**Figure S1.** DMSO decreases negative droplet intensity in RT-ddPCR assays. The RT-ddPCR reactions containing 0%, 2.5%, or 5% DMSO were performed for a Chelex-lowTE sample prepared with the ATCC SARS-CoV-2 virions and 293FT cells using N1 and cRPP30 (left panel) or N2 and RPP30 (right panel). The grey clusters represent negative droplets; blue and green clusters represent Fam and Hex positive droplets, respectively; and orange clusters represent double positive droplets.

**Figure S2.** SARS-CoV-2 prepared in different buffers used for RT-qPCR without RNA-extraction. (A) Samples were diluted in H$_2$O as indicated at the bottom. Expected Ct refers to Ct calculated based on Ct from extracted RNA normalized with added virion numbers after dilution using the ΔCt method. (B) Buffer compatibility in RT-qPCR. Sample RNA, not heated and 5 µl of which contained materials extracted from 6,250 virions. Other samples were heated in the presence of Chelex, of which undiluted samples also contained 6,250 virions per 5 µl. Samples were diluted in H$_2$O. Samples with undetermined Ct values were plotted as Ct 40. The NEB Luna RT-qPCR kit and NEB-Luna-Program I was used. NTC, no-template control.

**Figure S3.** Tris EDTA and DMSO containing buffers. (A) RT-qPCR of samples with heat-inactivated ATCC SARS-CoV-2 virions. 5 µl of samples were used for one reaction in RT-qPCR except that samples in MEM α were diluted 1:1 with H$_2$O. Samples with undetermined Ct values were plotted as Ct 40. The NEB Luna RT-qPCR kit and NEB-Luna-Program I was used. (B) RT-ddPCR of saliva samples with heat-inactivated ATCC SARS-CoV-2 virions. The Chelex was prepared in H$_2$O, lowTE or TED99.
(lowTE with 99% DMSO). RNA-kit refers to RNA extracted with the RNeasy Protect Saliva Mini Kit. NTC, no-template control.

**Figure S4.** Optimization of the NEB Luna RT-qPCR assay. Extracted RNA samples were serial diluted and assayed either using 2.5 µl sample in a 10 reaction volume or 5 µl in a 20 µl reaction, and using a longer PCR protocol (I: 10 seconds of denature and 40 seconds of annealing/extension) or a shorter PCR protocol (II: 5 seconds of denature and 20 seconds of annealing/extension). NTC, no-template control.
Figure S2

A

Prep_method

Heat

Heat_Chelex

Expected_Ct

Dilution before RT-qPCR

B

Target  N1  N2  dRPP30  RPP30
