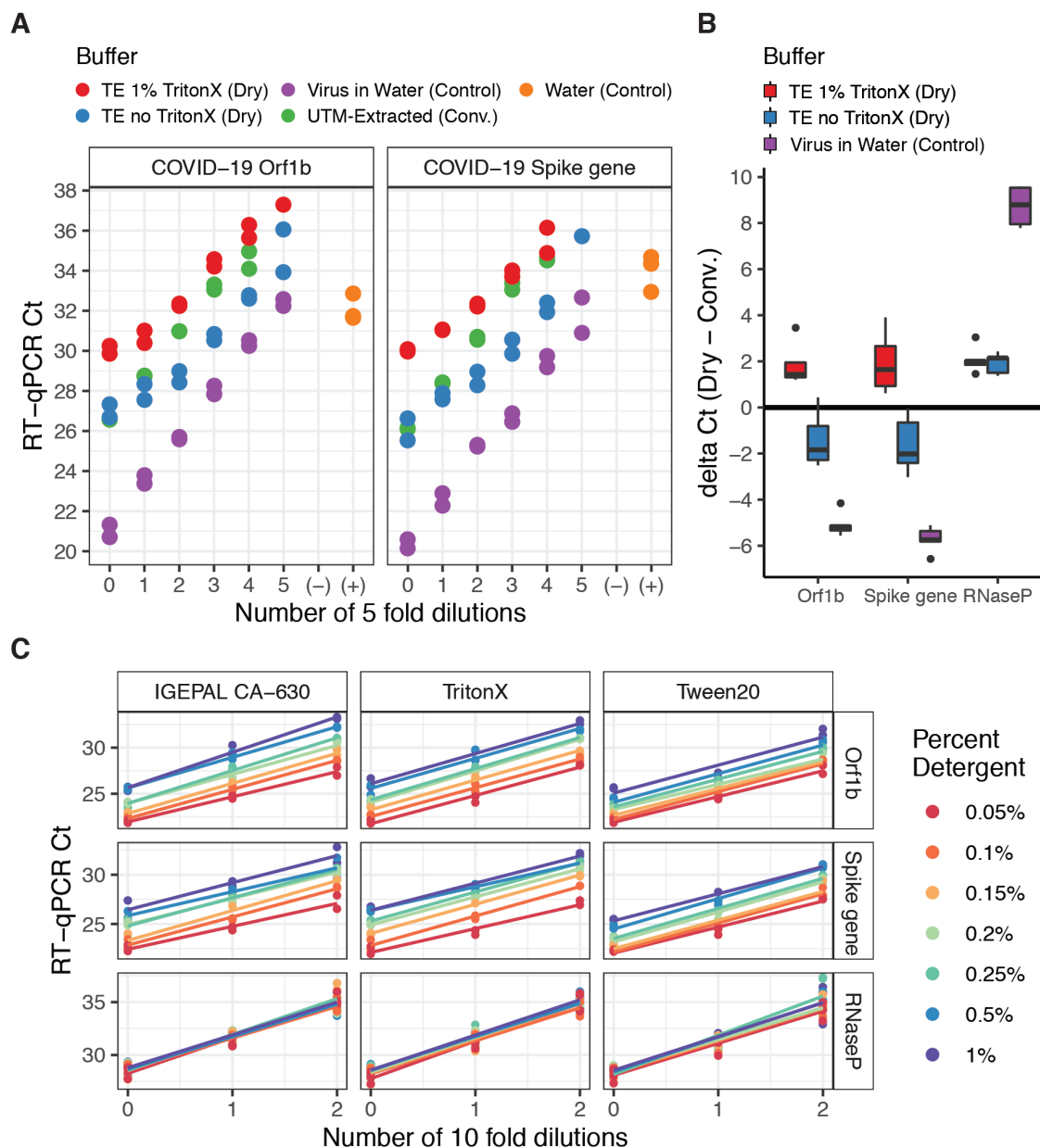
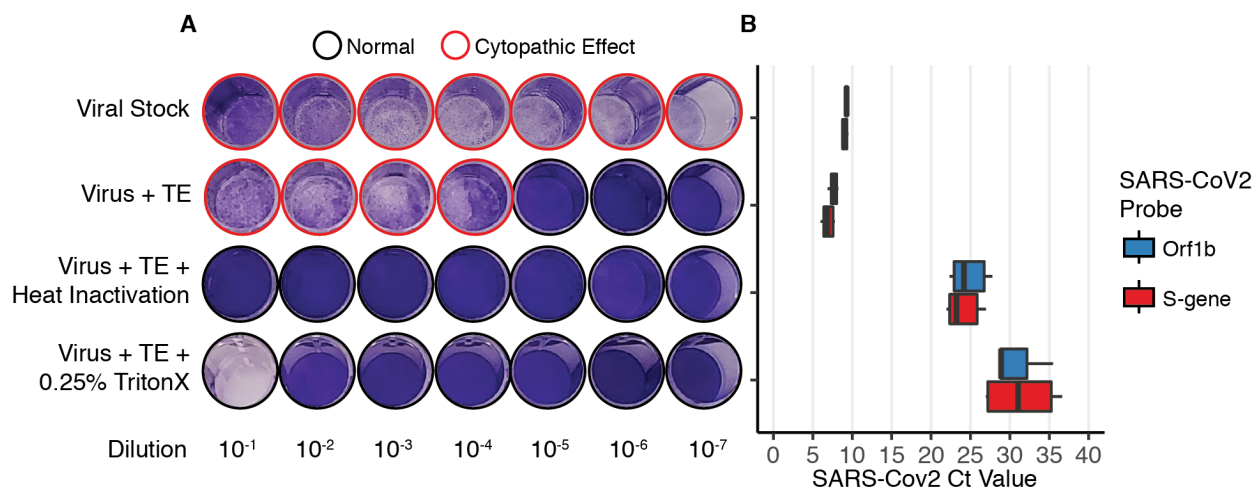


