

Appendix A.
Supplementary Data

For

Development of a Rapid Viability RT-PCR (RV-RT-PCR) Method to Detect Infectious SARS-CoV-2 from Swabs

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Table S1 RV-RT-PCR Results for SARS-CoV-2-Infected Vero E6 Cells with 2-hr Infection

Estimated PFU/Sample ^a	Sample Replicate	RT-PCR Replicate	C _T by Post-Infection Incubation Timepoint After 2-hr Infection ^b			Avg. ΔC _T (SD) ^c	
			T ₀	T ₁₂	T ₂₄	T ₁₂	T ₂₄
440	1	1	28.4	17.7	12.2	12.5 (0.1)	15.2 (0.1)
		2	28.7	17.5	12.0		
		3	28.6	17.9	12.2		
		Avg (SD)	28.6 (0.2)	17.7 (0.2)	12.1 (0.1)		
	2	1	29.0	15.4	12.6		
		2	28.7	15.6	12.6		
		3	29.0	15.5	12.6		
		Avg (SD)	28.9 (0.1)	15.5 (0.1)	12.6 (0.05)		
	3	1	29.1	15.8	16.2		
		2	29.0	15.9	16.3		
		3	28.9	15.8	16.2		
		Avg (SD)	29.0 (0.1)	15.8 (0.04)	16.2 (0.1)		
	Overall Avg. (SD)		28.8 (0.2)	16.3 (1.0)	13.6 (1.9)		
44	1	1	31.9	18.5	13.2	13.0 (4.1)	15.7 (4.4)
		2	32.4	18.5	13.3		
		3	32.7	18.6	13.4		
		Avg (SD)	32.3 (0.4)	18.5 (0.1)	13.3 (0.1)		
	2	1	27.9	18.5	17.8		
		2	27.8	18.6	17.8		
		3	27.8	18.5	17.8		
		Avg (SD)	27.9 (0.1)	18.5 (0.1)	17.8 (0.03)		
	3	1	34.6	18.9	16.6		
		2	34.8	18.8	16.7		
		3	34.1	18.8	16.7		
		Avg (SD)	34.5 (0.4)	18.8 (0.1)	16.6 (0.1)		
	Overall Avg. (SD)		31.6 (2.9)	18.6 (0.2)	15.9 (2.0)		
4	1	1	ND	19.4	13.7	23.0 (1.6)	23.6 (5.7)
		2	ND	19.5	13.6		
		3	ND	19.6	13.5		
		Avg (SD)	ND (NA)	19.5 (0.1)	13.6 (0.1)		
	2	1	ND	24.6	19.2		
		2	37.4	24.5	19.3		
		3	ND	24.5	19.3		
		Avg (SD)	ND (NA)	24.6 (0.1)	19.3 (0.1)		
	3	1	41.8	21.8	33.6		
		2	ND	21.8	27.1		
		3	ND	21.9	33.3		
		Avg (SD)	ND (NA)	21.8 (0.1)	31.3 (3.7)		
	Overall Avg. (SD)		ND (NA)	22.0 (2.2)	21.4 (8.0)		

^a Values are based on dilution of the SARS-CoV-2 titrated suspension (Avg. TCID₅₀ = 10^{5.5} per 0.1 mL or 2.2 × 10⁵ PFU/0.1 mL) with 0.1 mL used for each T₀ and T_f (total 0.2 mL). TCID₅₀/0.1 mL with SD = 2.19–4.56 × 10⁵ and PFU/0.1 mL with SD = 1.54–3.19 × 10⁵. Estimated PFU/Sample = TCID₅₀/Sample (corrected for dilution) × 0.7.

^b Avg. and SD are based on triplicate RT-PCR analyses. The overall Avg. and SD are based on three replicate samples. The overall SD is based on the following equation:

$$\text{Overall or joint SD} = \sqrt{\{(n_1 - 1)s_1^2 + (n_2 - 1)s_2^2 + (n_3 - 1)s_3^2 + (n_1 \times [X_1 - \bar{X}]^2) + (n_2 \times [X_2 - \bar{X}]^2) + (n_3 \times [X_3 - \bar{X}]^2)\} / (n_1 + n_2 + n_3 - 1)}$$

^c SD represents the pooled SD which equals the square root of the following: (SD for T₀ values squared plus the SD for the T_f values squared)/2, where T_f equals T₁₂ or T₂₄.

PFU = Plaque Forming Units; TCID₅₀ = 50% Tissue Culture Infectious Dose; C_T = Cycle Threshold; Avg. = Average; SD = Standard Deviation; ND = Not Detected; NA = Not Applicable.

Table S2 RV-RT-PCR Results for SARS-CoV-2-Infected Vero E6 Cells with 2-hr Infection – Replicate Experiment

Estimated PFU/Sample ^a	Sample Replicate	RT-PCR Replicate	C _T by Post-Infection Incubation Timepoint After 2-hr Infection ^b			Avg. ΔC _T (SD) ^c	
			T ₀	T ₁₂	T ₂₄	T ₁₂	T ₂₄
248	1	1	29.1	14.4	9.5	13.7 (0.6)	18.7 (0.6)
		2	29.4	14.4	9.6		
		3	29.2	14.4	9.6		
		Avg. (SD)	29.2 (0.1)	14.4 (0.01)	9.6 (0.1)		
	2	1	28.3	16.2	10.1		
		2	28.5	16.2	10.2		
		3	28.4	16.2	10.1		
		Avg. (SD)	28.4 (0.1)	16.2 (0.03)	10.1 (0.03)		
	3	1	29.4	15.4	11.0		
		2	29.1	15.3	11.0		
		3	29.4	15.4	11.2		
		Avg. (SD)	29.3 (0.2)	15.3 (0.04)	11.1 (0.1)		
	Overall Avg. (SD)		29.0 (0.4)	15.3 (0.8)	10.3 (0.7)		
25	1	1	32.6	18.4	12.8	12.3 (0.8) ^d	17.4 (0.8)
		2	32.1	18.5	12.6		
		3	31.6	18.5	12.8		
		Avg. (SD)	32.1 (0.5)	18.5 (0.1)	12.7 (0.1)		
	2	1	32.6	21.2	15.6		
		2	31.9	21.2	15.6		
		3	31.7	21.2	15.6		
		Avg. (SD)	32.1 (0.5)	21.2 (0.03)	15.6 (0.01)		
	3	1	32.4	34.4	16.0		
		2	32.2	34.8	15.9		
		3	32.1	35.2	15.8		
		Avg. (SD)	32.3 (0.1)	34.8 (0.4)	15.9 (0.1)		
	Overall Avg. (SD)		32.1 (0.3)	19.8 (1.4)^d	14.7 (1.5)		

