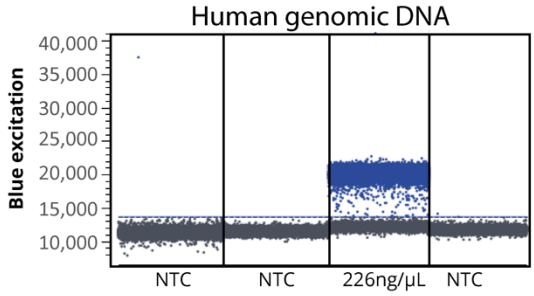
Supplementary Information list:

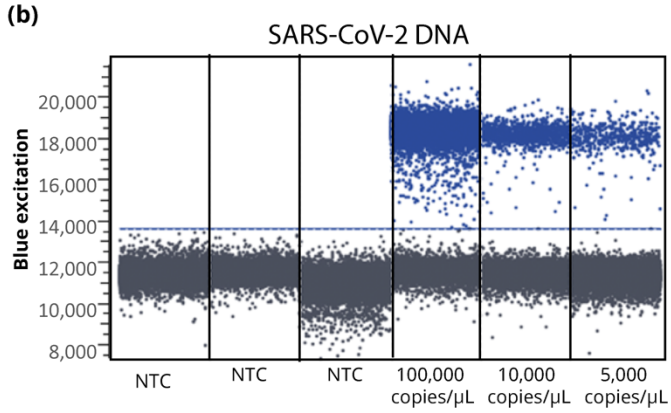
Supplementary information

(a)



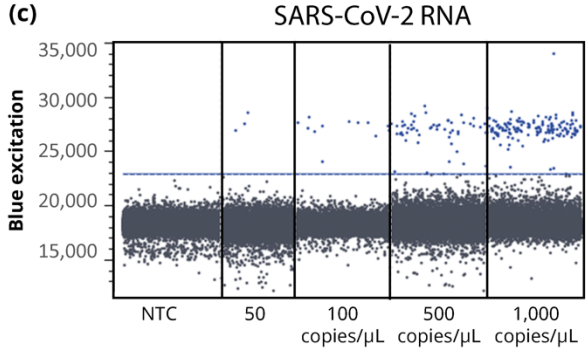
Human genomic DNA concentration	Theoretical total number (copies/µL)	Copies estimated by cdPCR in rxn (copies/µL)	% Estimated by cDPCR
226 ng/uL	77038.9	70450	91.4

(b)



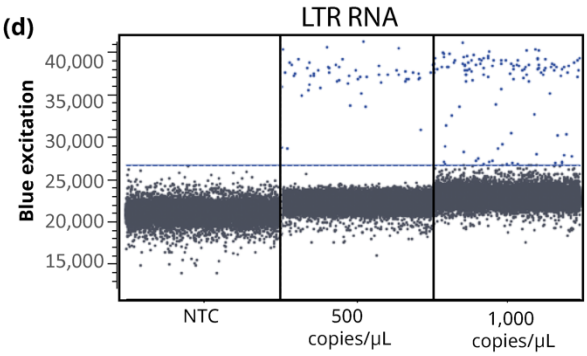
Concentration estimated by mass (copies/µL)	Copies estimated by cdPCR in rxn (copies/µL)	% Estimated by cdPCR
100,000	29725	29% (poor-quality image)
10,000	2172	21%
5,000	877	18%

(c)



Concentration estimated by mass (copies/µL)	Copies estimated by cdPCR in rxn (copies/µL)	% Estimated by cdPCR
50	9	18
100	20.5	20.5
500	90.25	18
1,000	260	26

(d)