**Supplementary Figure 1. Educational attainment and age range of 35 swab usability study participants. (A)** The highest level of education attained by recruited participants (teal) versus King County, Washington (golden). **(B)** Percentage of recruited study participants in the displayed age ranges.

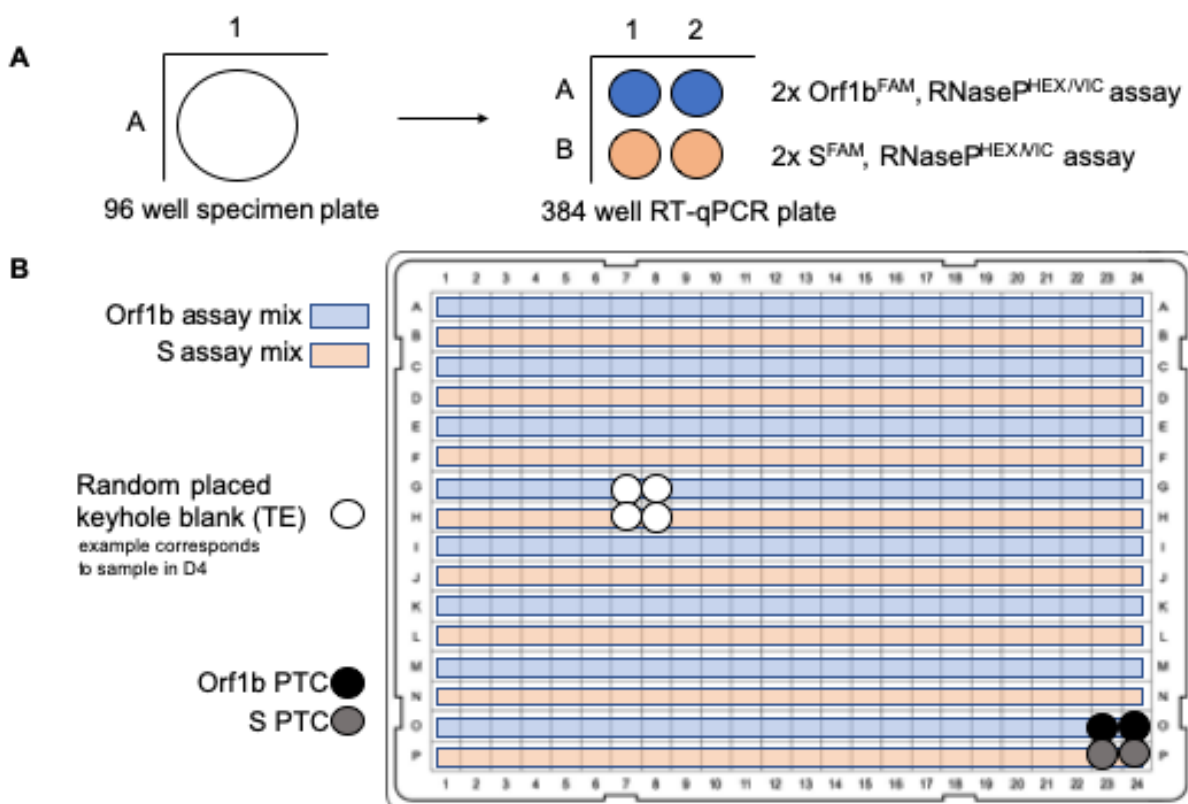


**Supplementary Figure 2. Comparison of RT-qPCR detection of inactivated virus from conventional and dry swabs.** (A) Crossing threshold (Ct) values shown for specimens comprising a self swab and inactivated SARS-CoV-2 virus for both the ORF1b primer-probe set (left) and the Spike gene primer-probe set (right). Colors correspond to unique combinations of extraction protocol or controls. All specimens were measured twice in independent RT-qPCR reactions. No template control (-) wells contained either buffer or water and positive control wells (+) contained synthetic template. (B) Delta Ct values between conventionally processed swabs and dry processed swabs at matched dilutions for this contrived experiment. (C) Ct values for three probes (rows: Orf1b, Spike, Rnase P) assayed in buffers containing one of three detergents (columns: IGEPAL CA-360, TritonX, Tween20) across ten-fold dilutions. Linear model (colored line) was fit for observations (colored points) at each detergent percent.