^a Values are based on dilution of the SARS-CoV-2 titrated stock suspension (Avg. TCID₅₀ = 10^{5.25} per 0.1 mL or 1.24 × 10⁵ PFU/0.1 mL) and based on 0.1 mL used for T₀ and 0.1 mL used for T_F (total 0.2 mL). TCID₅₀/0.1 mL with SD = 1.22–2.60 × 10⁵ and PFU/0.1 mL with SD = 0.85–1.82 × 10⁵. Estimated PFU/Sample = TCID₅₀/Sample (corrected for dilution) × 0.7.

^b Avg. and SD are based on triplicate RT-PCR analyses. The overall Avg. and SD are based on three replicate samples except where noted. The overall SD is based on the following equation:

$$\text{Overall or joint SD} = \sqrt{\{(n_1 - 1)s_1^2 + (n_2 - 1)s_2^2 + (n_3 - 1)s_3^2 + (n_1 \times [X_1 - \bar{X}]^2) + (n_2 \times [X_2 - \bar{X}]^2) + (n_3 \times [X_3 - \bar{X}]^2)\} / (n_1 + n_2 + n_3 - 1)}$$

^c SD represents the pooled SD which equals the square root of the following: (SD for T₀ values squared plus the SD for the T_F values squared)/2, where T_F equals T₁₂ or T₂₄.

^d Overall Avg. (SD) and Avg. ΔC_T (SD) are based on 2 positive replicate samples; one replicate had higher C_T values (~34-35) and was not included in the average and SD or pooled SD.

PFU = Plaque Forming Units; TCID₅₀ = 50% Tissue Culture Infectious Dose; C_T = Cycle Threshold; Avg. = Average; SD = Standard Deviation.

Table S3 RV-RT-PCR Results for SARS-CoV-2-Infected Vero E6 Cells with 1-hr Infection – Replicate Experiment

Estimated PFU/Sample ^a	Sample Replicate	RT-PCR Replicate	C _T by Post-Infection Incubation Timepoint After 1-hr Infection ^b			Avg. ΔC _T (SD) ^c	
			T ₀	T ₁₂	T ₂₄	T ₁₂	T ₂₄
248	1	1	29.2	17.6	11.4	11.5 (0.3)	16.8 (0.8)
		2	29.2	17.5	11.5		
		3	29.0	17.4	11.4		
		Avg. (SD)	29.1 (0.1)	17.5 (0.1)	11.4 (0.1)		
	2	1	29.5	18.3	13.9		
		2	29.6	18.3	14.0		
		3	29.4	18.3	13.8		
		Avg. (SD)	29.5 (0.1)	18.3 (0.02)	13.9 (0.1)		
	3	1	29.3	17.8	12.5		
		2	29.5	17.8	12.4		
		3	29.6	17.8	12.4		
		Avg. (SD)	29.5 (0.2)	17.8 (0.01)	12.4 (0.04)		
	Overall Avg. (SD)		29.4 (0.2)	17.9 (0.4)	12.6 (1.1)		
25	1	1	33.5	21.8	14.7	12.7 (2.1) ^d	21.3 (2.0) ^d
		2	33.8	22.2	14.6		
		3	34.1	22.3	14.6		
		Avg. (SD)	33.8 (0.3)	22.1 (0.3)	14.7 (0.1)		
	2	1	37.4	38.9	14.9		
		2	38.1	37.7	14.9		
		3	35.6	37.1	14.6		
		Avg. (SD)	37.1 (1.3)	37.9 (0.9)	14.8 (0.2)		
	3	1	36.2	24.4	36.3		
		2	39.0	24.7	34.6		
		3	36.1	24.2	34.4		
		Avg. (SD)	37.1 (1.7)	24.4 (0.3)	35.1 (1.1)		
	Overall Avg. (SD)		36.0 (2.0)	23.3 (1.0)^d	14.7 (0.1)^d		

^a Values are based on dilution of the SARS-CoV-2 titered stock suspension (Avg. TCID₅₀ = 10^{5.25} per 0.1 mL or 1.24 × 10⁵ PFU/0.1 mL) and based on 0.1 mL used for T₀ and 0.1 mL used for T_f (total 0.2 mL). TCID₅₀/0.1 mL with SD = 1.22–2.60 × 10⁵ and PFU/0.1 mL with SD = 0.85–1.82 × 10⁵. Estimated PFU/Sample = TCID₅₀/Sample (corrected for dilution) × 0.7.

^b Avg. and SD are based on triplicate RT-PCR analyses. The overall Avg. and SD are based on three replicate samples except where noted. The overall SD is based on the following equation:

$$\text{Overall or joint SD} = \sqrt{\{(n_1 - 1)s_1^2 + (n_2 - 1)s_2^2 + (n_3 - 1)s_3^2 + (n_1 \times [X_1 - \bar{X}]^2) + (n_2 \times [X_2 - \bar{X}]^2) + (n_3 \times [X_3 - \bar{X}]^2)\} / (n_1 + n_2 + n_3 - 1)}$$

^c SD represents the pooled SD which equals the square root of the following: (SD for T₀ values squared plus the SD for the T_f values squared)/2, where T_f equals T₁₂ or T₂₄.

