

Letter to the Editor

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Stability of SARS-CoV-2 respiratory samples in non-freezing condition: importance for tropical countries under heavy diagnostic demand

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To the Editor,

We read with great interest the article “SARS-CoV-2 RNA identification in nasopharyngeal swabs: issues in pre-analytics” by Basso et al. (2020), particularly regarding the findings on sample stability [1]. With those results in mind, we aimed to evaluate whether nasopharyngeal swab samples maintained viability for SARS-CoV-2 RNA detection through qRT-PCR following different storage periods in various conditions in southeastern Brazil.

It is universally acknowledged that timely transportation of the sample, with proper transport media, such as viral transport media or phosphate-buffered saline solution, and refrigeration, are among the gold standard parameters for optimal sample viability [2]. However, it is also known that this is not always possible, particularly in remote areas, hotter climates, and underdeveloped regions, not to mention in a period in which the diagnostics network is saturated such as in the COVID-19

pandemic. Therefore, accessing these pre-analytical issues such as establishing sample viability under different storage conditions in different countries is paramount for the improvement of the diagnostics process of a labile virus such as SARS-CoV-2.

To this end, 15 nasopharyngeal or oropharyngeal swab field samples were collected and sent to a private diagnostics laboratory in southeastern Brazil. Normal saline solution (0.9%) was used as transport and maintenance media of samples during the research. SARS-CoV-2 RNA investigation was performed by the qRT-PCR utilizing Allplex™ 2019-nCoV Assay System, which detects three viral gene segments (gene *E*, gene *N* and gene *RdRp/S*) as well as an internal control. Each of the 15 samples were aliquoted and stored in the following conditions: 25 °C (“environment” storage) for 5 consecutive days, in order to verify the stability of the samples in extreme situations of conditioning and transporting without refrigeration; 4 °C (refrigerated storage) for 5 consecutive days, in order to verify the stability of samples in situations of saturation of freezing availability; –20 °C (freezing storage) for 50 days (analyzed at days 0, 5, 10, 15, 20, 25, 30, 40 and 50), which simulates the ideal storage condition. Viral RNA detection followed manufacturer’s instructions, samples with qRT-PCR cycle threshold (Ct) up to 40 were considered positive and all amplification protocols had positive controls, which consisted of SARS-CoV-2-positive samples provided by the manufacturer, and negative controls with ultrapure water without template. The evaluation of storage conditions on 15 SARS CoV-2 positive samples were expressed in Ct variation for each of the investigated genes (*E*, *N* and *RdRp/S*).

For the environment temperature experiment, there was no significant variation in Ct for any of the three genes in the five days of analysis (Table 1). The coefficient of variation (CV) of observed Ct from samples maintained at environment temperature ranged from 1.3 to 5.3% for *E* gene; 0.4–6.8% for *RdRp/S* and 0.81–4.8% for *N* gene (data not shown). For the 4 °C temperature experiment, also carried

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Table 1: Comparison of Ct means over time according to gene and temperature.

	Mean Ct (SD; p-Value)	Mean Ct (SD; p-Value)	Mean Ct (SD; p-Value)
Condition	25 °C	4 °C	−20 °C
Gene <i>E</i>	24.24 (±3.75; 0.95)	24.00 (±4.24; 0.85)	25.25 (±4.39; <0.05) ^{a,c}
Gene <i>RdRp</i>	27.49 (±3.74; 0.88)	27.27 (±4.19; 0.74)	28.26 (±4.15; 0.81) ^d
Gene <i>N</i>	24.97 (±3.85; 0.74)	24.69 (±4.47; 0.63)	25.69 (±4.36; <0.05) ^{b,d}

Ct, cycle threshold; SD, standard deviation. ^aVariation occurred from D0 to D25; ^bvariation occurred from D0 to D30; ^cDurbin-Conover test; ^dTukey test; p<0.05 results are in bold letters.

on for five days, no variations in Ct were found for any of the three genes (Table 1). The CV of Ct from the samples kept at 4 °C temperature ranged from 1.4 to 3.2% for *E* gene; 1.9–5.3% for *RdRp/S* and 0.8–4.4% for *N* gene. Unlike the results from Basso and colleagues, we did not observe a spike in Ct values in any days of the analysis at environment temperature or at 4 °C, nor significant variation in mean Ct amongst different storage conditions.