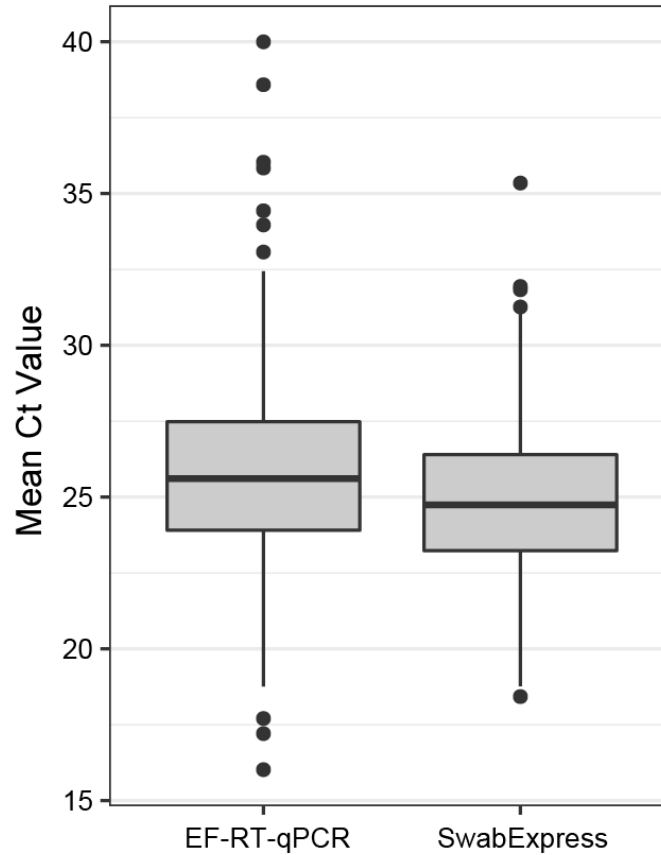


**Supplementary Figure 3. Comparison of heat treatment or detergent to inactivate**

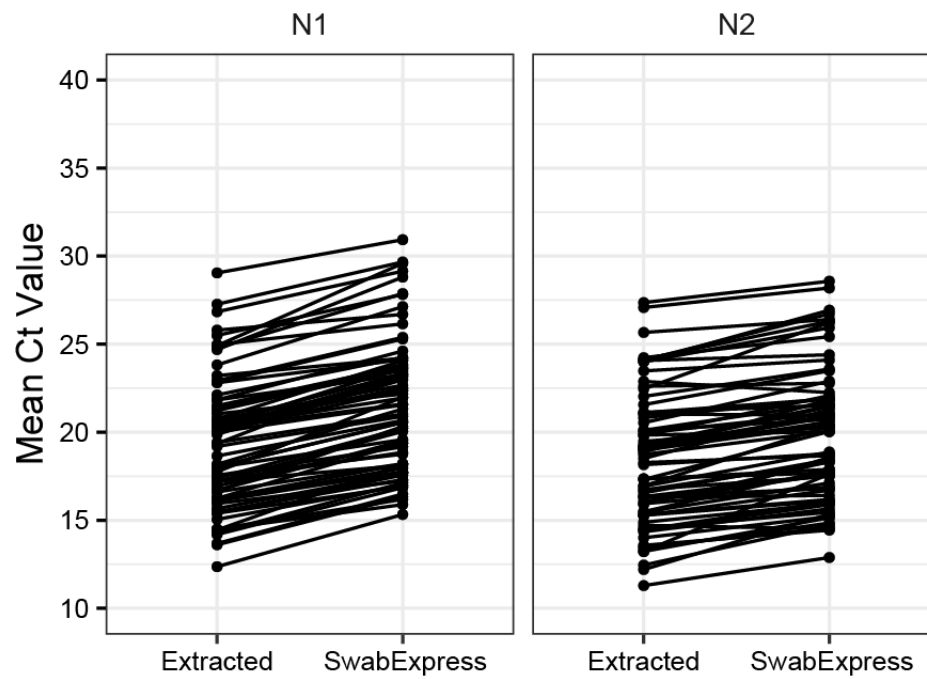
**SARS-CoV-2.** (A) Crystal violet staining shows the cytopathic effects of SARS-CoV-2 dilutions on Vero cells after incubation with TE, TE + heat inactivation at 65°C for 10 minutes or TE + 0.25% Triton. (B) RT-qPCR Ct values of SARS-CoV-2 RNA isolated from Vero cells. Purified viral particles were first incubated with TE, TE + heat inactivation at 65°C for 10 minutes or TE + 0.25% TritonX.



**Supplementary Figure S4. Plate layout of the Northwest Genomics Center SwabExpress RT-qPCR test.** (A) Each sample is loaded into 4 independent wells on a 96 well plate and tested in duplicate for the Orf1b and S-gene (Spike gene) primer/probes. (B) Included on every sample plate is a randomly positioned low-TE keyhole blank (white) and a positive template control (PTC) well containing synthetic SARS-CoV-2 template and human Hap1-RNA (black and grey).



**Supplementary Figure S5. SwabExpress is more sensitive for RNase P than extraction-free RT-qPCR.** 1222 samples were tested in parallel by extraction-free RT-qPCR (EF-RT-qPCR) and SwabExpress. Samples run through the SwabExpress protocol, which includes a Proteinase K digestion, had lower mean RNase P Cts than the same samples run on EF-RT-qPCR.



**Supplementary Figure 6. SwabExpress also works with the widely used CDC-N1 and CDC-N2 probesets.** Mean crossing threshold for 75 parallel specimens that were extracted or run through SwabExpress for the commonly used SARS-CoV-2 CDC N1 and N2 probe set.

**Table S1A. Age of study participants**

Age Groups	Number	%	Average Age (within group)