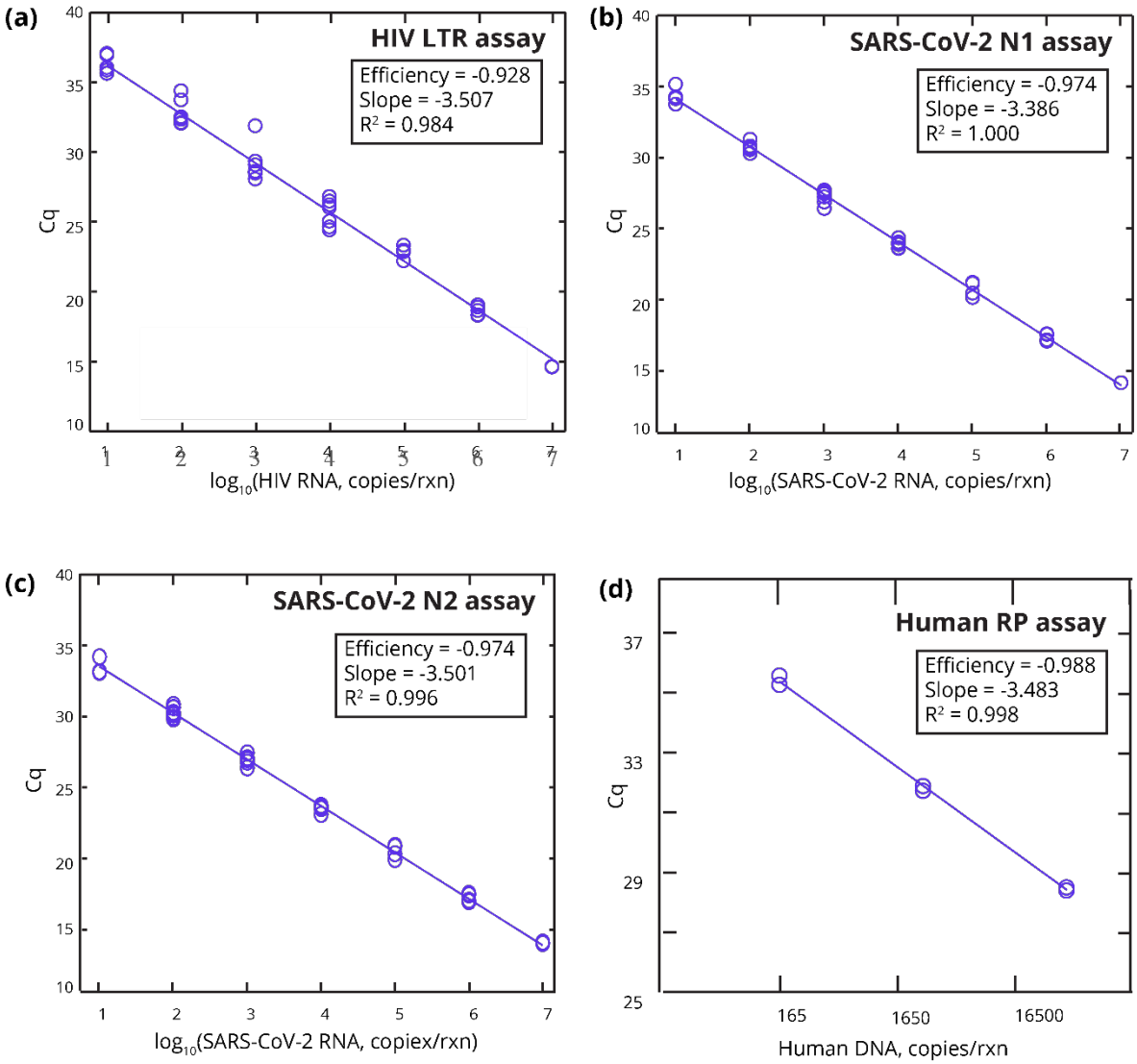


Concentration estimated by mass (copies/µL)	Copies estimated by cdPCR in rxn (copies/µL)	% Estimated by cdPCR
500	95.75	19.15
1000	200	20

