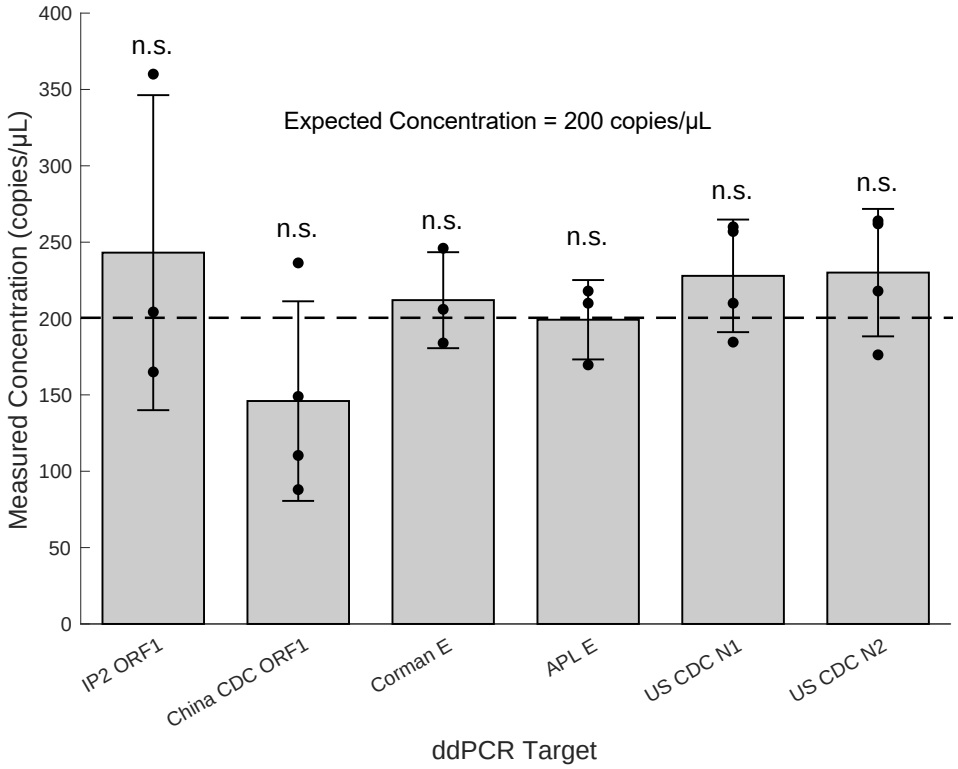
Supplemental Digital Content, Containing 4 Tables and 2 Figures.

The Supplemental Digital Content was not copyedited by *Archives of Pathology & Laboratory Medicine*.

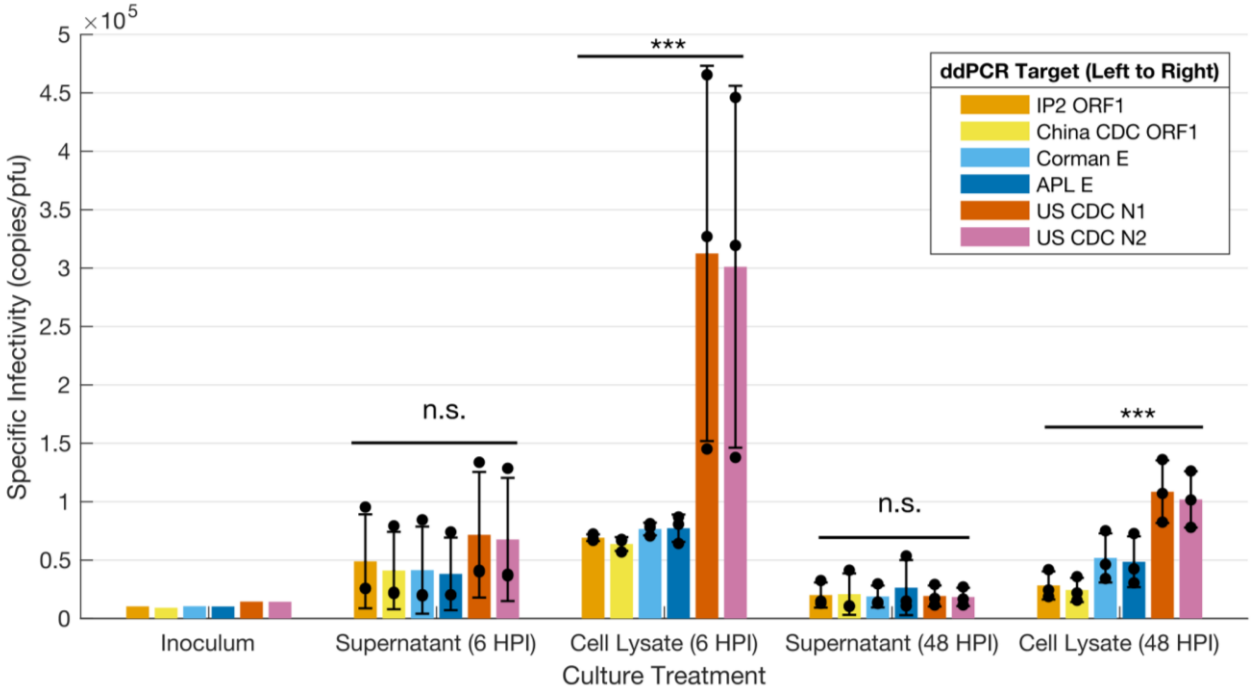
Supplemental Table 1. Cycle threshold values of clinical samples included in the study.