Between 18 months - 12 years old	6	17%	8.7
Between 13 and 18 years old	8	23%	15.1
Between 18 and 64 years old	14	40%	40.7
Over 65 years old	7	20%	68.3
TOTAL	35	100%	34.9
TOTAL	35	100%	34.9

**Table S1B. Educational attainment of study participants**

<b>Education Levels (of participants 18 &amp; over)</b>			
<b>"What is your highest level of education obtained?"</b>			
<b>Response</b>	<b>N</b>	<b>Participants (%)</b>	<b>King County (%)</b>
Less than high school graduate	0	0%	7%
Graduated high school/obtained GED	1	5%	15%
Some college (including vocational training, associate's degree)	3	14%	18%
Bachelor's degree	11	52%	31%
Advanced degree	6	29%	20%
<b>TOTAL</b>	<b>21</b>	<b>100%</b>	



**Table S1C. Household income of study participants**

<b>Household Income</b>			
<i>“Please choose the range that best represents your household income last year (before taxes). If you are still considered a dependent for tax purposes, choose the range that describes your parent/legal guardian’s household income.”</i>			
Response	N	Study participants (%)	King County (%)
Less than or equal to \$25,000	0	0%	12%
Between \$25,001 to \$50,000	0	0%	14%
Between \$50,001 to \$75,000	1	2.9%	14%
Between \$75,001 to \$100,000	7	20%	12%
Between \$100,001 to \$125,000	4	11.4%	19%
Between \$125,001 to \$150,000	3	8.6%	
Over \$150,000	16	45.7%	29%
Don't know	1	2.9%	
Prefer not to say	3	8.6%	
TOTAL	35	100%	