Supplementary figure 1. Characterization of *in vitro* transcribed RNA standards using Crystal digital PCR (cdPCR). (**a**) Quantification of human genomic DNA with known concentration (226ng/µl, Promega) shows accurate estimation of concentration by cdPCR (**b**) Estimation of HIV RNA concentration by cdPCR shows ~20% of concentration measured by mass using Nanodrop

instrument (ThermoFisher Scientific, US) (**c**) The measured concentrations based on the total mass of *in vitro* transcribed HIV RNA and COVID RNA was verified using cdPCR. We found that estimation of COVID RNA concentrations by cdPCR using N2 assay shows ~20% of concentration and thus we have corrected the concentration of the RNA stocks based on these cdPCR. The Naica Geode (cdPCR) was programmed to perform a PCR thermal cycling program: 95 °C for 10 minutes, followed by 55 cycles of 95 °C for 30 seconds and 60 °C for 30 seconds. Image acquisition was performed using the Naica Prism 3 reader in the blue channel (excitation range 415-480 nm and emission range 495-520 nm) at exposure time of 100 ms. Droplet counts were enabled by the detection of reference dye FITC in the blue channel and was performed by the Crystal Reader software. Extracted fluorescence value for each droplet was analyzed by Crystal Miner software. Blue threshold line was adjusted manually above the negative droplets of the NTC sample in the Crystal Miner software.

RT-qPCR optimization was performed to reliably detect 10 copies of RNA template on HIV LTR (**Supplementary figure 2a**) and SARS-CoV-2 N1 (**Supplementary figure 2b**) & N2 (**Supplementary figure 2c**) assays.

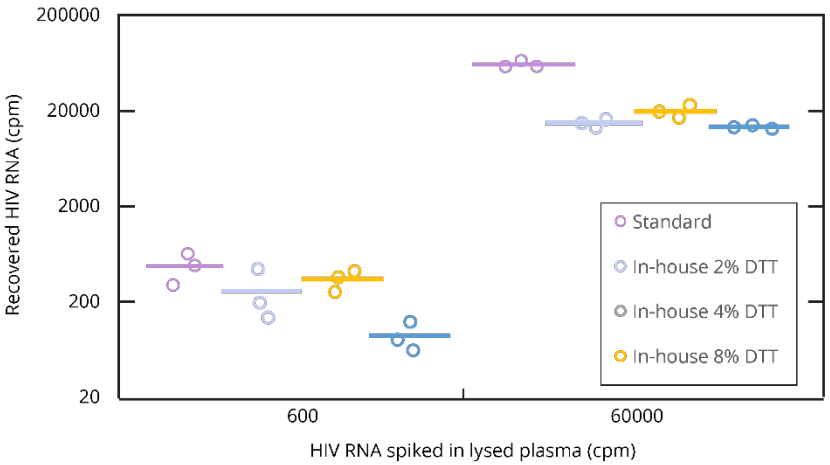


Supplementary figure 2. Standard curves of RT-qPCR assays (**a**) HIV LTR assay (**b**) SARS-CoV-2 N1 assay (**c**) SARS-CoV-2 N2 (**d**) human genomic DNA. For (**a**), (**b**), and (**c**), single-use stock of the standard RNA templates quantified by cdPCR (**Supplementary figure 1**) were diluted in nuclease-free water down to 2 - 2,000,000 copies/uL. 5µL of each RNA standard or water was added to 15µL of the qPCR master mix and subjected to RT-qPCR cycling as described in the methods section. Two replicate reactions were performed on each plate and data were pooled from four experiments on different days, accounting for a total of eight replicates. For human controls (**d**), two replicates/concentration were run in RT-qPCR. The concentrations were based on the reported values from the manufacturer (Promega). Note that to allow comparison across plates, we normalized the data of cycles 5-10 and used a fixed threshold of 200 RFU to determine the Cq

