Sample prep

Stability of SARS-CoV-2 in both raw and stabilized saliva samples extracted using the PureLink Viral RNA/DNA Mini Kit

Summary

- The PureLink Viral RNA/DNA Mini Kit successfully extracts highly pure nucleic acid from SARS-CoV-2 and HCoV-299E, a common respiratory virus, over an extended period of time from saliva samples.
- The extraction efficiency of the PureLink Viral RNA/DNA Mini Kit with both raw and preserved saliva samples is reproducible and reliable.

Introduction

Saliva is an emerging biological sample type for testing for SARS-CoV-2 and other respiratory pathogens, due to its relatively simple and noninvasive collection procedures [1,2]. One of the prerequisites for measuring SARS-CoV-2 and other human coronaviruses from saliva is that the viral nucleic acid needs to remain stable over an extended period in typical storage conditions. To protect the saliva sample from nucleic acid degradation, a stabilization buffer is commonly added; the stabilized sample is then transported to a lab for viral nucleic acid extraction and analysis. However, protocols that maintain the integrity and longevity of viral nucleic acid in raw saliva without the addition of stabilization buffer exist as well.

Using the Invitrogen™ PureLink™ Viral RNA/DNA Mini Kit, we show here that viral nucleic acid in SARS-CoV-2 remained stable in raw saliva stored at 4°C for up to 14 days. We also demonstrated that human coronavirus 229E (HCoV-229E), which causes a common upper respiratory tract infection [3], is also stable in raw saliva samples. We also compared the stability of viral nucleic acid in saliva preserved using the Thermo Scientific™ SpeciMAX™ Stabilized Saliva Collection Kit. The stabilization buffer from the SpeciMAX kit helped maintain the stability of nucleic acid of SARS-CoV-2 and HCoV-229E in preserved saliva for up to 14 days at room temperature.

Materials and methods

Sample collection and nucleic acid extraction

Saliva was collected from 12 individual donors, collected as raw samples and also preserved using the SpeciMAX kit. Inactivated SARS-CoV-2 was acquired from BEI Resources (Cat. No. NR-52287). The virus was spiked into 1 mL of raw saliva and saliva preserved with the SpeciMAX kit, to a concentration of 2,250 copies/mL, which is 9 times the limit of detection. All samples were also spiked with 5 µL of NATtrol™ Respiratory Panel (RP) Multimarker 2 controls (ZeptoMetrix), which contains inactivated HCoV-229E. Raw saliva samples were stored at 4°C, while the saliva samples preserved using the SpeciMAX kit were stored at 25°C. Samples were processed for nucleic acid extraction immediately after collection on day 1, after 7 days of storage, and after 14 days of storage, following the protocol for the PureLink Viral RNA/DNA Mini Kit. Nucleic acid was extracted from 200 µL of raw saliva samples and saliva samples preserved using the SpeciMAX kit. All samples were eluted in 50 µL of nuclease-free water.

Viral detection by PCR

Extraction efficiency was evaluated using quantitative real-time PCR (qPCR). For SARS-CoV-2 detection, 14 µL of sample was used with the Applied Biosystems™ TaqMan® SARS-CoV-2 with RNase P Assay 2.0 (Cat. No. A51121) and Applied Biosystems™ TaqPath™ 1-Step Multiplex Master Mix (No ROX™ dye, Cat. No. A28523), in a final reaction volume of 20 µL. Reactions were set up in duplicate. qPCR was performed on the Applied Biosystems™ QuantStudio™ 7 Flex Real-Time PCR System (384-well block). The cycling conditions used were: 1 cycle at 25°C for 2 min, 1 cycle at 53°C for 10 min, 1 cycle at 85°C for 10 min, 1 cycle at 95°C for 2 min, 40 cycles of (95°C for 3 sec, then 65°C for 30 sec). The optical filter settings for qPCR were selected according to the Applied Biosystems™ QuantStudio™ 7 Flex Real-Time PCR System user guide. The data were analyzed for SARS-CoV-2 targets by setting the threshold at 40,000. The automatic baseline for all reporter dyes was used for analyses, with a start cycle of 5. For interpretation of SARS-CoV-2 results, data were analyzed using JMP™ software 16.0 (SAS Institute Inc.). The HCoV-229E assay used 2.5 µL of sample with the Applied Biosystems™ TaqMan® Fast Virus 1-Step Master Mix (Cat. No. 4444436). The final volume of the qPCR reactions was 10 µL, and the cycling conditions used were: 1 cycle at 50°C for 5 min, 1 cycle at 95°C for 20 sec, 40 cycles of (95°C for 3 sec, then 60°C for 30 sec).