**Table S1D. Race of study participants**

<i>“How would you describe your race? Check all that apply”</i>			
Response	N	Study Participants (%)	King County (%)
American Indian or Alaska Native	0	0%	<1%
Asian	10	24%	17%
Native Hawaiian or Pacific Islander	0	0%	1%
Black or African American	0	0%	6%
White	30	73%	65%
Other	1	3%	10%
Prefer Not to Say	0	0%	

**Table S2A. Sample collection and packaging checklist**

<b>Sample Collection and Packaging Checklist and results</b>		
	Yes	%
Able to locate and identify swab and tube	35	100%
Used correct end of swab	35	100%
Sample both nostrils with same swab	34	97%
Swabbed 'fresh' nostril first	35	100%
Swabbed nostrils in correct order	33	94%
Swabbed at least 5 circles in each nostril	33	94%
Swabbed for at least 10 seconds in each nostril	29	83%
Wrote name & date on tube	34	97%
Put tube into biohazard bag	35	100%
Able to locate and identify swab and tube	35	100%
Used correct end of swab	35	100%
Swabbed 'fresh' nostril first	35	100%
Sample both nostrils with same swab	34	97%
Inserted entire tip of the swab into the nostrils	35	100%
Swabbed at least 5 circles in each nostril	33	94%
Swabbed for at least 10 seconds in each nostril	32	91%
Wrote name & date on tube	34	97%
Put tube into biohazard bag	35	100%
Put biohazard bag into box	34	97%
Put box into polmailer shipping bag	34	97%

**Table S2B. Observed errors or unwanted outcomes during sample collection or packaging**

<b>Did any of the following errors or reactions occur during observation?</b>		
	<b>N</b>	<b>%</b>
Contaminated end of swabs with hand/fingers	0	0%
Contaminated end of swabs by setting it down on a table or non-sanitary surface	0	0%
Did not put both tubes in biohazard bag	0	0%
Did not put biohazard bag in box	1	3%
Did not put box in polymailer shipping bag	1	3%
Did not write name on tube	0	0%
Did not write date on tube	0	0%
Nose bleed	0	0%
Sneezed during or after swabbing	4	11%
Expressed frustration or confusion about one or more of the steps	3	9%
Other*	2	6%

**Table S3. Summary of SwabExpress limit of detection (LoD) assay results.** Lowest concentration with 95% of the replicates detected for each probe is highlighted (in light grey) and bolded.

Molecules / 5uL Reaction	Assay	Percent Detected	Number of replicates	Number of replicates detected	Average Ct	Standard deviation	%Coefficient of variation
5	Spike	50.0%	20	10	36.03	0.51	1.40%
10	Spike	90.0%	20	18	34.22	0.65	1.90%
<b>20</b>	<b>Spike</b>	<b>95.4%</b>	<b>21</b>	<b>22</b>	<b>34.49</b>	<b>0.47</b>	<b>1.35%</b>
40	Spike	100%	24	24	33.76	0.94	2.78%
80	Spike	100%	24	24	31.83	0.31	0.97%
160	Spike	100%	24	24	30.36	0.23	0.76%
320	Spike	100%	24	24	30.08	0.15	0.51%
640	Spike	100%	24	24	28.86	0.27	0.92%
5	Orf1b	85.0%	20	17	36.87	0.76	2.06%
<b>10</b>	<b>Orf1b</b>	<b>100%</b>	<b>20</b>	<b>20</b>	<b>34.80</b>	<b>0.53</b>	<b>1.52%</b>
20	Orf1b	100%	22	22	34.56	0.48	1.38%
40	Orf1b	100%	24	24	33.94	0.50	1.48%
80	Orf1b	100%	24	24	32.15	0.26	0.79%
160	Orf1b	100%	24	24	31.01	0.24	0.77%
320	Orf1b	100%	24	24	30.74	0.31	1.01%
640	Orf1b	100%	24	24	29.56	0.28	0.94%

**Table S4. Concordance between extracted nucleic acids and SwabExpress for 67 previously positive specimens.** Cts are displayed for extracted specimens and sorted on the first Orf1b Ct. Concordance between extraction and SwabExpress is depicted by color.