Sample ID#	US CDC N2 gene RT-PCR Ct Value
1	19.04
2	20.76
3	21.89
4	23.82
5	25.8
7	27.11
6	27.16
9	29.46
11	30.21
8	30.28
10	31.95

United States Centers for Disease Control and Prevention (US CDC) N2 reverse transcription-PCR (RT-PCR) cycle threshold (Ct) values of the eleven frozen clinical nasopharyngeal swabs used in this study.



Supplemental Figure 1. Quantification of the Exact Diagnostics SARS-CoV-2 quantitative positive control by each SARS-CoV-2 droplet digital PCR (ddPCR) target. All ddPCR assays quantitated the standard not significantly (n.s.) different than the expected concentration (single sample t-test, H_0=200, *P*>.05). Error bars show ± one standard deviation from the mean. United States Centers for Disease Control and Prevention (US CDC) N1 (n=4, *P*=.23), US CDC N2 (n=4, *P*=.25), Institute Pasteur 2 (IP2) ORF1 (n=3, *P*=.54), China Centers for Disease Control and Prevention (CDC) ORF1 (n=4, *P*=.20), Alberta Precision Labs (APL) E (n=3, *P*=.96), and Corman E (n=3, *P*=.58).



Supplemental Figure 2. Specific infectivity of SARS-CoV-2 cultured in Vero CCL81 cells. Error bars show ± 1 standard deviation from the arithmetic mean of 3 replicates. An analysis of variance was conducted on each culture treatment with 17 total degrees of freedom (*** = P<.001, (non-significant) n.s. = P>.05). The supernatant 6 hours post-infection (HPI) treatment was tested with the Kruskal-Wallis test (P=.43). The cell lysate (6 HPI) was tested by one-way permutational (analysis of variance) ANOVA.[1] The two 48 HPI treatments were tested with one-way ANOVA (supernatant: P=.98, cell lysate: P<.001). The inoculum was not replicated (n=1). The mean counts from each culture treatment were used to make Figure 4B. US CDC: United States Centers for Disease Control and Prevention, IP2: Institute Pasteur 2, ORF1: open reading frame 1, China CDC: Chinese Centers for Disease Control and Prevention, APL: Alberta Precision Labs.

Supplemental Table 2. Details of droplet digital PCR assays used in this study.