^d Overall Avg. (SD) and Avg. ΔC_T (SD) are based on 2 positive replicate samples; one replicate had high C_T values (~34-36 or ~37-39) and was not included in the average and SD or pooled SD.

PFU = Plaque Forming Units; TCID₅₀ = 50% Tissue Culture Infectious Dose; C_T = Cycle Threshold; Avg. = Average; SD = Standard Deviation.

Table S4 Recovery Efficiency of SARS-CoV-2 from Swab Swatches

Estimated Starting PFU/ Swatch^a	Swatch Replicate	Recovered PFU/ Swatch^b	Percent Recovery (%)	PFU for RV-RT-PCR Analysis^c
514	1	111	21.5	44.3
	2	350	68.1	140.0
	3	197	38.3	78.7
	Avg. (SD)	219 (121)	42.7 (23.6)	87.7 (48.5)
257	1	197	76.6	78.7
	2	35	13.6	14.0
	3	62	24.2	24.9
	Avg. (SD)	98 (87)	38.2 (33.7)	39.2 (34.7)

^a Values are based on dilutions from the SARS-CoV-2 stock titrated for the experiment (TCID50 = $10^{5.17}/0.1$ mL or $\sim 1.03 \times 10^5$ PFU/0.1 mL), using 0.5 mL of either -3 or -3.3 log₁₀ dilution per swab swatch. TCID50/0.1 mL with SD = $0.99\text{--}2.17 \times 10^5$ and PFU/0.1 mL with SD = $0.69\text{--}1.52 \times 10^5$. PFU/Swatch = TCID50/Swatch (corrected for dilution) $\times 0.7$.

^b Values are based on TCID50 analysis of UF-retentates from the same swatch used for RV-RT-PCR analysis.

^c Values are based on TCID50 analysis of swatch UF-retentates using 0.2 mL as the total volume since 0.1 mL was used for T₀ and 0.1 mL was used T₉ for RV-RT-PCR analysis.

PFU = Plaque Forming Units; TCID50 = 50% Tissue Culture Infectious Dose; Avg. = Average; SD = Standard Deviation.

Table S5 RV-RT-PCR and TCID50 Results for SARS-CoV-2-Spiked Swab Swatches Processed and Used to Infect Vero E6 Cells with 2-hr Infection

Estimated PFU/Swatch ^a	Swatch Replicate	PFU for RV-RTPCR Analysis ^b	RT-PCR Replicate	C _T by Post-Infection Incubation Timepoint After 2-hr Infection ^c		Avg. (SD) ΔC _T ^d
				T ₀	T ₉	
514	1	44.3	1	32.4	21.4	11.2 (0.2)
			2	32.7	21.3	
			3	32.3	21.3	
			Avg. (SD)	32.5 (0.2)	21.3 (0.1)	
	2	140	1	32.1	22.4	9.8 (0.1)
			2	32.1	22.5	
			3	32.4	22.4	
			Avg. (SD)	32.2 (0.1)	22.4 (0.04)	
	3	78.7	1	31.8	21.3	10.5 (0.1)
			2	31.9	21.2	
			3	31.8	21.4	
			Avg. (SD)	31.8 (0.1)	21.3 (0.1)	
	Avg. (SD)	87.7 (48.5)	Overall Avg. (SD)	32.2 (0.3)	21.7 (0.6)	10.5 (0.6)
257	1	78.7	1	33.2	25.5	7.6 (0.1)
			2	32.9	25.5	
			3	33.1	25.5	
			Avg. (SD)	33.1 (0.2)	25.5 (0.04)	
	2	14.0	1	33.7	22.4	11.2 (0.1)
			2	33.8	22.5	
			3	33.4	22.5	
			Avg. (SD)	33.6 (0.2)	22.5 (0.03)	
	3	24.9	1	33.4	24.0	9.4 (0.2)
			2	33.4	23.8	
			3	33.0	23.9	
			Avg. (SD)	33.3 (0.2)	23.9 (0.1)	
	Avg. (SD)	39.2 (34.7)	Overall Avg. (SD)	33.3 (0.3)	24.0 (1.3)	9.4 (1.6)

^a Values are based on dilutions from a TCID50-titered SARS-CoV-2 stock (TCID50 = 10^{5.17}/0.1 mL or 1.03 × 10⁵ PFU/0.1 mL). Estimated PFU/Swatch = TCID50/Swatch (corrected for dilution) × 0.7.

^b Values are based on TCID50 analysis of swatch UF-retentates using 0.2 mL as the total volume since 0.1 mL was used for T₀ and 0.1 mL was used T₉ for RV-RTPCR analysis (see Table S4).

^c Avg. and SD are based on triplicate RT-PCR analyses. The overall Avg. and SD are based on three replicate swatches. The overall SD is based on the following equation:

$$\text{Overall or joint SD} = \sqrt{\{(n_1 - 1)s_1^2 + (n_2 - 1)s_2^2 + (n_3 - 1)s_3^2 + (n_1 \times [X_1 - \bar{X}]^2) + (n_2 \times [X_2 - \bar{X}]^2) + (n_3 \times [X_3 - \bar{X}]^2)\}/(n_1 + n_2 + n_3 - 1)}$$

^d SD represents the pooled SD which equals the square root of the following: (SD for T₀ values squared plus the SD for the T₉ values squared)/2.

PFU = Plaque Forming Units; TCID50 = 50% Tissue Culture Infectious Dose; C_T = Cycle Threshold; Avg. = Average; SD = Standard Deviation.