Key:

	Extraction-free sample remained positive
	Extraction-free sample became inconclusive (low positive)
	Extraction-free sample became negative

Sample ID	Orf1B- Ct-1	Orf1B- Ct-2	S- Ct-1	S- Ct-2	RnaseP Avg Extracted	RnaseP Avg SwabExpress
e01b13c8	14.62	14.65	13.58	13.6	21.97	25.84
2da728f7	14.8	14.68	14.24	13.95	24.06	27.19
1f2ebd6a	15.01	15	13.95	14.1	23.39	26.38
51a37bac	15.42	15.46	14.45	14.35	23.04	25.39
b537efd3	15.73	15.87	14.91	15.17	20.8	25.47
b2b6a2a2	15.79	15.97	15.05	15.21	20.61	25.63
9f966df1	16.91	17.01	16.01	15.75	24.07	26.84
cb477ce0	19.1	19.03	17.85	17.81	24.81	26.67
48931452	20.22	20.36	19.36	19.6	20.42	26.37
f74f502b	20.92	21.01	19.87	20.06	22.82	26.00
280825e0	21.05	21.05	20.54	20.42	28.66	31.08
044ff3c0	21.59	21.43	20.56	20.52	25.17	28.38
51ec40d2	22.46	22.62	21.86	22.24	22.96	27.04
3c2ec020	22.86	22.89	22.23	22.29	24.03	27.16
c8b3da3d	23.29	23.52	22.9	22.97	23.55	28.96
07e94a5c	24.8	24.83	23.86	24.28	24.99	30.45
f041d1a1	24.88	25.29	24.31	24.35	23.00	26.23
a7b9417b	25.29	25.16	24.73	24.69	22.91	24.38
798a56e4	25.72	25.73	25.35	25.59	23.96	28.65
4b911840	26	25.97	25.1	25.24	24.74	29.13
a55256ad	26.06	25.87	25	24.84	26.29	28.75
caab0ac2	26.12	26.1	25.91	25.81	25.07	27.64
1c059acb	26.45	26.31	25.63	25.62	23.65	26.31
d93f3a66	26.66	26.85	26.35	26.19	20.11	25.23
0b6c2d84	26.89	26.69	25.88	25.73	19.93	26.87
4f8e6372	27	27.13	26.34	26.22	21.24	23.46
8f579a44	27.38	27.51	26.03	26.57	25.22	27.20
4f7bdaf8	27.56	27.55	26.7	26.73	26.97	30.24
0b70294c	27.89	27.88	27.27	27.45	24.93	26.65
51ffc347	28	28.13	27.73	27.4	25.06	28.00

351128f0	28.03	28.08	26.88	26.94	24.76	27.61
dd2237f3	28.07	27.91	27.38	27.15	22.57	28.62
f20f729b	28.13	27.96	27.34	27.32	27.36	29.08
121170bd	28.23	28.28	27.58	27.67	23.14	26.28
bca72cde	28.27	28.11	27.59	27.9	24.67	28.57
3ec5746c	28.42	28.29	28.15	27.73	25.17	28.35
9652a0dc	28.62	28.71	28.28	28.16	23.42	28.35
cc9b3e0c	28.75	29.12	28.85	28.45	21.88	26.41
a9589b19	29.04	29.06	28.52	28.95	25.20	28.29
ed0e2c9a	29.1	29.27	27.85	28.11	19.25	26.24
22fab3d3	29.12	29.14	27.76	27.82	24.27	27.59
3f813b87	29.72	29.55	28.8	28.43	21.90	27.62
a30c9e54	30.29	30.18	28.99	29.47	20.88	27.34
a1494050	30.29	30.49	29.72	29.96	22.86	27.54
803bfb86	30.31	30.41	29.58	29.63	27.24	28.73
cf79667b	30.42	30.56	29.36	28.84	20.89	24.46
5200ef6a	30.46	30.37	29.49	29.68	23.39	27.74
9a00d505	30.7	30.5	29.47	29.34	20.15	26.86
39657634	31.23	30.97	31.41	31.75	19.54	23.94
f8f389df	31.41	31	30.63	30.7	23.43	29.20
63d028e6	31.52	31.53	30.82	30.62	25.46	28.81
355a78c4	31.65	31.23	31.32	30.47	23.39	28.17
97d353f1	31.68	32.41	30.78	31.01	28.49	31.37
474d55b3	32.02	31.33	30.56	31.27	22.17	28.63
39882ea0	32.16	32	31.94	32.21	22.83	26.42
f5fae9a7	32.18	32.21	31.44	31.41	25.17	27.93
895f8f73	32.76	32.22	32.24	31.32	21.01	27.37
8f31d7b3	32.78	33.35	32.62	32.63	25.96	29.60
b4777cdd	33.02	32.56	32.47	31.97	25.58	28.47
ff1c66fd	33.28	32.93	34.6	35.74	26.14	27.45
aa32401d	33.35	33.04	38.11	Undet	23.25	27.07
811bd6d9	33.36	32.98	31.88	31.76	22.54	26.06
b20645cd	33.71	34.27	32.08	33.28	23.70	28.43
928a60db	34.81	34.93	33.7	33.93	25.16	29.35
ab1ad199	34.92	33.7	37.34	33.43	24.05	27.20
d6eb2c9e	34.87	Undet	34.61	38.88	24.84	28.29
2491f402	36.32	35.26	Undet	34.25	25.83	30.73