Results

Raw saliva stored at 4°C showed excellent agreement with saliva preserved using the SpeciMAX kit and stored at room temperature for SARS-CoV-2 detection. Viral nucleic acid extracted from raw saliva samples remained stable for up to 14 days when stored at 4°C. Viral nucleic acid was also stable in saliva stabilized using the SpeciMAX kit. However, for 3 saliva donors whose saliva was stabilized using the SpeciMAX kit, the SARS-CoV-2 target *orf1b* and HCoV-229E were not detected after 7 and 14 days of room temperature storage (Figures 1 and 2). This is consistent with other data for donor-to-donor variability of saliva samples. Viral nucleic acid extracted from samples on days 1, 7, and 14 yielded similar C_t values at each time point, indicating stability of the viral nucleic acid. The ability to retain viable saliva samples for up to 14 days simplifies the workflow from sample collection to extraction and analysis, providing options for maintaining the usability of saliva samples even when immediate transport and processing are not feasible. Furthermore, the PureLink Viral RNA/DNA Mini Kit can be used to efficiently recover highly pure nucleic acid from saliva samples over an extended period of time.

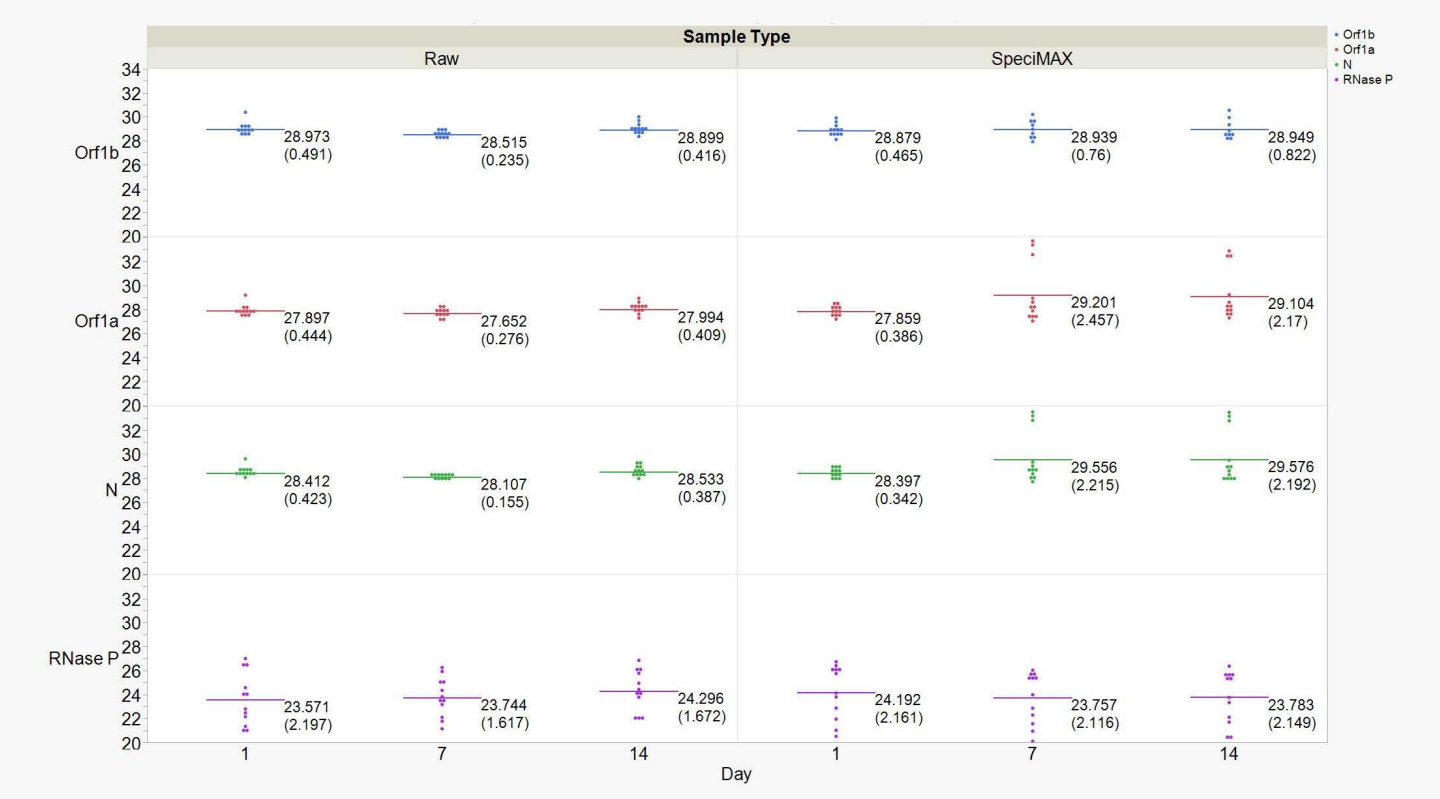

Figure 1. SARS-CoV-2 nucleic acid is stable for 14 days in raw saliva and saliva preserved using the SpeciMAX kit.
The numbers represent average C_t values, with standard deviation in parentheses.