Table S6 Recovery Efficiency of SARS-CoV-2 from Swab Swatches – Replicate Experiment

Estimated Starting PFU/ Swatch ^a	Swatch Replicate	Recovered PFU/ Swatch ^b	Percent Recovery (%)	PFU for RV-RT-PCR Analysis ^c
134	1	62.2	46.4	24.9
	2	>134	>100	>55.6
	3	62.2	46.4	24.9
	Avg. (SD)	62.2 (0)	46.4 (0)	24.9 (0)
67	1	35.0	52.2	14.0
	2	>67	>100	>28
	3	19.7	29.4	7.9
	Avg. (SD)	27.3 (10.8)	40.8 (16.2)	10.9 (4.3)

^a Values are based on dilutions from the SARS-CoV-2 stock titrated for the experiment (TCID50 10^{4.6}/0.1 mL or 2.68 × 10⁴ PFU/0.1 mL), using 0.5 mL of either -3 or -3.3 log₁₀ dilution per swab swatch. TCID50/0.1 mL with SD = 3.2–4.6 × 10⁴ and PFU/0.1 mL with SD = 2.2–3.25 × 10⁴. Estimated PFU/Swatch = TCID50/Swatch (corrected for dilution) × 0.7.

^b Values are based on TCID50 analysis of swatch UF-retentates from sample processing. TCID50 values showing >100% recovery were not included in calculation of the average recovery (see text below for explanation).

^c Values are based on TCID50 analysis of swatch UF-retentates using 0.2 mL as the total volume since 0.1 mL was used for T₀ and 0.1 mL was used T₉ for RV-RT-PCR analysis.

PFU = Plaque Forming Units; TCID50 = 50% Tissue Culture Infectious Dose; Avg. = Average; SD = Standard Deviation.

The recovery efficiency values ≥ 100% were not included in the calculation of average percent recovery (for 67 and 134 PFU/Swatch, one replicate for each viral level), because these values were inconsistent with the other swatch replicates, and likely represent technical error in swatch set-up or processing.

Table S7 RV-RT-PCR and TCID50 Results for SARS-CoV-2-Spiked Swab Swatches Processed and Used to Infect Vero E6 Cells with 2-hr Infection – Replicate Experiment

Estimated PFU/Swatch ^a	Swatch Replicate	PFU for RV-RTPCR Analysis ^b	RT-PCR Replicate	C _T by Post-Infection Incubation Timepoint After 2-hr Infection ^c		Avg. ΔC _T (SD) ^d
				T ₀	T ₉	
134	1	24.9	1	32.9	23.1	9.7 (0.09)
			2	32.7	23.2	
			3	33.0	23.1	
			Avg. (SD)	32.9 (0.1)	23.1 (0.04)	
	2	>55.6	1	34.1	26.3	7.6 (0.3)
			2	33.5	26.3	
			3	34.3	26.4	
			Avg. (SD)	34.0 (0.4)	26.3 (0.1)	
	3	24.9	1	33.4	26.7	6.8 (0.3)
			2	33.1	26.6	
			3	33.8	26.6	
			Avg. (SD)	33.5 (0.4)	26.6 (0.02)	
	Avg. (SD)	24.9 (0)	Overall Avg. (SD)	33.4 (0.5)	25.4 (1.7)	8.1 (1.8)
67	1	14.0	1	35.1	26.8	8.3 (0.1)
			2	35.2	26.7	
			3	35.0	26.9	
			Avg. (SD)	35.1 (0.1)	26.8 (0.1)	
	2	>28	1	35.4	23.7	11.4 (0.3)
			2	35.4	23.8	
			3	34.6	23.7	
			Avg. (SD)	35.1 (0.5)	23.7 (0.02)	
	3	7.9	1	35.5	35.9	-0.5 (0.2)
			2	35.6	36.2	
			3	35.4	35.8	
			Avg. (SD)	35.5 (0.1)	36.0 (0.2)	
	Avg. (SD)	10.9 (4.3)	Overall Avg. (SD) (3 Reps)	35.2 (0.3)	28.8 (5.5)	6.4 (5.3)
Overall Avg. (SD) (2 Reps)			35.1 (0.3)	25.3 (1.7)	9.8 (1.7)	

^a Values are based on dilutions from a TCID50-titered SARS-CoV-2 stock (TCID50 10^{4.6}/0.1 mL or 2.68 × 10⁴ PFU/0.1 mL). Estimated PFU/Swatch = TCID50/Swatch (corrected for dilution) × 0.7.

^b Values are based on TCID50 analysis of swatch UF-retentates using 0.2 mL as the total volume since 0.1 mL was used for T₀ and 0.1 mL was used T₉ for RV-RTPCR analysis (see Table S6), where PFU/Swatch = TCID50/Swatch (corrected for dilution) × 0.7. TCID50 values showing >100% recovery were not included in calculation of the average recovery.

^c Avg. and SD are based on triplicate RT-PCR analyses. The overall Avg. and SD are based on three replicate swatches unless specified as 2 or 3 replicates (Reps). The overall SD is based on the following equation:

$$\text{Overall or joint SD} = \sqrt{\{(n_1 - 1)s_1^2 + (n_2 - 1)s_2^2 + (n_3 - 1)s_3^2 + (n_1 \times [X_1 - \bar{X}]^2) + (n_2 \times [X_2 - \bar{X}]^2) + (n_3 \times [X_3 - \bar{X}]^2)\} / (n_1 + n_2 + n_3 - 1)}$$

^d SD represents the pooled SD which equals the square root of the following: (SD for T₀ values squared plus the SD for the T₉ values squared)/2.

PFU = Plaque Forming Units; TCID50 = 50% Tissue Culture Infectious Dose; C_T = Cycle Threshold; Avg. = Average; SD = Standard Deviation; Reps = Replicates.

