling	8.86	1	8.86
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 10uF	P10425CT	Power stabilizing	0.147	3	0.44
Optics	Capacitor 0.1uF	P01425CT	Power stabilizing	0.147	1	0.15
Optics	Voltage Regulator	AP7312-3.3/1.8	Power supplier	0.8	1	0.80
Optics	I2C Multiplexer	TCA9548A	I2C expender for sensor	1.10	1	1.10
Enclosure	ABS Filament (Black)	-	3D platform material	69.00	0.05	3.45
Enclosure	Screws (M2 cap screw)	91290A015	Assembly	0.14	10	1.40
			Total Cost			**$63.54**

Table S3. RT-qPCR primer set targeting HIV-1

Primer	Sequence (5’ → 3’)
Forward	TACTGACGCTCTCGCACC
Reverse	TCTCGACGCAGGACTCG
Probe	ATTO425-CTCTCTCCTTCTAGCCTC

Table S4. RT-qPCR primer set targeting HCV

Primer	Sequence (5’ → 3’)
Forward	GCAGAAAGCGTCTAGCCATG
Reverse	CGCAAGCACCCTATCAGG
Probe	HEX-CATAGTGGTCTGCGGAACCG

Table S5. RT-qPCR primer set targeting Rnase P

Primer	Sequence (5' → 3')
Forward	AGATTTGGACCTGCGAGCG
Reverse	GAGCGGCTGTCTCCACAAGT
Probe	Cy5-TTCTGACCTGAAGGCTCTGCGCG

Table S6. Reverse-transcriptase sub-region targeting HIV-1

Sequence (5' → 3')
tctcgacgcaggactcggcttgctgaagcgcgcacggcaagaggcgaggggcggcgactggtgagtacgccaaaaattttgactagcggaggct agaaggagagagatgggtgcgagagcgtcagta

Table S7. Reverse-transcriptase sub-region targeting HCV

Sequence (5' → 3')
gcagaaagcgtctagccatggcgttagtatgagtgtcgtgcagcctccaggacccccccctcccgggagagccatagtggtctgcg gaaccggtgagtacaccggaattgccaggacgaccgggtcctttcttggataaaacccgctcaatgcctggagatttgggcgtgcc cccgcaagactgctagccgagtagtgttgggtcgcgaaaggccttgtggtactgcctgatagggtgcttgcg

T for RT = 5 min, T for qPCR Activation =3 min

T for denaturation / extension	HIV - 1	HCV	RNase P
3s / 3s	3/3	3/3	3/3
2s / 2s	3/3	3/3	3/3
* 1s / 1s *	3/3	3/3	3/3

T for qPCR Activation =3 min, T for denaturation / extension = 1s / 1s

T for RT	HIV - 1	HCV	RNase P
4 min	3/3	3/3	3/3
* 3 min *	3/3	3/3	3/3
2 min	3/3	0/3	3/3

T for RT = 2 min, T for denaturation / extension = 1s / 1s

T for qPCR Activation	HIV - 1	HCV	RNase P
2 min	3/3	3/3	3/3
* 1 min *	3/3	3/3	3/3
30 s	3/3	0/3	3/3

Table S8. This table summarizes experimental results across three parameter sets: Set 1 varies denaturation/extension times; Set 2 varies RT time; Set 3 varies qPCR activation time, with other conditions held constant in each set. It shows detection successes and failures for HIV-1, HCV, and RNase P, identifying minimal operational standards. Highlighted results indicate optimal conditions of 3 min RT, 1 min qPCR activation, and 1s/1s denaturation/extension times for rapid and accurate PCR diagnostics.