<table>
<tr><th>ddPCR Target</th><th>Primer/Probe Set Author</th><th>ddPCR Thermocycling Profile Used</th><th>Primer/Probe</th><th>Sequence (5'→3')</th></tr>
<tr><td rowspan="3">N1</td><td rowspan="8">United States Centers for Disease Control and Prevention US CDC[2]</td><td rowspan="8">
<table>
<tr><th>Cycling Step</th><th>Temperature (°C)</th><th>Time</th><th>Number of Cycles</th></tr>
<tr><td>Reverse Transcription</td><td>50</td><td>60 min</td><td>1</td></tr>
<tr><td>Enzyme activation</td><td>95</td><td>10 min</td><td>1</td></tr>
<tr><td>Denaturation</td><td>94</td><td>30 sec</td><td rowspan="2">40</td></tr>
<tr><td>Annealing/Extension</td><td>55</td><td>1 min</td></tr>
<tr><td>Enzyme Deactivation</td><td>98</td><td>10 min</td><td>1</td></tr>
<tr><td>Hold and Droplet Stabilization</td><td>4</td><td>∞</td><td>1</td></tr>
</table>
From [3].
</td><td>N1 Forward</td><td>GACCCCAAAATCAGCGAAAT</td></tr>
<tr><td>N1 Reverse</td><td>TCTGGTTACTGCCAGTTGAATCTG</td></tr>
<tr><td>N1 Probe</td><td>FAM – ACCCCGCATTACGTTTGGTGGACC – BHQ1</td></tr>
<tr><td rowspan="2">N2</td><td>N2 Forward</td><td>TTACAAACATTGGCCGCAAA</td></tr>
<tr><td>N2 Reverse</td><td>GCGCGACATTCCGAAGAA</td></tr>
<tr><td rowspan="3">RNase P</td><td>N2 Probe[A]</td><td>FAM or HEX – ACAATTTGCCCCCAGCGCTTCAG – BHQ1</td></tr>
<tr><td>RNase P Forward</td><td>AGATTTGGACCTGCGAGCG</td></tr>
<tr><td>RNase P Reverse</td><td>GAGCGGCTGTCTCCACAAGT</td></tr>
<tr><td>RNase P Probe</td><td>FAM – TTCTGACCTGAAGGCTCTGCGCG – BHQ-1</td></tr>
<tr><td rowspan="3">E gene</td><td rowspan="3">Corman et al.[4]</td><td rowspan="3">
<table>
<tr><th>Cycling Step</th><th>Temperature (°C)</th><th>Time</th><th>Number of Cycles</th></tr>
<tr><td>Reverse Transcription</td><td>50</td><td>60 min</td><td>1</td></tr>
<tr><td>Enzyme activation</td><td>95</td><td>10 min</td><td>1</td></tr>
<tr><td>Denaturation</td><td>95</td><td>30 sec</td><td rowspan="2">45</td></tr>
<tr><td>Annealing/Extension</td><td>58</td><td>1 min</td></tr>
<tr><td>Enzyme Deactivation</td><td>98</td><td>10 min</td><td>1</td></tr>
<tr><td>Hold and Droplet Stabilization</td><td>4</td><td>∞</td><td>1</td></tr>
</table>
</td><td>Corman E Forward</td><td>ACAGGTACGTTAATAGTTAATAGCGT</td></tr>
<tr><td>Corman E Reverse</td><td>ATATTGCAGCAGTACGCACACA</td></tr>
<tr><td>Corman E Probe</td><td>FAM – ACACTAGCCATCCTTACTGCGCTTCG – BBQ</td></tr>
<tr><td rowspan="2">E gene</td><td rowspan="2">Alberta Precision Labs (APL)[5]</td><td rowspan="2">
<table>
<tr><th>Cycling Step</th><th>Temperature (°C)</th><th>Time</th><th>Number of Cycles</th></tr>
</table>
</td><td>APL E Forward</td><td>GAGACAGGTACGTTAATAGTTAATAGCG</td></tr>
<tr><td>APL E Reverse</td><td>CAATATTGCAGCAGTACGCACAC</td></tr>
</table>

		Reverse Transcription	50	60 min	1	APL E Probe	FAM – CTAGCCATCCTTACTGCG – MGB
		Enzyme activation	95	10 min	1		
		Denaturation	95	30 sec	41		
		Annealing/Extension	60	1 min			
		Enzyme Deactivation	98	10 min	1		
		Hold and Droplet Stabilization	4	∞	1		
ORF1ab (nsp10)	China Centers for Disease Control and Prevention (CDC)[6]	**Cycling Step**	**Temperature (°C)**	**Time**	**Number of Cycles**	China CDC ORF1 Forward	CCCTGTGGGTTTTACACTTAA
		Reverse Transcription	50	60 min	1	China CDC ORF1 Reverse	ACGATTGTGCATCAGCTGA
		Enzyme activation	95	10 min	1	China CDC ORF1 Probe	FAM – CCGTCTGCGGTATGTGGAAAGGTTATGG – BHQ1
		Denaturation	95	30 sec	45		
		Annealing/Extension	60	1 min			
		Enzyme Deactivation	98	10 min	1		
		Hold and Droplet Stabilization	4	∞	1		
IP2 (ORF1, also known as RdRP)	Institute Pasteur, Paris, France[7]	**Cycling Step**	**Temperature (°C)**	**Time**	**Number of Cycles**	IP2 ORF 1 Forward	ATGAGCTTAGTCCTGTTG
		Reverse Transcription	50	60 min	1	IP2 ORF 1 Reverse	CTCCCTTTGTTGTGTTGT
		Enzyme activation	95	10 min	1	IP2 ORF1 IP2 Probe	HEX – AGATGTCTTGTGCTGCCGGTA – BHQ-1
		Denaturation	94	30 sec	45		
		Annealing/Extension	52.2	1 min			
		Enzyme Deactivation	98	10 min	1		
		Hold and Droplet Stabilization	4	∞	1		

Primer and probe concentrations were used as per kit instructions. The thermocycling ramp rate used for all assays was 2 ºC/sec. **A)** The United States Centers for Disease Control and Prevention (US CDC) N2 probe was a mixture of FAM and HEX as this was run as a triplex ddPCR assay along with N1 and RNase P [3].