**Table S5. Concordance of 619 AN specimens between KingFisher nucleic acids extraction (KF) and EF-RT-qPCR (EF).**

	Plate 1 COV119		Plate 2 RIP107		Plate 3 COV120		Plate 4 RIP108		Plate 5 RIP109		Plate 6 RIP112		Plate 7 COV179	
<b>Swab collection type</b>	Observed		At-home		Observed		At-home		At-home		At-home		Observed , at-home	
<b>Date</b>	Oct 26		Oct 26		Oct 26		Oct 27		Oct 27		Oct 28		Nov 8	
<b>Operator</b>	SC	SC	EM	EM	EM	EM	WZ	WZ	WZ	WZ	BB	BB	BB	BB
<b>QuantStudio</b>	1	2	1	2	1	2	1	2	1	2	2	1	1	2
<b>Clinical result</b>	KF	EF	KF	EF	KF	EF	KF	EF	KF	EF	KF	EF	KF	EF
<b>Positive</b>	0	0	4	4	0	0	3	2	0	0	1	1	1	1
<b>Low- positive (inconclusive )</b>	1	0	0	0	0	0	0	2	1	0	0	0	0	0
<b>Negative</b>	87	88	83	83	87	88	87	86	89	90	85	84	89	89
<b>RNase P failures</b>	0	0	0	0	1	0	0	0	0	0	0	1	0	0
<b>Total</b>	88	88	87	87	88	88	90	90	90	90	86	86	90	90



**Table S6. Concordance of prospectively collected swab specimens with or without extraction**

		<b>SARS-CoV-2 test with KingFisher nucleic acids extraction</b>			
		positive	inconclusive	negative	test fail
<b>SwabExpress</b>	positive	8	0	0	0
	inconclusive	1	0	1	0
	negative	0	2	605	1
	test fail	0	0	1	0
Total		9	2	607	1
positive agreement (9/9)		100%			
inconclusive agreement (0/2)		0%			
negative agreement (605/606)		99.8%			
failed due to poor quality (1/619)		0.16%			

**Table S7. Comparison of Ct values for SARS-CoV-2 targets from specimens by the SARS-CoV-2 test with KingFisher extraction (KF) and SwabExpress (EF)**

	KF - Orf1b	KF Spike	EF - Orf1b	EF - S gene	KF	EF	ΔCt	ΔCt
Clinical Sample	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Clinical Result	Clinical Result	Orf1b	Spike
87359aac	36.85 *	38.32 *	undet	undet	incon	neg	N/A	N/A
7bd7b5e1	27.41 ± 0.11	25.42 ± 0.08	29.92 ± 0.00	29.05 ± 0.03	pos	pos	2.51	3.63
d0023767	26.07 ± 0.01	25.48 ± 0.18	27.92 ± 0.11	27.15 ± 0.13	pos	pos	1.85	1.67
4757675b	29.62 ± 0.11	27.95 ± 0.10	30.25 ± 0.02	28.61 ± 0.42	pos	pos	0.63	0.66
7a22a1f3	27.88 ± 0.13	27.1 ± 0.21	28.67 ± 0.14	27.51 ± 0.06	pos	pos	0.79	0.41
57f5bb53	13.50 ± 0.11	12.55 ± 0.45	15.66 ± 0.05	15.04 ± 0.35	pos	pos	2.16	2.49
19344233	20.18 ± 0.04	19.31 ± 0.23	21.66 ± 0.03	21.25 ± 0.11	pos	pos	1.48	1.94
f343051c	35.22 ± 0.92	34.82 ± 0.03	36.03 ± 0.36	undet	pos	incon	0.81	NA
cc80131a	undet	undet	undet	39.28 ± 0.60	neg	incon	NA	NA
aef02e4c	36.6 *	39.05 *	undet	undet	incon	neg	NA	NA
20e0f036	25.44 ± 0.16	24.21 ± 0.08	29.71 ± 0.29	29.50 ± 0.02	pos	pos	4.27	5.29
6b496def	16.92 ± 0.03	16.08 ± 0.13	20.07 ± 0.00	19.61 ± 0.38	pos	pos	3.15	3.53