Table S8 Recovery Efficiency of SARS-CoV-2 from Swabs

Estimated Starting PFU/ Swab ^a	Swab Replicate	Recovered PFU/ Swab ^b	Percent Recovery (%)	Estimated Avg. PFU for RV-RT-PCR Analysis ^c
6200	1	6220	100	NA
	2	1970	31.6	
	3	1970	31.6	
	Avg. (SD)	3390 (2460)	54 (40)	
620	1	350	56.2	~220
	2	111	17.8	
	3	197	31.6	
	Avg. (SD)	219 (121)	35 (20)	
Avg. Percent Recovery			44.8	
310		Not Determined	Not Determined	~139
62		Not Determined	Not Determined	~28

^a Values are based on dilutions from the SARS-CoV-2 stock titrated for the experiment (TCID50 10^{5.25}/0.1 mL or 1.24 × 10⁵ PFU/0.1 mL), using 0.5 mL of either -2 or -3 log₁₀ dilution per swab, 6200 or 620 PFU, respectively. TCID50/0.1 mL with SD = 1.32–2.40 × 10⁵ and PFU/0.1 mL with SD = 0.92–1.68 × 10⁵. Estimated PFU/Swab = TCID50/Swab (corrected for dilution) × 0.7.

^b Values are based on TCID50 analysis of UF-retentates from replicate swabs. Avg. and SD are based on three replicate PFU/Swab values for each viral dilution tested.

^c Values are based on actual measurement of recovered virus by TCID50 analysis for swabs spiked with 6200 and 620 PFU and calculated for swabs spiked with 310 or 62 PFU using an average recovery efficiency (determined from the other virus dilutions). Recovered PFU in ~0.2 mL were split between T₀ and T₉ swab sample wells.

NA: This viral dilution was not used to spike swab samples.

Not Determined: Recovery efficiency was not determined directly from these viral dilutions.

PFU = Plaque Forming Units; TCID50 = 50% Tissue Culture Infectious Dose; Avg. = Average; SD = Standard Deviation; NA = Not Applicable.

Table S9 RV-RT-PCR Results (N1 Assay) for SARS-CoV-2-Spiked Swabs Processed and Used to Infect Vero E6 Cells with 2-hr Infection

Estimated Starting PFU/Swab ^a	Estimated Avg. PFU Recovered ^b	Swab Replicate	RT-PCR Replicate	C _T by Post-Infection Incubation Timepoint After 2-hr Infection ^c		Avg. ΔC _T (SD) ^d
				T ₀	T ₉	
620	220	1	1	31.0	20.7	10.2 (0.1)
			2	30.9	20.8	
			3	31.1	20.9	
			Avg. (SD)	31.0 (0.1)	20.8 (0.1)	
		2	1	30.7	19.0	11.6 (0.1)
			2	30.6	19.2	
			3	30.6	19.0	
			Avg. (SD)	30.7 (0.1)	19.0 (0.1)	
		3	1	31.4	20.5	11.1 (0.2)
			2	31.3	20.3	
			3	31.3	19.9	
			Avg. (SD)	31.4 (0.1)	20.2 (0.3)	
		Overall Avg. (SD)			31.0 (0.3)	20.0 (0.8)
310	139	1	1	32.1	21.1	11.0 (0.1)
			2	31.9	21.1	
			3	32.1	21.0	
			Avg. (SD)	32.0 (0.2)	21.0 (0.1)	
		2	1	32.3	21.5	10.9 (0.2)
			2	32.4	21.0	
			3	31.9	21.5	
			Avg. (SD)	32.2 (0.2)	21.3 (0.3)	
		3	1	31.8	21.0	10.9 (0.1)
			2	31.8	20.9	
			3	31.9	20.9	
			Avg. (SD)	31.9 (0.1)	21.0 (0.08)	
		Overall Avg. (SD)			32.0 (0.2)	21.1 (0.2)

^a Values are based on dilutions from a TCID50-titered SARS-CoV-2 stock (TCID50 10^{5.25}/0.1 mL or 1.24 × 10⁵ PFU/0.1 mL). Estimated PFU/Swab = TCID50/Swab (corrected for dilution) × 0.7.

^b Avg. values are based on SARS-CoV-2 recovery determined from replicate swab samples (see Table S8).

^c Avg. and SD are based on triplicate RT-PCR analyses. The overall Avg. and SD are based on three replicate swabs. The overall SD is based on the following equation:

$$\text{Overall or joint SD} = \sqrt{\{(n_1 - 1)s_1^2 + (n_2 - 1)s_2^2 + (n_3 - 1)s_3^2 + (n_1 \times [X_1 - \bar{X}]^2) + (n_2 \times [X_2 - \bar{X}]^2) + (n_3 \times [X_3 - \bar{X}]^2)\} / (n_1 + n_2 + n_3 - 1)}$$

^d SD represents the pooled SD, which equals the square root of the following: (SD for T₀ values squared plus the SD for the T₉ values squared)/2.

PFU = Plaque Forming Units; TCID50 = 50% Tissue Culture Infectious Dose; C_T = Cycle Threshold; Avg. = Average; SD = Standard Deviation.

