

Supplementary Materials for:

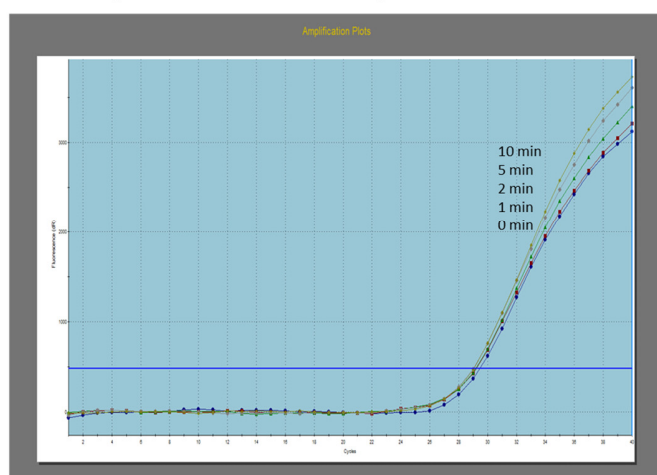
# A sensitive, portable microfluidic device for SARS-CoV-2 detection from self-collected saliva

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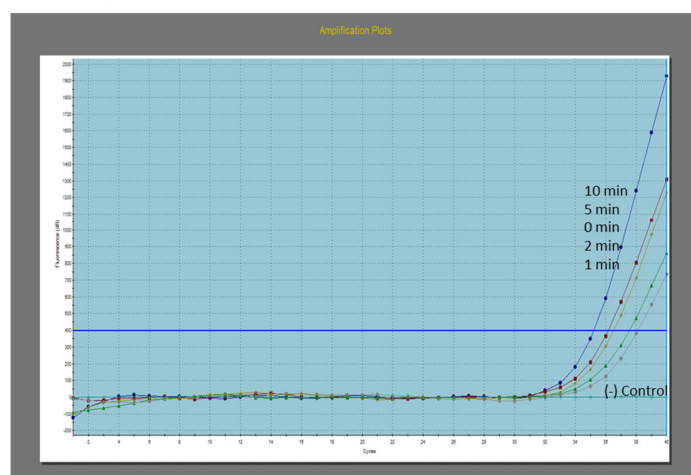
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Insert figure

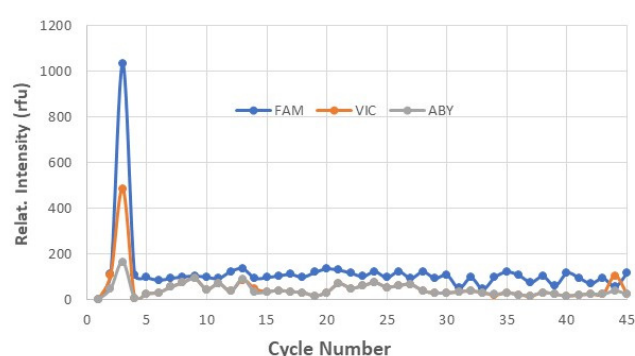
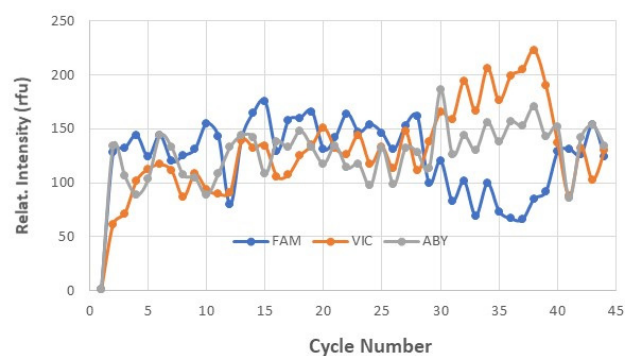
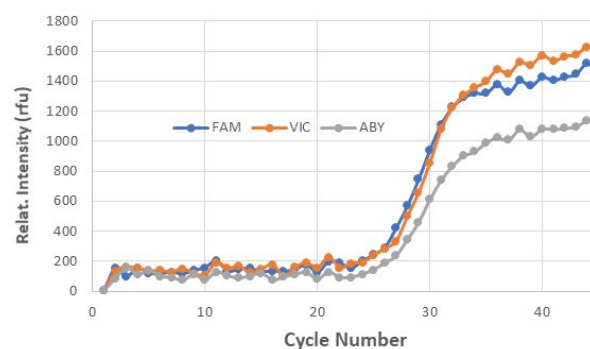
A: Saliva/CoV2 virions at 200 copies



