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

**Garrett A. Perchetti,[a] Meei-Li Huang,[a] Vikas Peddu,[a] Keith R. Jerome,[a,b] Alexander L. Greninger[a,b]**

[a]Department of Laboratory Medicine, Virology Division, University of Washington, Seattle, Washington, USA
[b]Vaccine and Infectious Disease Division, Fred Hutchinson Cancer Research Center, Seattle, Washington, USA

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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 $C_T$s over time at the higher viral concentration. For lower titers, $C_T$s increased by 2.1 $C_T$s for N1 and 2.6 $C_T$s for N2 over the 28 days. At −20°C, lower titers of virus fluctuated slightly more, increasing by ≥3 $C_T$s. 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 $C_T$s 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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Address correspondence to Alexander L. Greninger, agrening@uw.edu.

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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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