Table S9 RV-RT-PCR Results (N1 Assay) for SARS-CoV-2-Spiked Swabs Processed and Used to Infect Vero E6 Cells with 2-hr Infection (Cont'd)

Estimated Starting PFU/Swab ^a	Estimated Avg. PFU Recovered ^b	Swab Replicate	RT-PCR Replicate	C _T by Post-Infection Incubation Timepoint After 2-hr Infection ^c		Avg. ΔC _T (SD) ^d
				T ₀	T ₉	
62	28	1	1	35.4	22.2	13.4 (0.4)
			2	35.1	22.2	
			3	36.1	22.2	
			Avg. (SD)	35.5 (0.5)	22.2 (0.03)	
		2	1	35.4	22.5	12.5 (0.3)
			2	35.2	22.5	
			3	34.4	22.6	
			Avg. (SD)	35.0 (0.5)	22.5 (0.1)	
		3	1	35.2	23.3	12.2 (0.4)
			2	36.1	23.2	
			3	35.0	23.2	
			Avg. (SD)	35.4 (0.6)	23.2 (0.04)	
		Overall Avg. (SD)			35.3 (0.5)	22.7 (0.5)

^a Values are based on dilutions from a TCID50-titered SARS-CoV-2 stock (TCID50 10^{5.25}/0.1 mL or 1.24 × 10⁵ PFU/0.1 mL). Estimated PFU/Swab = TCID50/Swab (corrected for dilution) × 0.7.

^b Avg. values are based on SARS-CoV-2 recovery determined from replicate swab samples (see Table S8).

^c Avg. and SD are based on triplicate RT-PCR analyses. The overall Avg. and SD are based on three replicate swabs. The overall SD is based on the following equation:

$$\text{Overall or joint SD} = \sqrt{\{(n_1 - 1)s_1^2 + (n_2 - 1)s_2^2 + (n_3 - 1)s_3^2 + (n_1 \times [X_1 - \bar{X}]^2) + (n_2 \times [X_2 - \bar{X}]^2) + (n_3 \times [X_3 - \bar{X}]^2)\} / (n_1 + n_2 + n_3 - 1)}$$

^d SD represents the pooled SD, which equals the square root of the following: (SD for T₀ values squared plus the SD for the T₉ values squared)/2.

PFU = Plaque Forming Units; TCID50 = 50% Tissue Culture Infectious Dose; C_T = Cycle Threshold; Avg. = Average; SD = Standard Deviation.

Table S10 RV-RT-PCR Results (N2 Assay) for SARS-CoV-2-Spiked Swabs Processed and Used to Infect Vero E6 Cells with 2-hr Infection

Estimated Starting PFU/Swab ^a	Estimated Avg. PFU Recovered ^b	Swab Replicate	RT-PCR Replicate	C _T by Post-Infection Incubation Timepoint After 2-hr Infection ^c		Avg. ΔC _T (SD) ^d
				T ₀	T ₉	
620	220	1	1	30.1	19.4	10.7 (0.1)
			2	30.2	19.4	
			3	30.2	19.5	
			Avg. (SD)	30.1 (0.1)	19.4 (0.1)	
		2	1	29.9	17.8	12.0 (0.1)
			2	29.9	17.9	
			3	29.8	17.8	
			Avg. (SD)	29.9 (0.1)	17.8 (0.04)	
		3	1	31.0	19.2	11.7 (0.2)
			2	30.7	19.2	
			3	30.8	18.9	
			Avg. (SD)	30.8 (0.2)	19.1 (0.2)	
		Overall Avg. (SD)		30.3 (0.4)	18.8 (0.7)	11.5 (0.6)
310	139	1	1	31.3	19.9	11.3 (0.2)
			2	31.3	20.1	
			3	31.1	19.7	
			Avg. (SD)	31.2 (0.1)	19.9 (0.2)	
		2	1	31.6	20.7	11.0 (0.1)
			2	31.6	20.5	
			3	31.7	20.6	
			Avg. (SD)	31.6 (0.04)	20.6 (0.1)	
		3	1	31.2	19.8	11.4 (0.1)
			2	31.1	19.7	
			3	31.2	19.7	
			Avg. (SD)	31.1 (0.1)	19.8 (0.05)	
		Overall Avg. (SD)		31.3 (0.2)	20.1 (0.4)	11.2 (0.2)

^a Values are based on dilutions from a TCID50-titered SARS-CoV-2 stock (TCID50 10^{5.25}/0.1 mL or 1.24 × 10⁵ PFU/0.1 mL). Estimated PFU/Swab = TCID50/Swab (corrected for dilution) × 0.7.

^b Avg. values are based on SARS-CoV-2 recovery determined from replicate swab samples (see Table S8).

^c Avg. and SD are based on triplicate RT-PCR analyses. The overall Avg. and SD are based on three replicate swabs. The overall SD is based on the following equation:

$$\text{Overall or joint SD} = \sqrt{\{(n_1 - 1)s_1^2 + (n_2 - 1)s_2^2 + (n_3 - 1)s_3^2 + (n_1 \times [X_1 - \bar{X}]^2) + (n_2 \times [X_2 - \bar{X}]^2) + (n_3 \times [X_3 - \bar{X}]^2)\} / (n_1 + n_2 + n_3 - 1)}$$

^d SD represents the pooled SD, which equals the square root of the following: (SD for T₀ values squared plus the SD for the T₉ values squared)/2.

PFU = Plaque Forming Units; TCID50 = 50% Tissue Culture Infectious Dose; C_T = Cycle Threshold; Avg. = Average; SD = Standard Deviation.

Table S10 RV-RT-PCR Results (N2 Assay) for SARS-CoV-2-Spiked Swabs Processed and Used to Infect Vero E6 Cells with 2-hr Infection (Cont'd)

Estimated Starting PFU/Swab ^a	Estimated Avg. PFU Recovered ^b	Swab Replicate	RT-PCR Replicate	C _T by Post-Infection Incubation Timepoint After 2-hr Infection ^c		Avg. ΔC _T (SD) ^d
				T ₀	T ₉	
62	28	1	1	34.6	21.1	13.7 (0.3)
			2	34.3	20.9	
			3	35.2	21.0	
			Avg. (SD)	34.7 (0.4)	21.0 (0.1)	
		2	1	34.3	21.3	12.9 (0.1)
			2	34.0	21.2	
			3	34.2	21.3	
			Avg. (SD)	34.2 (0.2)	21.3 (0.1)	
		3	1	34.8	22.4	13.0 (0.4)
			2	35.9	22.4	
			3	35.5	22.3	
			Avg. (SD)	35.4 (0.6)	22.4 (0.1)	
		Overall Avg. (SD)			34.7 (0.7)	21.6 (0.6)

^a Values are based on dilutions from a TCID50-titered SARS-CoV-2 stock (TCID50 10^{5.25}/0.1 mL or ~1.24 × 10⁵ PFU/0.1 mL). Estimated PFU/Swab = TCID50/Swab (corrected for dilution) × 0.7.