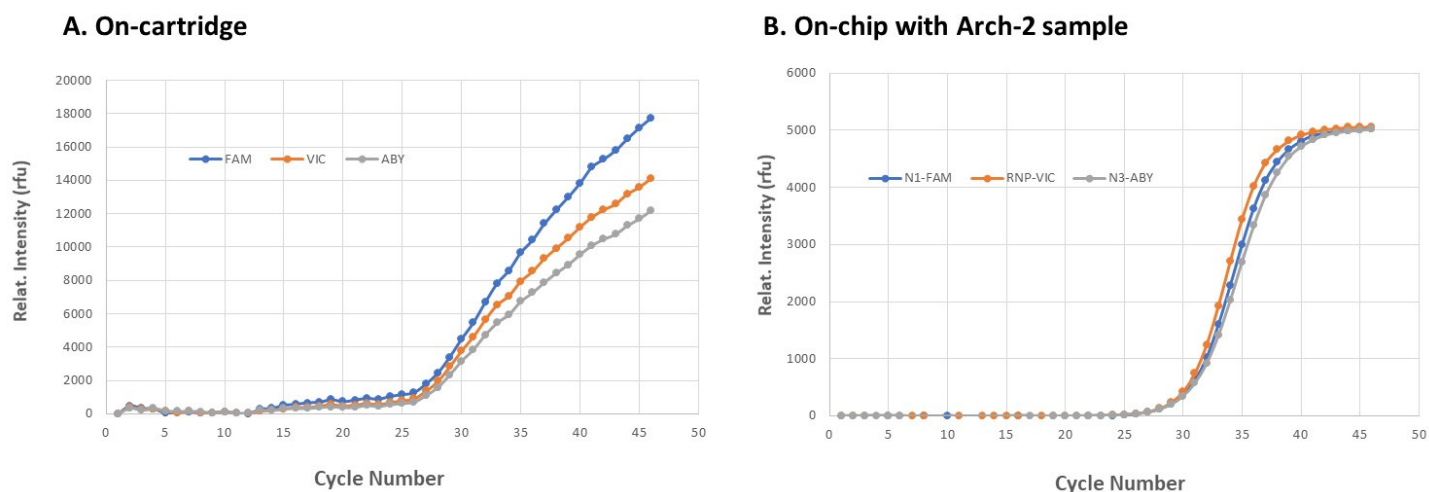
B: Saliva/CoV2 virions at 2 copies



**Figure S1. Heating time course of saliva samples spiked with SARS-CoV-2 virions.** Saliva samples spiked with SARS-CoV-2 virions at 200 copies (A) and 2 copies (B) were heated at 100°C for 10min, 5min, 2min, 1min, and 0min (no heating). The 1-Step RT-qPCR with N1 primer was performed on benchtop (Stratagene Mx3005P) and the amplification curves were plotted.

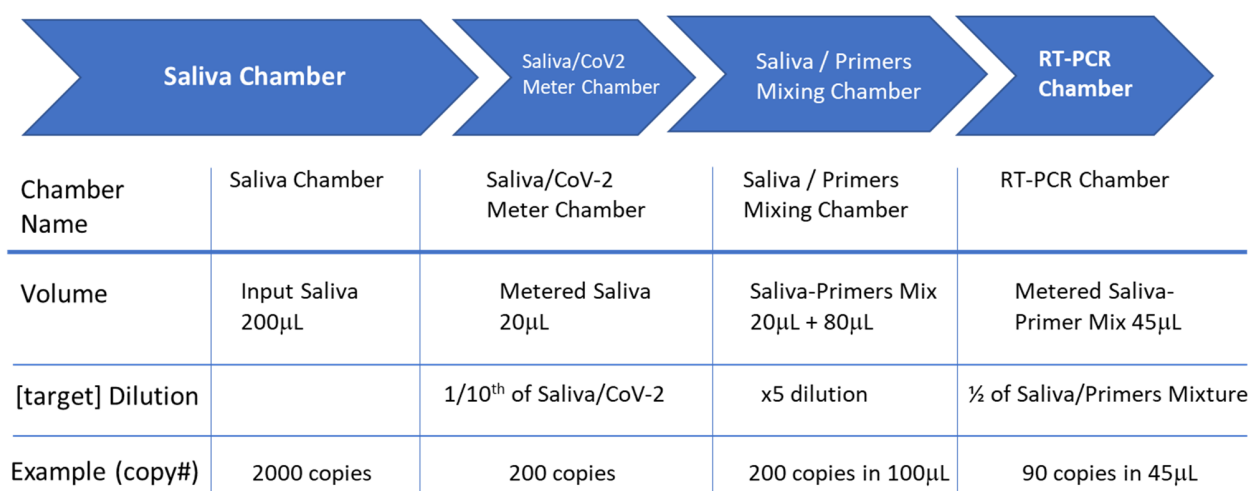
**A. On-Cartridge RT-qPCR****B. On-Chip RT-qPCR with Arch-2 Sample****C. On-Chip RT-qPCR with Arch-1 Sample**

