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## Effect of Environmental Conditions on SARS-CoV-2 Stability in Human Nasal Mucus and Sputum

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We found that environmental conditions affect the stability of severe acute respiratory syndrome coronavirus 2 in nasal mucus and sputum. The virus is more stable at low-temperature and low-humidity conditions, whereas warmer temperature and higher humidity shortened half-life. Although infectious virus was undetectable after 48 hours, viral RNA remained detectable for 7 days.

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is shed predominantly in upper and lower airway secretions (1), and transmission likely occurs predominantly through respiratory droplets, and potentially through direct contact and fomites. We describe SARS-CoV-2 stability in human nasal mucus and sputum under different environmental conditions.

We acquired pooled human nasal mucus and sputum commercially (Lee BioSolutions Inc., <https://www.leebio.com>) and mixed it with SARS-CoV-2 (SARS-CoV-2/human/USA/USA-WA1/2020) (2). We aliquoted 50  $\mu$ L of each fluid containing  $1 \times 10^5$  50% tissue culture infective dose/mL SARS-CoV-2 into sealed tubes (liquid setting) or onto polypropylene disks (surface setting), as previously described (3). We assessed stability under 3 environmental conditions: 4°C/40% relative humidity (RH), 21°C/40% RH, and 27°C/85% RH (RH applies only to exposed surface samples). We collected samples at specified timepoints and analyzed them for infectious virus by using endpoint titration. We extracted aliquots of collected surface samples by using the QIAGEN QIAamp Viral RNA Mini Kit (QIAGEN, <https://www.qiagen.com>) and analyzed them for the presence of viral RNA by using a quantitative reverse transcription PCR assay targeting the E gene (4). We fit linear regression models to  $\log_{10}$ -transformed titer data, calculated SARS-CoV-2 half-life ( $t_{1/2}$ ) for each condition, and tested differences by using analysis of covariance. We report all experimental measurements as means of 3 replicates with SE. We considered differences with  $p$  values  $\leq 0.05$  statistically significant.

We observed no significant differences in SARS-CoV-2  $t_{1/2}$  between environmental conditions in liquid nasal mucus. In surface nasal mucus, SARS-CoV-2  $t_{1/2}$  was significantly shorter at 27°C/85% RH compared with 21°C/40% RH ( $p = 0.0023$ ) and 4°C/40% RH ( $p = 0.0007$ ). At 27°C/85% RH, SARS-CoV-2  $t_{1/2}$  also was significantly shorter in surface compared with liquid nasal mucus ( $p = 0.0101$ ). Other comparisons of nasal mucus did not demonstrate significant differences in SARS-CoV-2  $t_{1/2}$  (Table; Figure, panel A, B).

SARS-CoV-2  $t_{1/2}$  was significantly longer in liquid sputum at 4°C than at 21°C ( $p = 0.0006$ ) and 27°C ( $p < 0.0001$ ). In surface sputum, SARS-CoV-2  $t_{1/2}$  also was significantly longer at 4°C/40% RH than at 21°C/40% RH ( $p = 0.0042$ ) and 27°C/85% RH ( $p = 0.0002$ ). In addition, SARS-CoV-2  $t_{1/2}$  was significantly longer at 21°C/40% RH than 27°C/85% RH ( $p = 0.0027$ ) in surface sputum. We observed no significant differences in SARS-CoV-2  $t_{1/2}$  between

liquid and surface sputum (Table; Figure, panel C, D). SARS-CoV-2 RNA remained detectable for  $\geq 7$  days in all surface samples, with a gradual increase in cycle threshold value (decrease in detected RNA) occurring only in nasal mucus at 27°C/85% RH (Figure, panel B, D).

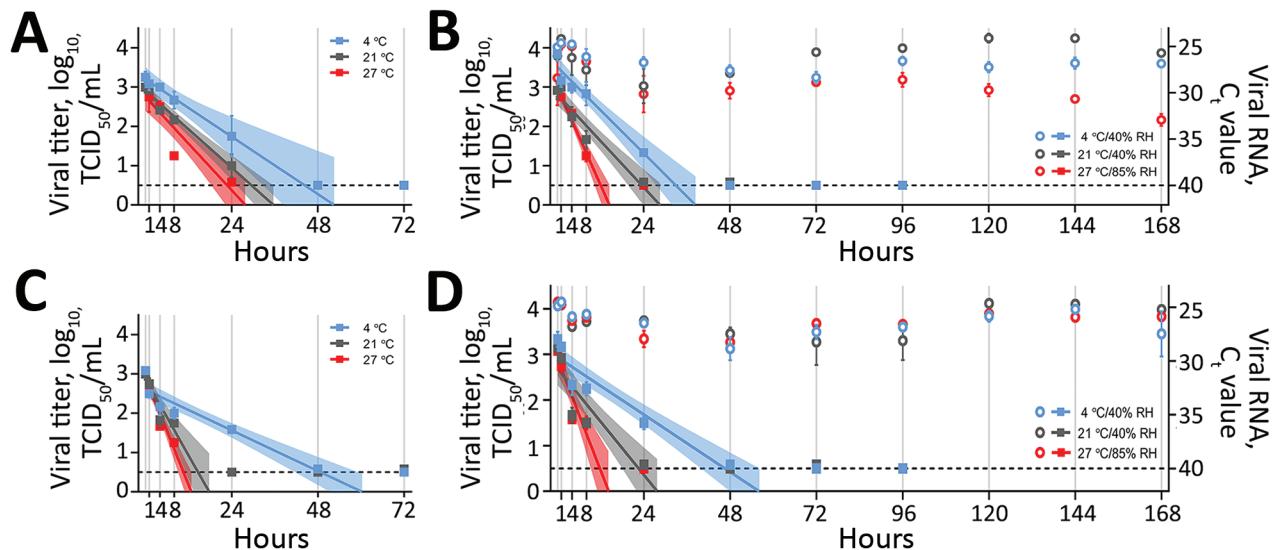
We previously reported on the surface stability of SARS-CoV-2 in culture media at 21°C/40% RH (3). However, SARS-CoV-2 stability is affected by its surrounding matrix and environmental conditions. The  $t_{1/2}$  we report here for SARS-CoV-2 in surface nasal mucus and sputum at 21°C/40% (Table) is considerably shorter than what we found in culture media under similar conditions ( $t_{1/2}$  6.8 [95% CI 5.6–8.2] hours) (3). In addition, we set out to determine SARS-CoV-2 stability in nasal mucus and sputum under environmental conditions that approximate temperate winter (4°C/40% RH), climate-controlled (21°C/40% RH), and temperate summer or tropical (27°C/85% RH) settings. SARS-CoV-2 was generally more stable at cooler temperatures and lower RH, and less stable at warmer temperatures and higher RH. Nevertheless, with our experimental protocol and initial titer, we predicted that SARS-CoV-2 would remain infectious in nasal mucus and