^b Avg. values are based on SARS-CoV-2 recovery determined from replicate swab samples (see Table S8).

^c Avg. and SD are based on triplicate RT-PCR analyses. The overall Avg. and SD are based on three replicate swabs. The overall SD is based on the following equation:

$$\text{Overall or joint SD} = \sqrt{\{(n_1 - 1)s_1^2 + (n_2 - 1)s_2^2 + (n_3 - 1)s_3^2 + (n_1 \times [X_1 - \bar{X}]^2) + (n_2 \times [X_2 - \bar{X}]^2) + (n_3 \times [X_3 - \bar{X}]^2)\} / (n_1 + n_2 + n_3 - 1)}$$

^d SD represents the pooled SD, which equals the square root of the following: (SD for T₀ values squared plus the SD for the T₉ values squared)/2.

PFU = Plaque Forming Units; TCID50 = 50% Tissue Culture Infectious Dose; C_T = Cycle Threshold; Avg. = Average; SD = Standard Deviation.

Table S11 Recovery Efficiency of SARS-CoV-2 from Swabs – Replicate Experiment

Estimated Starting PFU/Swab ^a	Swab Replicate	Recovered PFU/Swab ^b	Percent Recovery (%)	Estimated Avg. PFU for RV-RT-PCR Analysis ^c
3500	1	1970	56	NA
	2	3500	100	
	3	>3500	>100	
	Avg. (SD)	2730 (1080)	78 (31)	
350	1	197	56	NA
	2	111	32	
	3	>350	>100	
	Avg. (SD)	154 (61)	44 (17)	
Avg. Percent Recovery			61	
175		Not Determined	Not Determined	~107
35		Not Determined	Not Determined	~21
18		Not Determined	Not Determined	~11

^a Values are based on dilutions from the SARS-CoV-2 stock titrated for the experiment (TCID50 10⁵/0.1 mL or ~7 × 10⁴ PFU/0.1 mL), using 0.5 mL of either -2 or -3 log₁₀ dilution per swab, 3500 or 350 PFU, respectively. TCID50/0.1 mL with SD = 0.71–1.42 × 10⁵ and PFU/0.1 mL with SD = 4.95–9.90 × 10⁵. Estimated PFU/Swab = TCID50/Swab (corrected for dilution) × 0.7.

^b Values are based on TCID50 analysis of UF-retentates from replicate swabs. Avg. and SD are based on two replicate PFU/Swab values for each viral dilution tested. TCID50 values showing >100% recovery were not included in calculation of the average recovery.

^c Values are based on actual measurement of recovered virus by TCID50 analysis for swabs spiked with 3500 and 350 PFU and calculated for swabs spiked with 175, 35 or 18 PFU using an average recovery efficiency (determined from the other virus dilutions). Recovered PFU were split between T₀ and T₉ swab sample wells.

NA: This viral dilution was not used to spike swab samples.

Not Determined: Recovery efficiency was not determined directly from these viral dilutions.

PFU = Plaque Forming Units; TCID50 = 50% Tissue Culture Infectious Dose; Avg. = Average; SD = Standard Deviation; NA = Not Applicable.

As for swab swatches, individual swab sample replicates with percent recovery determined to be ≥ 100% were not included in the calculation of an average % recovery.

Table S12 RV-RT-PCR Results (N1 Assay) for SARS-CoV-2-Spiked Swabs Processed and Used to Infect Vero E6 Cells with 2-hr Infection –Replicate Experiment

Estimated Starting PFU/Swab ^a	Estimated Avg. PFU Recovered ^b	Swab Replicate	RT-PCR Replicate	C _T by Post-Infection Incubation Timepoint After 2-hr Infection ^c		Avg. ΔC _T (SD) ^d
				T ₀	T ₉	
175	107	1	1	29.8	19.1	10.9 (0.1)
			2	30.1	19.1	
			3	30.0	19.0	
			Avg. (SD)	30.0 (0.2)	19.1 (0.03)	
		2	1	30.3	17.8	12.5 (0.1)
			2	30.4	17.8	
			3	30.2	17.9	
			Avg. (SD)	30.3 (0.1)	17.8 (0.06)	
		3	1	29.9	19.1	10.8 (0.1)
			2	29.8	19.2	
			3	29.9	18.9	
			Avg. (SD)	29.9 (0.04)	19.1 (0.1)	
Overall Avg. (SD)			30.0 (0.2)	18.7 (0.6)	11.4 (0.8)	
35	21	1	1	32.9	20.2	12.9 (0.2)
			2	33.3	20.1	
			3	32.9	20.3	
			Avg. (SD)	33.1 (0.2)	20.2 (0.1)	
		2	1	33.6	20.1	13.6 (0.2)
			2	34.1	20.2	
			3	33.6	20.2	
			Avg. (SD)	33.8 (0.3)	20.2 (0.04)	
		3	1	32.7	19.4	13.5 (0.3)
			2	32.7	19.3	
			3	33.4	19.5	
			Avg. (SD)	32.9 (0.4)	19.4 (0.1)	
Overall Avg. (SD)			33.3 (0.5)	19.9 (0.4)	13.3 (0.4)	
18	11	1	1	32.8	36.3	-2.8 (0.5)
			2	33.7	36.0	
			3	33.9	36.5	
			Avg. (SD)	33.5 (0.6)	36.3 (0.3)	
		2	1	32.6	35.0	-2.0 (0.2)
			2	33.0	34.9	
			3	32.8	34.5	
			Avg. (SD)	32.8 (0.2)	34.8 (0.3)	
		3	1	33.5	35.8	-4.5 (1.5)
			2	33.0	40.0	
			3	33.8	37.9	
			Avg. (SD)	33.4 (0.4)	37.9 (2.1)	
Overall Avg. (SD)			33.2 (0.5)	36.3 (1.7)	-3.1 (1.4)	

