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Ambient temperature stabilization of SARS-CoV-2 viral RNA in OMNIgene®•ORAL (OME-505) collected saliva samples

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Introduction

SARS-CoV-2 is the virus that causes coronavirus disease 2019 (COVID-19). Currently, RT-qPCR diagnostic tests for COVID-19 are commonly performed on samples collected from the upper respiratory tract with the use of nasopharyngeal or oropharyngeal swabs. However, these samples require collection by a trained healthcare professional, which can limit access to testing and creates an exposure risk to the person collecting the sample. Additionally, the potential for long wait times at testing sites and the discomfort caused by collecting these types of samples may deter some people from getting tested for COVID-19.

There is growing evidence that the SARS-CoV-2 virus can be reliably detected in saliva, a less invasive alternative to current sample collection methods.[1-3] Several studies have compared saliva to upper respiratory tract samples and demonstrated similar performance in terms of detection rate and viral load.[4-7] Given this evidence for saliva as a viable sample type for COVID-19 testing, this study aims to demonstrate that SARS-CoV-2 viral RNA is stabilized by and reliably detected in saliva collected using OMNIgene®•ORAL (OME-505). The OMNIgene•ORAL (OME-505) collection device allows for ambient temperature shipping and storage of saliva samples, making it suitable for at-home sample collection. The availability of a non-invasive at-home collection solution will improve access to COVID-19 testing. For this reason, it will play an important role in responding to the need for expanded testing to help slow the global spread of the virus. In addition to its advantages as an at-home testing solution, OMNIgene•ORAL (OME-505) has been previously shown to inactivate the virus, which improves safety for laboratory staff during sample processing.[8] The current study evaluates the performance of the OMNIgene•ORAL (OME-505) collection device, specifically for the stabilization of SARS-CoV-2 viral RNA during ambient temperature shipping and storage.

Methods

Sample collection

Thirty saliva samples (15 per experiment) were collected from healthy participants into OMNIgene•ORAL (OME-505) collection devices. In the absence of positive clinical samples, the experiments were conducted on contrived positive samples. Heat inactivated SARS-CoV-2 was diluted in sterile saline to 500,000 copies (cp)/mL and spiked into saliva samples at the specified concentrations. This reagent was deposited by the Centers for Disease Control and Prevention and obtained through BEI Resources, NIAID, NIH: SARS-Related Coronavirus 2, Isolate USA-WA1/2020, Heat Inactivated, NR-52286.

Nucleic acid extractions

OMNIgene•ORAL (OME-505) stabilized saliva samples were extracted with the MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit (Applied Biosystems™) according to DNA Genotek's recommendations for sample processing.[9] Extracted nucleic acid samples were stored at -80°C until needed.

RT-qPCR

RT-qPCR was performed on extracted nucleic acid samples using the 2019-nCoV RUO Kit (Integrated DNA Technologies) for the detection of SARS-CoV-2 RNA. RT-qPCR reactions were set up in 20 µL reactions with GoTaq® Probe 1-Step RT-qPCR System (Promega). 5 µL of extracted nucleic acids were added to each reaction. A CFX96 Touch™ Real-Time PCR Detection System (Bio-Rad Laboratories) was used with the following cycling conditions: 1) 45°C for 15 minutes, 2) 95°C for 2 minutes, 3) 45 cycles of: i) 95°C for 15 seconds, ii) 55°C for 1 minute.

Results

SARS-CoV-2 RNA is stable and detectable in OMNIgene•ORAL (OME-505) collected saliva samples during storage at room temperature for 21 days.

To determine whether SARS-CoV-2 RNA could be reliably detected in OMNIgene•ORAL (OME-505) collected saliva samples following room temperature storage, stability testing was performed on samples from 15 participants using an internally verified RUO assay optimized for the detection of SARS-CoV-2 RNA in saliva. Samples were spiked with heat inactivated SARS-CoV-2 at two different concentrations representing a low viral spike (500 cp/mL) and a high viral spike (2,500 cp/mL). Samples were stored at room temperature (23 ± 3°C) for 21 days and extracted at baseline and at 7- and 21-day time points. The SARS-CoV-2 nucleocapsid gene (targets N1 and N2) was used to evaluate viral RNA stability by RT-qPCR, where samples with a C_q of < 40 were determined to have detectable viral RNA. The human RNase P gene (RP) was included as a control for human nucleic acid detection. An unspiked control sample was tested for each participant in parallel to screen for the presence of SARS-CoV-2, and 100% (15/15) of these samples had no detectable SARS-CoV-2 viral RNA (data not shown).