**Figure S2. Evaluation of the earlier design of cartridge with flow-through mixing module.** Saliva sample was spiked with SARS-CoV-2 virions 9000 copies and processed on the saliva cartridge. The results of 1-step triplex, RT-qPCR with N1-FAM, N3-ABY, and RNP-VIC were presented. The microfluidic flow sequence was: saliva sample from Saliva/CoV2 Meter Chamber (20 $\mu$ L of heated sample) flowing through  $\rightarrow$  Primers Mix Chamber, picking up the primer mix (80 $\mu$ L) and ending at  $\rightarrow$  Saliva/Primers Mixing Chamber, diffusion-mix for 1min, then flowing toward PCR Chamber  $\rightarrow$  RT-PCR Chamber metering (45 $\mu$ L) and the excess of saliva/primers mixture was archived in Arch-2 chamber. The failed on-cartridge RT-qPCR (A) was evaluated by on-chip RT-qPCR with samples retrieved from Archive-2 (B) and Archive-1 chambers (C) of the same cartridge. No amplification was achieved from the on-chip 1-Step RT-qPCR with Archive-2 sample which contained a mixture of on-cartridge flow-through mixed saliva and primers. A successful on-chip 1-Step RT-qPCR was achieved with Archive-1 sample, in which the on-cartridge heated saliva sample was mixed with primers in tube before loaded onto the PCR chip, indicating the insufficient on-cartridge sample/primers mixing was the cause for failed on-cartridge PCR.

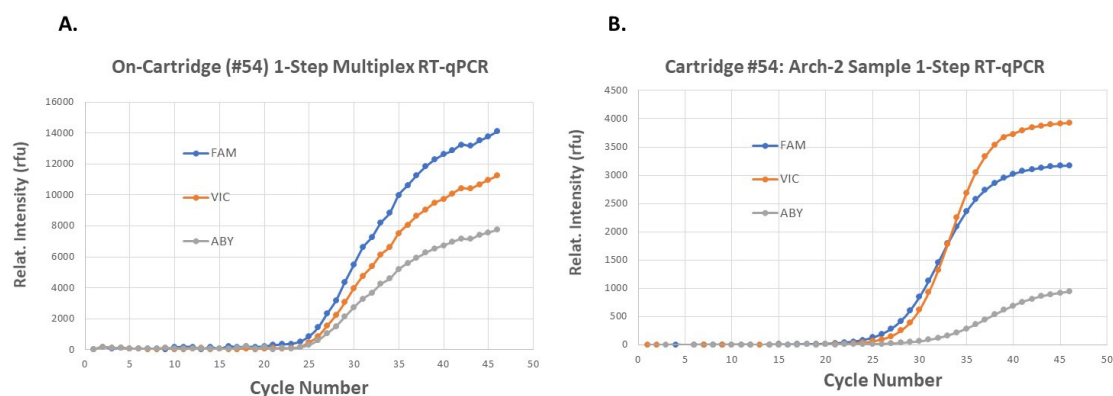


**Figure S3. Evaluation of on-cartridge “bubble” mixing module.** After several rounds of modification and testing, the on-cartridge flow-through mixing was replaced with “bubble” mixing. The microfluidic flow sequence was: primer mix (80 $\mu$ L) from Primers Mix Chamber flowing through  $\rightarrow$  Saliva/CoV2 Meter Chamber (20 $\mu$ L of heated sample) and ending at  $\rightarrow$  Saliva/Primers Mixing Chamber and bubble-mixing for 1min, then flowing toward PCR Chamber  $\rightarrow$  RT-PCR Chamber metering (45 $\mu$ L) and the excess of saliva/primers mixture was archived in Arch-2 chamber. SARS-CoV-2 RNA 9000 copies were spiked into the saliva sample. After the on-cartridge “bubble” mixing, successful multiplex RT-qPCR amplification was achieved in both on-cartridge (A) and on-chip with sample retrieved from Arch-2 chamber (B).