\* only 1 replicate; pos - Positive; neg - Negative; incon - Inconclusive; undet - Undetermined Ct > 40

**Table S8. Summary of RNase P detection failure by sample (SwabExpress)**

<b>RNase P reactions:</b>	<b>Number of Specimens in group</b>
Failed 1 RNase P reaction	563
Failed 2 RNase P reactions	151
Failed 3 RNase P reactions	38
Failed 4 RNase P reactions	194

**Table S9. Counts of Spurious (Ct < 30) SARS-CoV-2 amplification from EF-RT-qPCR**

<b>Target</b>	<b>Number of specimens with one well of spurious amplification</b>
Orf1b	35
S gene	194

**Table S10. Comparison of RNase P detection failure with and without Proteinase K (PK) digestion**

		<b>EF-RT-qPCR (-PK) RNase P amplification</b>	<b>SwabExpress (+PK) RNase P amplification</b>
<b>Plate</b>	<b>Instance Number</b>	<b># wells amplified/total # wells</b>	<b># wells amplified/total # wells</b>
COV320	1	3/4	4/4
COV326	1	3/4	4/4
COV331	1	1/4	4/4
	2	0/4	4/4
	3	4/4	3/4
	4	3/4	4/4
COV351	1	3/4	4/4
	2	3/4	4/4
	3	3/4	4/4
	4	3/4	4/4
	5	3/4	4/4
COV352	1	2/4	4/4
	2	1/4	4/4
	3	2/4	4/4
	4	3/4	4/4
	5	3/4	4/4
	6	3/4	4/4
COV358	1	3/4	4/4
	2	1/4	4/4
COV360	1	4/4	3/4
total		27/4888 (0.55%)	2/4888 (0.04%)

**Table S11. Concordance of clinical results for specimens with total nucleic acids extraction (Kingfisher Flex), heat treatment (EF-RT-qPCR) or Proteinase K digestion plus heat treatment (SwabExpress)**

	Sample Prep Method		
Qualitative Result	KingFisher Flex	EF-RT-qPCR	SwabExpress
# Positive	23	24	28
# Inconclusive	3	3	0
# Negative	4	3	2

**Table S12. Concordance of prospectively collected swab specimens from participants with or without extraction**

		<b>SARS-CoV-2 test with KingFisher nucleic acids extraction</b>			
		positive	inconclusive	negative	test fail
<b>SwabExpress</b>	positive	9	1	0	0
	inconclusive	0	0	1	0
	negative	0	0	1157	1
	test fail	0	0	0	0
Total		9	1	1158	1
positive agreement (9/9)		100%			
inconclusive agreement (0/1)		0%			
negative agreement (1157/1158)		99.91%			
failed due to poor quality (1/1169)		0.08%			

**Table 13. Mean  $\pm$  SD of 75 positive specimens extracted on the Roche Magna Pure 96 (MP96) or processed SwabExpress (SE) protocol and amplified using the N1 and N2 CDC probe sets.**

Probe	MP96 Mean $\pm$ SD	SwabExpress Mean $\pm$ SD	$\Delta$ Ct [SE-MP96]
N1	19.22 $\pm$ 3.67	21.79 $\pm$ 4.33	2.57
N2	18.31 $\pm$ 3.73	19.80 $\pm$ 3.72	1.49



**Table S14. SwabExpress per sample cost breakdown.** Values displayed in US dollars.

<b>Item</b>	<b>SwabExpress</b>
US Cotton #3 Swab	\$0.29
Transport Tube	\$0.61
Barcode Stickers	\$0.50
Low TE	\$0.05
Proteinase K	\$0.14
RT-qPCR Reagents	\$2.67
Lab Consumables	\$1.72