**Table.** Half-life ( $t_{1/2}$ ) for SARS-CoV-2 in human nasal mucus and sputum under different environmental conditions\*

Sample and exposure type	Environment	Half-life, h (95% CI)
<b>Nasal mucus</b>		
Liquid	4°C	4.9 (3.5–8.7)
	21°C	3.7 (3.1–4.7)
	27°C	3.1 (2.3–4.4)
Surface	4°C/40% RH	3.3 (2.6–4.4)
	21°C/40% RH	3.1 (2.5–4.1)
	27°C/85% RH	1.5 (1.2–1.9)
<b>Sputum</b>		
Liquid	4°C	7.0 (5.8–8.9)
	21°C	1.9 (1.3–3.2)
	27°C	1.3 (1.1–1.7)
Surface	4°C/40% RH	5.8 (4.8–7.3)
	21°C/40% RH	3.1 (2.3–4.6)
	27°C/85% RH	1.5 (1.1–2.4)

\*RH, relative humidity; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

sputum on surfaces for  $\geq 10$ –12 hours even in warm, humid conditions. However, the amount of infectious SARS-CoV-2 shed and virus stability in relationship to infectious dose for humans are currently unknown, hampering conclusions regarding infectious duration and transmission. The general similarity in SARS-CoV-2 stability between liquid and surface samples suggests that in general temperature factored more heavily than humidity.



**Figure.** Stability of severe acute respiratory syndrome coronavirus 2 over time in human nasal mucus and sputum under different environmental conditions: liquid nasal mucus (A), surface nasal mucus (B), liquid sputum (C), and surface sputum (D). For panels B and D, the squares correspond to viral titer on the left y-axis, and the circles correspond to viral RNA ( $C_t$  value) on the right y-axis. We collected samples in 1 mL media for each condition at 0, 1, 4, 8, and 24 hours, then daily for 7 days and performed end-point titrations in quadruplicate on Vero E6 cells and made calculations using the Spearman-Kärber method. We  $\log_{10}$ -transformed and fit titers with linear regression models, including 95% CIs (shaded area around lines of best fit), by using GraphPad Prism 8 (<https://www.graphpad.com>). We extracted aliquots of collected surface samples by using the QIAamp Viral RNA Mini Kit (QIAGEN, <https://www.qiagen.com>) and analyzed them for the presence of viral RNA by using quantitative reverse transcription PCR targeting the E gene. For both viral titers and  $C_t$  values, plots show means of 3 replicates with SE. The limit of detection for each experimental condition was  $10^{0.5}$  TCID<sub>50</sub>/mL for viral titer and 40 for  $C_t$  value and is indicated by the dashed line. Relative humidity is not applicable to liquid samples (panels A and C), which were in sealed tubes.  $C_t$ , cycle threshold; RH, relative humidity; TCID<sub>50</sub>/mL, 50% tissue culture infective dose/mL.

Community transmission of SARS-CoV-2 is widespread (5) and might be explained by contact with asymptomatic or presymptomatic (6) infected persons. Because of the surface stability of SARS-CoV-2, fomite transmission might also play a role. In addition, reduced surface stability of SARS-CoV-2 in human nasal mucus and sputum in warmer and more humid conditions might result in decreased virus transmission, and climatic influence on SARS-CoV-2 transmission rates might eventually drive seasonal outbreak dynamics in a postpandemic period (7), similar to other respiratory viruses (e.g., influenza A virus or human coronavirus OC43).

SARS-CoV-2 RNA has been detected on surfaces throughout clinical settings (8,9) and aboard a cruise ship for extended periods (10), but any correlation to infectious virus was previously unknown. In our study, infectious virus persisted in both nasal mucus and sputum on surfaces for  $\approx 24$  hours under climate-controlled conditions. However, viral RNA was consistently detectable for  $\geq 7$  days under various conditions in both nasal mucus and sputum on surfaces. These findings suggest that inferences regarding the presence of infectious virus from quantitative reverse transcription PCR data alone should be made with caution.

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