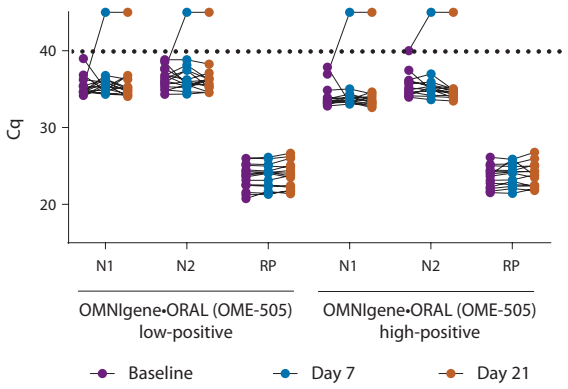

Figure 1: C_q values for the N1, N2 and RP RT-qPCR targets at baseline (purple), on day 7 (blue) and on day 21 (orange) in low-positive (500 cp/mL, N = 15) and high-positive (2,500 cp/mL, N = 14) spiked saliva samples. *Dotted line indicates detection limit. When no amplification was detected, C_q was set to 45 (maximum C_q) for visualization purposes. Statistical significance was determined using a two-way ANOVA with Sidak's test for multiple comparisons.*

After 21 days of storage at room temperature, 93% (14/15) of the samples with the low viral spike (Table 1) and 93% (13/14) of the samples with the high viral spike (Table 2) were positive for both viral targets. One participant was excluded from the high viral spike dataset due to a processing error, making the total cohort size 14 donors. Examination of the C_q values for both viral targets by participant (Figure 1) revealed there was no significant difference ($P > 0.05$)

Table 1: Summary of C_q values and detection rates at baseline (T0), day 7 (T7) and day 21 (T21) for room temperature stability samples that received the low viral spike (500 cp/mL). *Samples with no amplification were excluded from the mean and standard deviation (SD) calculations.*

Low viral spike (500 cp/mL)	N1			N2			RP		
	T0	T7	T21	T0	T7	T21	T0	T7	T21
Mean C_q	35.29	35.39	34.94	36.44	36.17	35.90	23.62	23.71	23.69
SD	1.26	0.76	0.82	1.34	1.32	1.07	1.65	1.57	1.63
Detection rate	15/15 (100%)	14/15 (93%)	14/15 (93%)	15/15 (100%)	14/15 (93%)	14/15 (93%)	15/15 (100%)	15/15 (100%)	15/15 (100%)

Table 2: Summary of C_q values and detection rates at baseline (T0), day 7 (T7) and day 21 (T21) for room temperature stability samples that received the high viral spike (2,500 cp/mL). *Samples with no amplification were excluded from the mean and standard deviation (SD) calculations.*

High viral spike (2,500 cp/mL)	N1			N2			RP		
	T0	T7	T21	T0	T7	T21	T0	T7	T21
Mean C_q	33.97	33.74	33.58	35.51	35.07	34.48	23.75	23.98	24.06
SD	1.50	0.52	0.60	1.57	0.83	0.50	1.50	1.49	1.53
Detection rate	14/14* (100%)	13/14* (93%)	13/14* (93%)	13/14* (93%)	13/14* (93%)	13/14* (93%)	14/14* (100%)	14/14* (100%)	14/14* (100%)

**One participant was excluded from the dataset due to a processing error. Total number of participants was 14.*

in SARS-CoV-2 RNA abundance between any of the time points for either the high or low viral spike samples despite one participant's sample that had no detectable viral RNA at day 7 or day 21. The human RP target was stable over time and was detected in 100% (15/15) of the samples. This result demonstrates that even at low viral loads (i.e., 500 cp/mL), SARS-CoV-2 RNA is reliably detected in OMNIgene•ORAL (OME-505) collected saliva samples after 21 days of room temperature storage. This data supports the use of this product for sample collection for downstream COVID-19 testing.

SARS-CoV-2 RNA is stable in OMNIgene•ORAL (OME-505) collected samples during simulated shipping and handling conditions.

Sample stability during shipping is essential for an at-home collection device since the sample may experience extreme temperature fluctuations during shipping from a subject's home to a processing laboratory. To determine whether SARS-CoV-2 RNA could be reliably detected in an OMNIgene•ORAL (OME-505) collected saliva sample following shipping, spiked samples collected from 15 participants were cycled through extreme temperatures expected to occur during shipping. OMNIgene•ORAL (OME-505) collected saliva samples were spiked with heat inactivated SARS-CoV-2 (2,500 cp/mL) and subjected to three cycles of -20°C for ≥ 3 hours to 50°C for ≥ 3 hours. During the freeze-thaw cycles, samples were held overnight at each of the temperatures at least once. Samples were extracted before (pre-shipping) and after (post-shipping) exposure to simulated shipping conditions. The RT-qPCR assay for the detection of SARS-CoV-2 RNA was performed on pre- and post-shipping samples as described above. An unspiked control sample was extracted for each participant in parallel to screen for the presence of SARS-CoV-2, and 100% (15/15) of these samples had no detectable viral RNA (data not shown).