^a Values are based on dilutions from a TCID₅₀-titered SARS-CoV-2 stock (TCID₅₀ 10⁵/0.1 mL or ~7 × 10⁴ PFU/0.1 mL). Estimated PFU/Swab = TCID₅₀/Swab (corrected for dilution) × 0.7.

^b Avg. values are based on SARS-CoV-2 recovery determined from replicate swab samples (see Table S11).

^c Avg. and SD are based on triplicate RT-PCR analyses. The overall Avg. and SD are based on three replicate swabs. The overall SD is based on the following equation:

$$\text{Overall or joint SD} = \sqrt{\{(n_1 - 1)s_1^2 + (n_2 - 1)s_2^2 + (n_3 - 1)s_3^2 + (n_1 \times [X_1 - \bar{X}]^2) + (n_2 \times [X_2 - \bar{X}]^2) + (n_3 \times [X_3 - \bar{X}]^2)\} / (n_1 + n_2 + n_3 - 1)}$$

^d SD represents the pooled SD, which equals the square root of the following: (SD for T₀ values squared plus the SD for the T₉ values squared)/2.

PFU = Plaque Forming Units; TCID₅₀ = 50% Tissue Culture Infectious Dose; C_T = Cycle Threshold; Avg. = Average; SD = Standard Deviation.

Table S13 RV-RT-PCR Results (N2 Assay) for SARS-CoV-2-Spiked Swabs Processed and Used to Infect Vero E6 Cells with 2-hr Infection – Replicate Experiment

Estimated Starting PFU/Swab ^a	Estimated Avg. PFU Recovered ^b	Swab Replicate	RT-PCR Replicate	C _T by Post-Infection Incubation Timepoint After 2-hr Infection ^c		Avg. ΔC _T (SD) ^d
				T ₀	T ₉	
175	107	1	1	28.3	17.3	11.1 (0.1)
			2	28.3	17.2	
			3	28.4	17.0	
			Avg. (SD)	28.3 (0.1)	17.2 (0.1)	
		2	1	28.4	15.6	12.8 (0.1)
			2	28.6	15.7	
			3	28.6	15.8	
			Avg. (SD)	28.5 (0.1)	15.7 (0.07)	
		3	1	28.5	17.0	11.5 (0.04)
			2	28.4	16.9	
			3	28.5	17.0	
			Avg. (SD)	28.5 (0.04)	16.9 (0.04)	
		Overall Avg. (SD)			28.4 (0.1)	16.6 (0.7)
35	21	1	1	31.5	18.0	13.7 (0.1)
			2	31.6	17.9	
			3	31.8	17.9	
			Avg. (SD)	31.6 (0.2)	17.9 (0.1)	
		2	1	32.5	17.8	14.4 (0.3)
			2	31.8	17.8	
			3	32.4	17.8	
			Avg. (SD)	32.2 (0.4)	17.8 (0.02)	
		3	1	31.5	17.3	14.2 (0.2)
			2	31.0	17.0	
			3	31.4	17.1	
			Avg. (SD)	31.3 (0.3)	17.1 (0.1)	
		Overall Avg. (SD)			31.7 (0.5)	17.6 (0.4)
18	11	1	1	31.7	34.2	-3.1 (0.5)
			2	31.6	35.5	
			3	31.7	34.8	
			Avg. (SD)	31.7 (0.1)	34.8 (0.7)	
		2	1	31.0	33.7	-2.6 (0.2)
			2	30.9	33.5	
			3	31.3	33.7	
			Avg. (SD)	31.1 (0.2)	33.6 (0.2)	
		3	1	31.7	34.4	-3.3 (1.1)
			2	31.8	37.0	
			3	32.0	34.1	
			Avg. (SD)	31.8 (0.1)	35.1 (1.6)	
		Overall Avg. (SD)			31.5 (0.4)	34.5 (1.1)

^a Values are based on dilutions from a TCID50-titered SARS-CoV-2 stock (TCID50 10⁵/0.1 mL or ~7 × 10⁴ PFU/0.1 mL). Estimated PFU/Swab = TCID50/Swab (corrected for dilution) × 0.7.

^b Avg. values are based on SARS-CoV-2 recovery determined from replicate swab samples (see Table S11).

^c Avg. and SD are based on triplicate RT-PCR analyses. The overall Avg. and SD are based on three replicate swabs. The overall SD is based on the following equation:

$$\text{Overall or joint SD} = \sqrt{\{(n_1 - 1)s_1^2 + (n_2 - 1)s_2^2 + (n_3 - 1)s_3^2 + (n_1 \times [X_1 - \bar{X}]^2) + (n_2 \times [X_2 - \bar{X}]^2) + (n_3 \times [X_3 - \bar{X}]^2)\} / (n_1 + n_2 + n_3 - 1)}$$

^d SD represents the pooled SD, which equals the square root of the following: (SD for T₀ values squared plus the SD for the T₉ values squared)/2.

PFU = Plaque Forming Units; TCID₅₀ = 50% Tissue Culture Infectious Dose; C_T = Cycle Threshold; Avg. = Average; SD = Standard Deviation.