The amplification intensity was lower in the on-chip RT-qPCR from the Arch-2 sample, which was attributed to time lag between the sample retrieval from the Arch-2 chamber and running the RT-qPCR control. In general, the archived samples were retrieved from the cartridges and stored at -20 $^{\circ}$ C till needed to test for on-chip RT-qPCR on the same MiDAS instrument.



**Figure S4. A simplified diagram illustrating on-cartridge microfluidic dilutions of the input target.** The target concentration in the initial input sample was different from the target concentration ended in the RT-PCR chamber, due to the dilution and volume changes. During on-cartridge process, only 20μL (one tenth) of the heated saliva sample was metered in the Saliva/CoV-2 Meter Chamber and mixed with 80μL of Primer Mix (five-fold dilution), then only 45% of the mixture from Saliva/Primer Mixing Chamber was metered in the RT-PCR Chamber for on-cartridge 1-Step Multiplex RT-qPCR. Therefore, the system could detect 1000 copies/mL of SARS-CoV-2 virions in saliva samples and the on-cartridge RT-qPCR could detect as low as 9 copy /45μL.



**Figure S5. One example of on-cartridge SARS-CoV-2 virion detection from saliva.** The Arch-2 samples retrieved from the same cartridge were routinely checked for multiplex 1-step RT-qPCR on an isolated PCR chip. The overall amplification intensity for all three primer sets was decreased in the retrieved Arch-2 samples (B) as compared to the on-cartridge amplification (A). This was attributed to time lag between the sample retrieval from the Arch-2 chamber and running the RT-qPCR control. The efficacy of PCR reagents in the mixture retrieved from Arch-2 would decrease before running the on-chip RT-qPCR control. Samples retrieved from Arch-2 chambers, which contained both saliva/CoV-2 template and primers, were stored at -20°C and RT-qPCR control runs were performed at a later time.

Table S1. A brief cost comparison of major FDA-approved POC instruments commercially available for SARS-CoV-2 diagnostic test				
POC Equipment	Sample Type	Turnaround Time	Cost per Test (\$)	Resources for The Cost
Abbott ID NOW (Abbott)	saliva	20 min	43	<a href="https://www.sdbor.edu/mediapubs/Documents/Abbott%20ID%20Now%20Universities%20TRS%202020.pdf">https://www.sdbor.edu/mediapubs/Documents/Abbott%20ID%20Now%20Universities%20TRS%202020.pdf</a>
Xpert Xpress SARS-CoV-2 Device (Cepheid)	NP swab	20 min	20	<a href="https://www.msf.org/diagnostic-company-cepheid-charging-more-it-should-covid-19-tests">https://www.msf.org/diagnostic-company-cepheid-charging-more-it-should-covid-19-tests</a>
Accula SARS-CoV-2 POCT (Mesa Biotech)	NP swab	30 min	20	<a href="https://cen.acs.org/analytical-chemistry/diagnostics/Rapid-COVID-19-testing-breaks/98/web/2020/08">https://cen.acs.org/analytical-chemistry/diagnostics/Rapid-COVID-19-testing-breaks/98/web/2020/08</a>
Mobilefuge	Saliva	n/a*	n/a	Reference [32]
Sherlock	saliva	55 - 120 min	n/a	Reference [25]
WREN Laboratory	NP swab / saliva	24 - 48 hours	80-100	<a href="https://www.wrencovidtesting.com">https://www.wrencovidtesting.com</a>
Urgent Care / Clinical Lab test	NP swab	24 - 72 hours	100 - 200	<a href="https://www.nbcnews.com/health/urgent-care/clinical-lab-test-covid-19-test-kits-5c9a6a6c-2020-08">How to shop for FDA-authorized home Covid test kits: A guide (nbcnews.com)</a>
At-home Test / Shipping to Test Centers	NP swab / Saliva	24 - 72 hours	109 - 155	<a href="https://www.nbcnews.com/health/urgent-care/clinical-lab-test-covid-19-test-kits-5c9a6a6c-2020-08">How to shop for FDA-authorized home Covid test kits: A guide (nbcnews.com)</a>
Emergency Rooms	NP swab	varies	varies	
* n/a = not available				