Following simulated shipping conditions, 100% (15/15) of spiked samples were positive for both N1 and N2 viral targets post-shipping (Table 3). Examination of the C_q values for viral targets in individual participants (Figure 2) revealed that while simulated shipping increased viral RNA detection for some donors, overall there was no significant difference ($P > 0.05$) in SARS-CoV-2 RNA abundance between pre- and post-shipping samples. The human RP target was also stable during simulated shipping and was detected in 100% (15/15) of the post-shipping samples. This result demonstrates that SARS-CoV-2 RNA is stable in OMNIgene•ORAL (OME-505) collected saliva samples through the extreme temperature fluctuations that can be experienced during shipping and supports the use of this collection device for at-home sample collection.

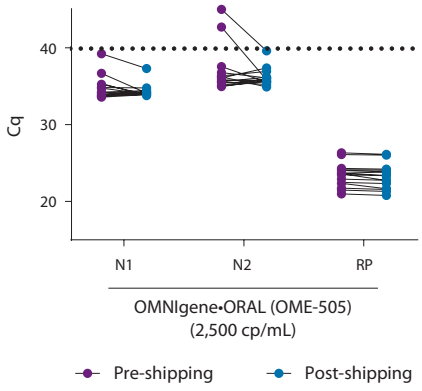

Figure 2: C_q values for N1, N2 and RP RT-qPCR targets pre-shipping (purple) and post-shipping (blue) in spiked saliva samples (2,500 cp/mL, N = 15). *Dotted line indicates detection limit. When no amplification was detected, C_q was set to 45 (maximum C_q). Statistical significance was determined using a two-way ANOVA with Sidak's test for multiple comparisons.*

Table 3: Summary of C_q values and detection rates for simulated shipping on high viral spiked samples (2,500 cp/mL). *Samples with no amplification were excluded from the mean and standard deviation (SD) calculations.*

Simulated shipping (2,500 cp/mL)	N1		N2		RP	
	Pre-shipping	Post-shipping	Pre-shipping	Post-shipping	Pre-shipping	Post-shipping
Mean C_q	34.73	34.35	36.36	36.15	23.43	23.21
SD	1.50	0.86	1.96	1.13	1.52	1.58
Detection rate	15/15 (100%)	15/15 (100%)	13/15 (87%)	15/15 (100%)	15/15 (100%)	15/15 (100%)

Conclusions

The experiments presented here evaluated the stability of SARS-CoV-2 RNA in OMNIgene•ORAL (OME-505) collected saliva samples spiked with heat inactivated SARS-CoV-2. The room temperature stability experiment showed viral RNA detection in 93% of the samples tested following storage for 21 days at room temperature and no change in viral RNA abundance over time, even for viral loads as low as 500 cp/mL (supporting low viral load use cases). This 21-day window from sample collection to processing ensures that reliable results will be obtained despite delays that may occur either in transit or in sample processing. The simulated shipping stability experiment showed detection of viral RNA in 100% of the post-shipping samples tested, indicating that the extreme temperature fluctuations that may occur during shipping do not affect nucleic acid stability in OMNIgene•ORAL (OME-505) collected saliva samples. Together, these results demonstrate that SARS-CoV-2 RNA is stable in OMNIgene•ORAL (OME-505) collected saliva samples and highlights the potential for OMNIgene•ORAL (OME-505) to be used for at-home as well as on-site sample collection.

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OME-505 is for IVD, Rx and for use under Emergency Use Authorization.
OME-505 collection device has not been FDA cleared or approved.
OME-505 collection device has been authorized by FDA under an EUA.
OME-505 collection device has been authorized only to collect, stabilize, and maintain during transport, saliva specimens suspected of containing SARS-CoV-2 ribonucleic acid (RNA), not for any other viruses or pathogens.
OME-505 collection device is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of medical devices during the COVID-19 outbreak under Section 564(b)(1) of the Federal Food, Drug, and Cosmetic Act, 21 U.S.C. § 360bbb-3(b)(1), unless the authorization is terminated or revoked sooner.

Some DNA Genotek products may not be available in all geographic regions.
®OMNIgene•ORAL (OME-505) is CE marked for In Vitro Diagnostic Use.