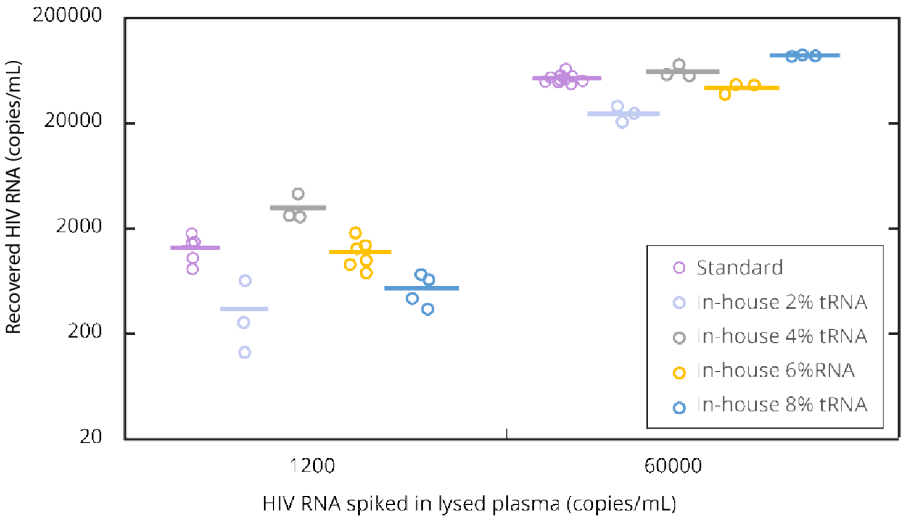
values. All replicates were plotted along with the fitted linear regression. Corresponding Pearson's R values were reported along with the RT-PCR efficiency ($1\text{-}10^{-1/\text{slope of fitted linear regression line}}$).

Optimizing the lysis buffer with DTT and tRNA using HIV LTR RT-qPCR assay

To optimize the lysis buffer for RNA extraction , variable DTT concentrations (2-8%) were tested. The LTR RNA spiked plasma samples were mixed with lysis buffer (added 2M DTT, 2-8% v/v) and extracted with in-house RNA extraction kit. The extracted RNA recovery was measured by RT-qPCR assay for LTR gene. **Supplementary figure 3** showed the effect of 2%, 4% and 8% (v/v) DTT in the lysis buffer on the extraction recovery of RNA as compared to standard method. 2% to 4% (v/v) of 2M DTT added in the in-house lysis buffer improved the extraction recovery of RNA copies as detected by RT-qPCR. However, recovery of RNA copies was significantly less (~30%) as compared to standard extraction method. We hypothesized that this reduced recovery was due to uninhibited RNAse activity in the samples. To further improve the RNA extraction recovery, tRNA was added in the lysis buffer with 4% v/v (2M) DTT. **Supplementary figure 4** showed measured HIV RNA input copies against actual HIV copies spiked in lysed plasma. Recovered HIV copies in 4-6% v/v yeast tRNA groups were comparable to standard method (1% tRNA in lysis buffer, Qiagen RNA extraction kit). This suggested that in-house lysis buffer can be used as an alternative extraction buffer for HIV RNA.