Supplemental Table 3. Details of reverse transcriptase-PCR assays used in this study.

RT-PCR Target	Reaction Mix Setup	Thermocycling Profile
United States Centers for Disease Control and Prevention (US CDC) N1[8]	8.5 µL nuclease free water, 1.5 µL combined primer/probe mix (Integrated DNA Technology), 5 µL TaqPath 1-Step RT-qPCR Master Mix (4X), 5 µL RNA sample.	**Temperature (°C) / Time / Number of Cycles** 25 ºC — 2 min — 1 50 ºC — 15 min 95 ºC — 2 min 95 ºC — 3 sec — 45 55 ºC — 30 sec
US CDC N2[8]		
Corman E[9]	11.25 µL nuclease free water, 1 µL forward primer (10 µM), 1 µL reverse primer (10 µM), 0.5 µL probe (10 µM), 6.25 µL TaqPath 1-Step RT-qPCR Master Mix (4X), 5 µL RNA sample[A].	**Temperature (°C) / Time / Number of Cycles** 55 ºC — 10 min — 1 94 ºC — 3 min 94 ºC — 15 sec — 45 58 ºC — 30 sec
Alberta Precision Labs (APL) E[5]	1.5 µL nuclease free water, forward primer 0.4 µL (20 µM stock), reverse primer 0.4 µL (20 µM stock), probe 0.2 µL (10 µM stock), Taqman Fast Virus One-Step RT-PCR Master Mix (4X), 5 µL RNA sample.	**Temperature (°C) / Time / Number of Cycles** 50 — 5 min — 1 95 — 20 sec 95 — 5 sec — 45 60 — 30 sec
China Centers for Disease Control and Prevention (CDC) ORF1[6]	7.5 µL nuclease free water, 1 µL forward primer (10 µM stock), 1 µL reverse primer (10 µM stock), 0.5 µL probe (10 µM stock), 5 µL Taqman Fast Virus One-Step RT-PCR Master Mix (4X), 5 µL RNA sample.	
Institute Pasteur 2 (IP2) ORF1[7]	11.25 µL of nuclease free water, 1 µL forward primer (10 µM stock), 1 µL reverse primer (10 µM stock), 0.5 µL probe (10 µM stock), 6.25 µL Taqman Fast Virus One-Step RT-PCR Master Mix (4X), 5 µL RNA sample.	**Temperature (°C) / Time / Number of Cycles** 55 ºC — 20 min — 1 95 ºC — 3 min 95 ºC — 15 sec — 50 58 ºC — 30 sec 40 ºC — 30 sec — 1

The same primers and probes used for droplet digital PCR (ddPCR) were used for RT-PCR with the exception of the United States Centers for Disease Control and Prevention (US CDC) N2 probe which was used entirely as a FAM probe for RT-PCR (singleplex detection for N1 and N2). All RT-PCR reactions were ran on a Bio-Rad CFX-96 real-time thermocycler. **A)** Many samples had less than 5 µL of RNA added to them (due to insufficient quantities remaining) and the RNA went through an extra freeze-thaw step compared to the other RT-PCR assays.

Supplemental Table 4. Relative abundance of each droplet digital PCR (dd-PCR) target for the inoculum for SARS-CoV-2 cultures.

Inoculum						
						US CDC N2
					1.01	US CDC N1
				0.71	0.72	APL E
			1.02	0.73	0.73	Corman E
		0.88	0.89	0.64	0.64	China CDC ORF1
	1.13	0.99	1.01	0.72	0.73	IP2 ORF1
IP2 ORF1	China CDC ORF1	Corman E	APL E	US CDC N1	US CDC N2	

For each sample, the copies of each target, indicated in the vertical column, was divided by the copies of each target, indicated in the horizontal row (n=1). US CDC: United States Centers for Disease Control and Prevention, IP2: Institute Pasteur 2, ORF1: open reading frame 1, China CDC: Chinese Centers for Disease Control and Prevention, APL: Alberta Precision Labs.

References

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2. Centers for Disease Control and Prevention. Real-time RT-PCR Primers and Probes for COVID-19. CDC. cdc.gov. https://www.cdc.gov/coronavirus/2019-ncov/lab/rt-pcr-panel-primer-probes.html. Published 2020. Accessed November 3, 2020.
3. Bio-Rad. *Bio-Rad SARS-CoV-2 DdPCR Test Qualitative Assay for Use on the QX200 ™ and QXDx ™ Droplet Digital ™ PCR Systems Instructions For Use*. Vol 12013769.; 2020.
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9. Corman VM, Landt O, Kaiser M, et al. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. *Eurosurveillance*. 2020;25(3):2000045. doi:10.2807/1560-7917.ES.2020.25.3.2000045