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Stability of SARS-CoV-2 in Phosphate-Buffered Saline for Molecular Detection

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RNA viruses often require "cold chains" of transportation to prevent the breakdown of genetic material. The logistics of getting samples from the patient to a diagnostic laboratory, sometimes thousands of miles away from the original collection site, can be complex and resource intensive (1, 2). Nucleic acid degradation of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) RNA can compromise the accuracy of molecular detection methods. It has been demonstrated that nasopharyngeal specimens containing SARS-CoV-2 can be stored in phosphate-buffered saline (PBS) as a substitute for viral transport medium (VTM) for up to 7 days (3). Here, we evaluate the stability of differing viral loads of SARS-CoV-2 over 28 days stored at room temperature, 4°C, −20°C, or −80°C.

We used the first SARS-CoV-2-positive nasopharyngeal swab detected in our laboratory for spike-in material. PBS spiked with this SARS-CoV-2 specimen was stored in quadruplicates and divided into two concentrations, namely, 5,000 to 10,000 copies/ml (high titer) and 500 to 1,000 copies/ml (low titer), as determined by droplet digital reverse transcriptase PCR (RT-PCR). Nucleic acids were isolated on the Roche MagNA Pure96 system (Basel, Switzerland). Qualitative RT-PCR (qRT-PCR) was performed on 448 samples using our CDC-based laboratory-developed test, as described previously (4, 5).

For the high concentration of SARS-CoV-2, regardless of storage conditions, 100% of samples were detected by qRT-PCR through day 28. At room temperature, median cycle threshold (C_T) values for lower titers for both N1 and N2 targets remained consistent through day 28, fluctuating less than 1 median C_T (Table 1). For lower concentrations of virus, storage at room temperature was associated with reductions of positivity beginning at day 7, and by day 28, 0% of samples were detected for N1. Storage at room temperature was the least stable of all environmental conditions tested, with 54.2% of negative PCR results.

At 4°C, there was minimal change in C_Ts over time at the higher viral concentration. For lower titers, C_Ts increased by 2.1 C_Ts for N1 and 2.6 C_Ts for N2 over the 28 days. At −20°C, lower titers of virus fluctuated slightly more, increasing by ≥3 C_Ts. Storage of SARS-CoV-2 in PBS at −20°C was the second least stable condition, accounting for 37.5% of negative PCR results. Storage at −80°C showed the greatest stability, with all samples detected throughout the 28 days and ≤1.5 median C_Ts for both N1 and N2 targets.

Here, we show that the stability of SARS-CoV-2 can be maintained at 4°C for up to a month when −80°C storage is not available (6, 7). At viral loads of >5,000 copies/ml—corresponding to >75% of positive samples recovered in our clinical lab to

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TABLE 1 Median C_T values for N1 and N2 targets[a]

Storage condition by target and titer	Median C_T value						
	Day 0	Day 1	Day 2	Day 7	Day 14	Day 21	Day 28
N1							
5,000–10,000 copies/ml							
Room temp	31.4	31.0	31.5	31.2	31.6	31.4	31.3
4°C	30.8	30.9	31.3	31.5	31.7	31.5	31.4
−20°C	30.9	31.4	31.7	31.2	31.7	31.8	31.8
−80°C	30.7	31.1	31.4	31.0	31.0	30.9	31.8
500–1,000 copies/ml							
Room temp	33.4	36.8	36.3	36.1 (3/4)[b]	35.8 (3/4)	35.2 (1/4)	NDET (0/4)
4°C	34.4	34.9	35.1	35.9	35.9	35.8 (3/4)	36.5
−20°C	34.2	35.2	36.0	36.5	37.5 (1/4)	37.4	37.3 (3/4)
−80°C	35.1	34.4	35.3	34.9	36.2	36.4	35.3
N2							
5,000–10,000 copies/ml							
Room temp	30.6	30.4	30.9	30.9	31.1	31.1	31.2
4°C	30.4	30.2	30.5	30.6	31.3	30.6	31.1
−20°C	30.6	30.5	30.9	30.6	31.6	30.9	31.2
−80°C	30.6	30.5	30.6	30.6	30.6	30.5	31.2
500–1,000 copies/ml							
Room temp	32.8	35.0	35.8	36.6 (3/4)	37.0	38.3 (2/4)	38.3 (3/4)
4°C	33.8	34.2	34.7	34.9 (3/4)	36.0	35.4	36.4
−20°C	33.7	35.3	36.0	37.8 (3/4)	36.7	36.3 (3/4)	37.0 (3/4)
−80°C	34.4	33.7	34.8	35.7	35.8	34.7	35.9

[a]C_T, cycle threshold; NDET, not detected. N1 and N2 are amplicons within the nucleocapsid gene of SARS-CoV-2.
[b]Parentheticals denote the number of samples that were detected at a condition only if the 4 replicates were not all detected.

date—different storage temperatures did not have a substantial impact on our ability to detect SARS-CoV-2 when stored in PBS.

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