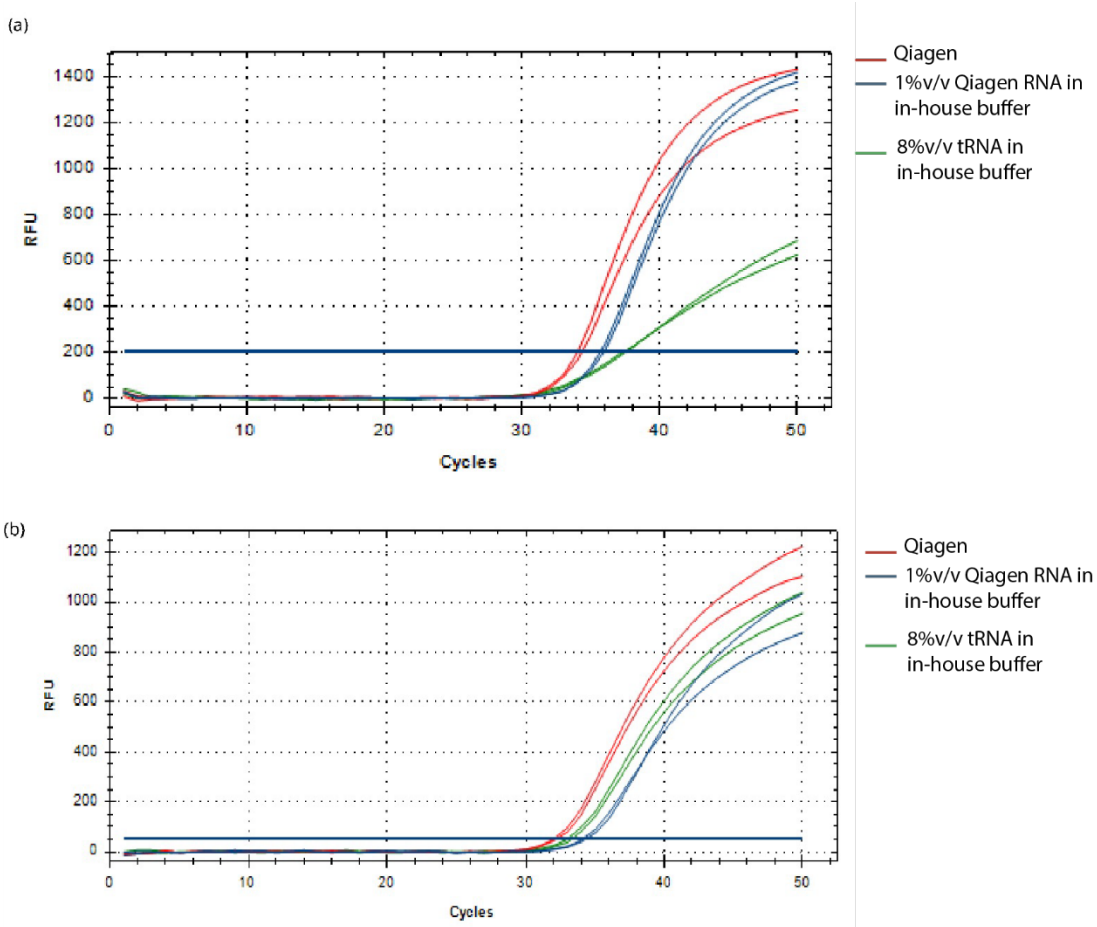
Supplementary figure 3. Effect of DTT concentrations in in-house lysis buffer (4M GuSCN, 10mM MES pH 5.5) The in-house RNA lysis buffer containing 2 - 8% (v/v) of 2M DTT (corresponding to final concentrations of 40-160 mM DTT) or the standard lysis buffer (Qiagen ViralAmp kit) was used to lyse HIV negative human plasma. After 10min (Qiagen protocol) and 15min (in-house protocol) incubation, HIV RNA was spiked into the samples either at 0, 600, or 60000 copies/mL. The samples were then proceeded with the rest of the extraction protocol and analyzed using RT-qPCR. The Cq values from each condition were converted to recovered copies and plotted (mean±SE, *n = 3*).



Supplementary figure 4. Effect of carrier RNA in in-house lysis buffer (4M GuSCN, 10mM MES pH 5.5, 4% v/v DTT). The in-house RNA lysis buffer containing 2 - 8% (v/v) 1mg/mL tRNA (corresponding to 11.2 – 44.8 µg tRNA per 560µL lysis buffer) or the standard lysis buffer (Qiagen ViralAmp kit) was used to lyse HIV negative human plasma. After 10 minutes (standard protocol, Qiagen) and 15 minutes (in-house protocol) incubation, HIV RNA was spiked into the samples either at 0, 1200, or 60000 copies/mL, followed by rest of extraction protocol. 5µl of extracted sample was added to 15µl of RT-qPCR reaction mix and analyzed using RT-qPCR. The Cq values from each condition was converted to recovered HIV RNA copies and plotted against spiked-in HIV RNA copies (mean ± SE, *n = 3).* Addition of 4% v/v and 6% v/v carrier RNA (tRNA) in in-house lysis buffer showed recovered HIV copies greater than or equivalent to standard extraction method at 1200 and 60000 copies/ml, respectively. This suggested that 4-6% v/v of carrier RNA in the in-house lysis buffer is optimum. 2% v/v carrier RNA was not enough to protect spike-in HIV RNA from RNases in plasma. On the other hand, 8% v/v carrier RNA competed with HIV RNA for amplification, especially when HIV copies was present at a low concentration (1200 copies/ml).