Finally, in the freezer experiment, conducted for 50 days, there were significant differences for genes *E* (p<0.05) and *N* (p<0.05) (Table 1). Tukey's test indicated significant Ct differences (p<0.05) comparing day 0 to day 25 onward for *E* gene, day 5 compared to day 30 and 50 for *RdRp/S* gene, and day 0 compared to day 5, 30 and 50 for *N*

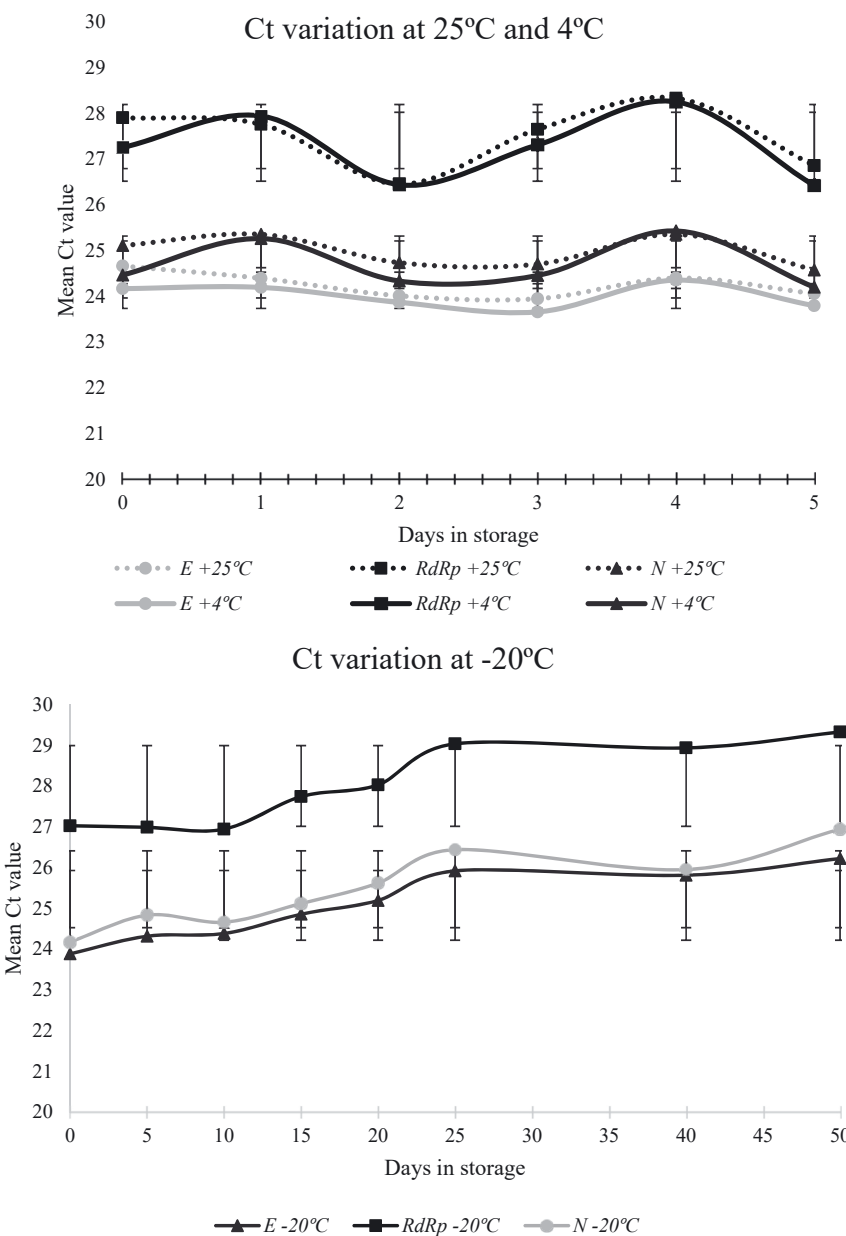


Figure 1: The effects of storage temperature and duration on SARS-CoV-2 genes *E*, *N* and *RdRp/S* mean Ct. The upper panel shows the mean threshold cycles (Ct) of SARS-CoV-2 detection stored at environment temperature (+25 °C) and at +4 °C for genes *E*, *N* and *RdRp/S* over five consecutive days. The lower panel shows mean Ct values of the same three genes of aliquots stored at −20 °C for 50 days.

gene. The CV of Ct from samples kept at freezer temperature ranged from 1.6 to 9.8% for *E* gene; 1.9–8.0% for *RdRp/S* and 2.5–9.8% for *N* gene. Figure 1 summarizes mean Ct findings for all three genes in different storage conditions.

The results obtained showed that, within the studied experimental domain, for environment and refrigerator conditions, there was no loss of stability of the collected material for up to five days, at 95% confidence, which is consistent with findings from Ott et al. [3]; while at -20°C there was a significant decrease in viral load observed between days D0 and D25. Considering the guidelines issued by the CDC for storage of COVID-19 samples, which recommends storage at $2-8^{\circ}\text{C}$ for up to 72 h and -70°C for longer delays [4], our results are relevant for places without access to ultrafreezers, as samples maintain stability and diagnostic viability for at least 5 days at 25°C or 4°C and 25 days at -20°C , warranting the possibility of retesting samples within that time frame without significant Ct variability or diminished diagnostic precision.

These results, as well as those reported by Basso et al. [1], can be used to provide subsidies for a better understanding regarding time and appropriate conditions for storage of samples in the laboratory in different scenarios. As establishing sample viability up to 5 days at environment temperature and at -20°C for 25 days has large implications

for settings such as: geographically large territories with few and/or distant diagnostic facilities as well as low infrastructure, rural and hotter climate areas.

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