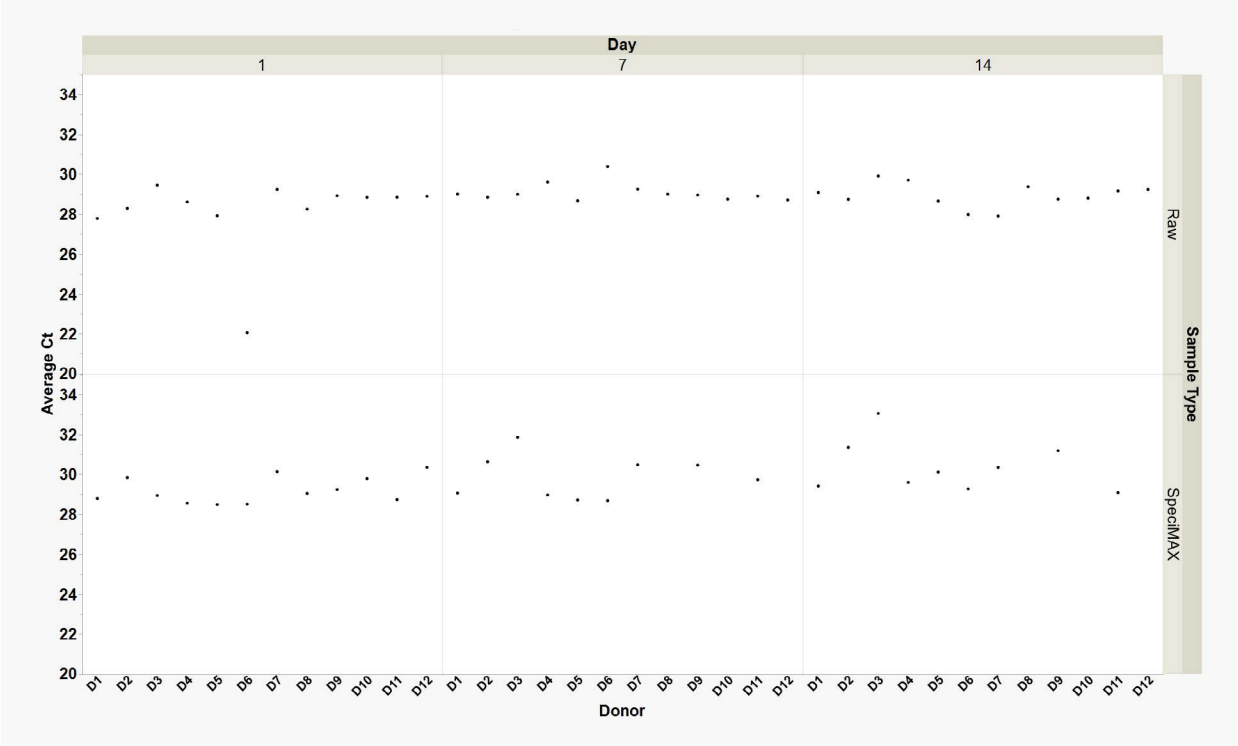

Figure 2. HCoV-229E nucleic acid is stable for 14 days in raw saliva and saliva preserved using the SpeciMAX kit.

Conclusion

The SpeciMAX Stabilized Saliva Collection Kit demonstrated effective stabilization and storage of saliva samples for downstream detection of SARS-CoV-2 and other respiratory viruses, preventing the degradation of viral nucleic acid in the saliva samples. Furthermore, the PureLink Viral RNA/DNA Mini Kit was effective in extracting nucleic acid from samples of raw saliva as well as saliva preserved using the SpeciMAX kit, for the detection of viral nucleic acid of SARS-CoV-2 and HCoV-229E. This study showed a simple and reliable method that includes saliva collection, storage, and nucleic acid extraction for detection of SARS-CoV-2 and other respiratory viruses.

References

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