.

.



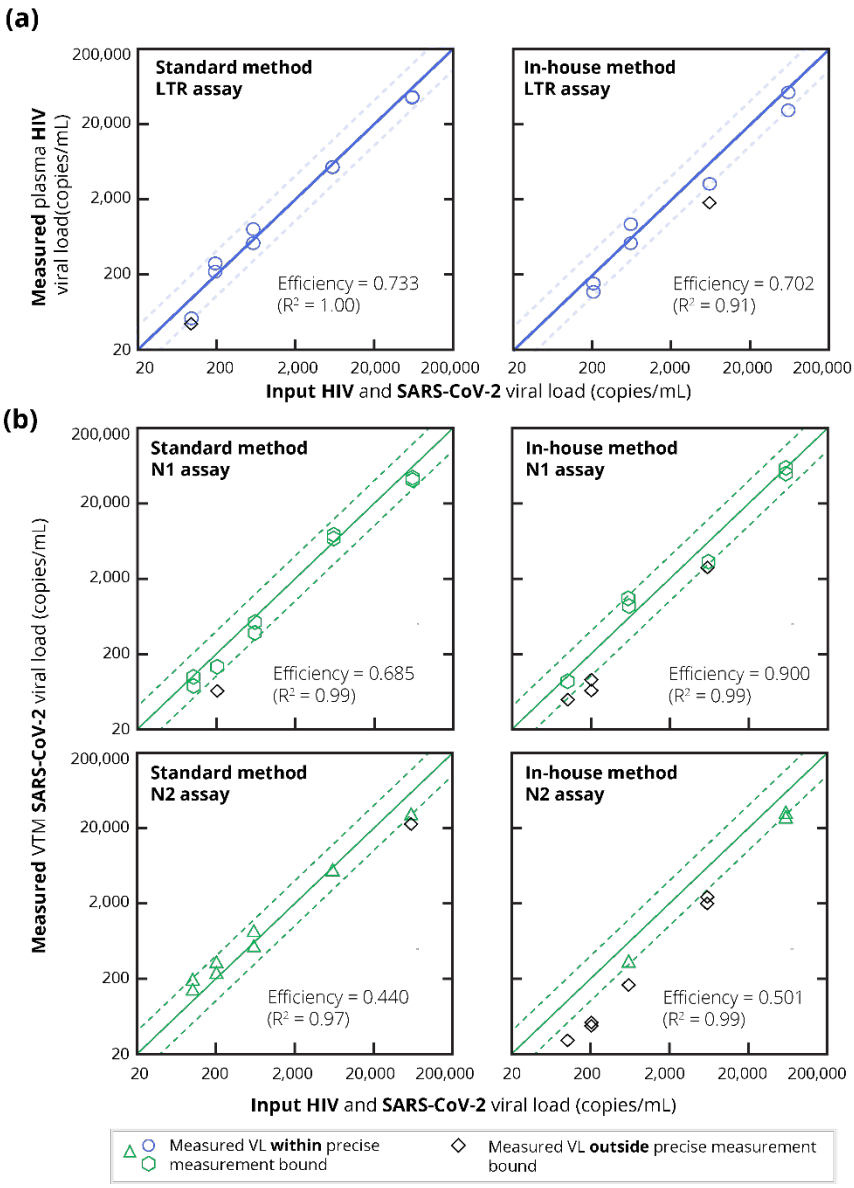
Supplementary figure 5. Impact of carrier tRNA concentration on RT-qPCR

(**a**) Amplification curves of 5µL extracted RNA (**b**) Amplification curves of 2µL extracted RNA from HIV RNA 1000 copies/mL using either standard extraction protocol (Qiagen, red lines), carrier tRNA condition (1%v/v) in in-house buffer (blue lines), or 8%v/v tRNA in in-house buffer (green lines). Each condition was performed in duplicates and individually plotted. To further understand that there is any competition during amplification between carrier tRNA and HIV RNA, we conducted experiment with 2 and 5µl volumes of the extracted RNA in the net 20 µl RT-qPCR reaction. We expect that increased in the sample volume would increase the carrier RNA in the reaction mix that competes with HIV RNA for amplification, mainly, at a low concentration (1000 copies/ml).

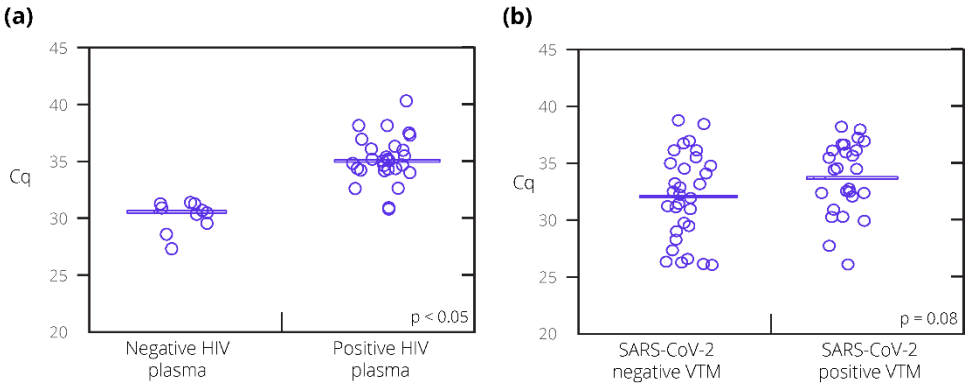
Addition of 8%v/v carrier RNA in in-house lysis buffer and 5µL extracted RNA sample in the reaction mix inhibited amplification of HIV RNA (green lines, **Supplementary figure 5a), however,** 1%(v/v) of carrier RNA overcame the RT-qPCR inhibition (blue lines, **Supplementary figure 5a**) to a certain extent but differed by about 2 cycles from the standard extraction protocol (red lines, **Supplementary figure 5a**).

Surprisingly, 8% v/v carrier RNA with 2µL extracted sample in the reaction mixture did not show any inhibition on amplification of HIV RNA (green lines, **Supplementary figure 5b**). Recovered HIV copies was higher at 8% v/v than 1% v/v carrier RNA (blue lines, **Supplementary figure 5b**), but still lower than standard Qiagen protocol (red lines, **Supplementary figure 5b**). The difference between in-house method with 8%(v/v) tRNA and standard Qiagen protocol was about 1 cycle.

Based on the results of supplementary figures 4 and 5, 4-6% (v/v) carrier RNA in in-house extraction protocol with 5µL extracted sample volume in the reaction mix was used for HIV detection assay.



Supplementary figure 6. **Correlation plots of measured VL from co-extraction of contrived plasma/VTM samples.** Measured VL of (**a**) HIV and (**b**) SARS-CoV-2 targets in contrived plasma/VTM samples (lysed plasma/VTM sample and spiked with synthetic HIV and SARS-CoV-2 RNAs at 0, 100, 200, 600, 6000, or 60000 copies/mL). All individual sample data is plotted. Diagonal lines represent 100% recovery with the dash lines indicating precise measurement bound of ±0.3 log10 (VL input, copies/mL).



Supplementary figure 7. Levels of RP gene in clinical specimens

(a) Human plasma. Compared to HIV-seronegative plasma specimens (purchased from Innovative Research, Inc, MI, USA), HIV-seropositive plasma specimens showed lower RP gene levels. Positive plasma specimens were collected from treatment naive individuals in Mexico City. **(b)** NS. A non-significant difference ($p>0.05$) was observed between Rp levels fromSARS-CoV-2 positive and negative specimens, collected from individuals in the US with respiratory symptoms. All specimens were stored at –80 °C until test. Averages of the positive and negative groups were assessed for statistical difference using Student's t-test (two-tailed).

Supplementary table 1. Primers and probe sequences
nCoV-N control: severe acute respiratory syndrome coronavirus 2 isolate Wuhan-Hu-1, complete genome (GenBank: NC_045512.2)[31, 42]

Name	Sequence (5' to 3')	Base positions mapped to reference sequences	Final concentration in RT-qPCR mixture
2019-nCoV_N1-F (N1 forward primer)	GAC CCC AAA ATC AGC GAA AT	28287 - 28306 nCoV-2	500 nM
2019-nCoV _N1-R (N1 reverse primer)	TCT GGT TAC TGC CAG TTG AAT CTG	28335 - 28358 nCoV-2	500 nM
2019-nCoV_N1-P (N1 probe)	FAM-ACC CCG CAT /ZEN/ TAC GTT TGG TGG ACC-3IABkFQ	28309 - 28332 nCoV-2	125 nM
2019-nCoV_N2-F (N2 forward primer)	TTA CAA ACA TTG GCC GCA AA	29164 - 29183 nCoV-2	500 nM
2019-nCoV_N2-R (N2 Reverse primer)	GCG CGA CAT TCC GAA GAA	29213 - 29230 nCoV-2	500 nM
2019-nCoV_N2-P (N2 probe)	FAM-ACA ATT TGC /ZEN/ CCC CAG CGC TTC AG-3IABkF	29188 - 29210 nCoV-2	125 nM
LTR Forward primer	GCCTCAATAAAGCTTGCCTTGA	522 - 543 HXB2	250 nM
LTR Reverse primer	GGCGCCACTGCTAGAGATTTT	622 - 642 HXB2	250 nM
LTR Probe	/56-FAM/CTGGTAACTAGAGATCCCT/3MGBEC/	581 - 599 HXB2	250 nM
RP-F (RP forward primer)	AGA TTT GGA CCT GCG AGC G	28 - 46 Human RP gene (NM_006413.5)	500 nM
RP-R (RP reverse primer)	GAG CGG CTG TCT CCA CAA GT	73 - 92 Human RP gene	500 nM
RP-P (RP probe)	FAM-TTC TGA CCT /ZEN/ GAA GGC TCT GCG CG-3IABkFQ	49 - 71 Human RP gene	125 nM

Supplementary table 2. Cost estimate for the RNA extraction kit

Consumables/reagents	**Source**	**Unit**	**Cost**
GuSCN	VWR, 97061-524	500g	$252.96
MES buffer	Sigma, 76039	1M, 0.5L	$116
Tris-HCl buffer	Fisher, 15568025	1M, 1L	$95.25
DTT	Promega, C4H10O2S2	5g	$139.10
Collection tubes	Qiagen, 19201	1000 tubes	$166
Spin column tubes	Epoch Life Science, 1920	250 tubes	$100
tRNA	Fisher, AM7119	5mg	$95.25
Buffer (50mL stock)			
Lysis buffer	4M GuSCN, 10mM MES	50mL	$13.66
Wash buffer 1	1M GuSCN, 10mM Tris-HCl	50mL	$3.18
Wash buffer 2	10mM Tris-HCl	50mL	$0.05
Cost/kit (140µL sample)			
Epoch column			$0.40
Collection tubes			$0.51 (3 x $0.17)
Lysis buffer			$0.15
tRNA			$0.64
DTT			$0.10
Wash buffer 1			$0.05
Wash buffer 2			negligible
Total cost			**$1.85**