APPLICATION NOTE

SARS-CoV-2 detection in saliva using bead-beating homogenization and an RT-PCR kit.

Authors

Caleb Proctor M.S.
Gabriella Ryan M.S.
Rodney J Nash, Ph.D.

Revvity, Inc.

Background

With over 14.8 million individuals infected in the United States since March 2020, there has been a significant need for the development of additional modalities to assist in removing some of the stress placed on the supply chain [1]. Currently, the most widely used test for SARS-CoV-2 is nasopharyngeal swab-based PCR detection [1]. Whilst the sensitivity and specificity of this method is considered upward of 95 % in both categories [1], many view the sample collection as invasive and uncomfortable, reducing the number of individuals willing to get tested. This reluctance to be tested has the potential to greatly hinder public health responses.

The use of saliva sampling for subsequent testing has been proposed as a more pleasant sample collection experience, while maintaining similar levels of sensitivity and specificity. Recently published articles show the utility of the Omni Bead Ruptor Elite™ bead mill homogenizer in homogenizing saliva samples for effective PCR based SARS-CoV-2 detection [2]. Using the Omni Bead Ruptor Elite bead mill homogenizer and a commercially-available SARS-CoV-2 RT-PCR detection kit, we have demonstrated an 100 % agreement in detecting SARS-CoV-2 from saliva in samples with recently confirmed SARS-CoV-2 infections as detected by standard nasopharyngeal swab PCR methods.

Omni Bead Ruptor Elite bead mill homogenizer

For research use only. Not for use in diagnostic procedures.

Materials and methods

Materials

- Omni Bead Ruptor Elite bead mill homogenizer (Cat # 19-042E)
- Omni Bead Ruptor Elite 2 mL Tube Carriage (Cat # 19-373)
- Hard Tissue Homogenizing Mix 2.8 mm Ceramic (2 mL) (Cat # 19-628)

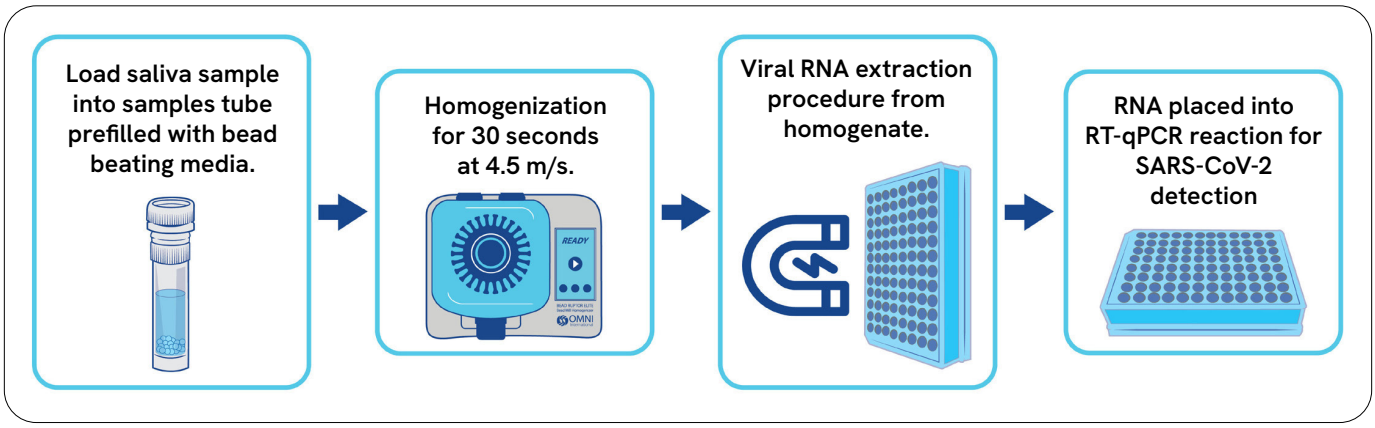

Figure 1: Graphical depiction of SARS-CoV-2 saliva protocol using the Omni Bead Ruptor Elite bead mill homogenizer for sample preparation and RT-qPCR for viral gene detection.

Saliva sample collection

Saliva samples were collected in accordance with IntegReview IRB protocol number 2020-QXD-1. A total of sixty samples were obtained, 30 positive and 30 negatives. SARS-CoV-2 positivity had previously been confirmed by a commercial CLIA laboratory offering RT-qPCR of SARS-CoV-2 within 48 hours of sample collection via nasopharyngeal swabs. All samples used in this study were either asymptomatic or mildly symptomatic, no hospitalizations were included in this study.

Each saliva sample was deposited into a specimen collection cup and 250 µL of the sample was pipetted into a 2 mL screw capped tube pre-filled with bead-beating media (Cat # 19-628) and 250 µL of viral transport media (Fisher Scientific Cat. No. A48750BA). A 1:1 mixture ratio of saliva to VTM was used for processing samples in the method described in this document.

Sample processing

The saliva filled 2 mL screw capped tubes were loaded into the Omni Bead Ruptor Elite bead mill homogenizer (Cat # 19-042E) and processed at 4.5 m/s for 30 s. Following homogenization, 300 µL of saliva homogenate underwent RNA extraction using a commercially available viral RNA extraction kit followed per manufacturer's instructions.

Viral detection

5 µL of extracted viral RNA was added to a commercially-available SARS-CoV-2 RT-PCR kit and loaded into a Bio-Rad CFX Connect Real-Time Instrument (Bio-Rad Cat. No. 1855201) for detection of SARS-CoV-2 N1/N2 genes. SARS-CoV-2 positive samples were defined as any sample with a Cq value < 40 for both the N1/N2 genes and the RNase P internal control.

Results

When testing saliva for SARS-CoV-2, we found all 30 samples in the SARS-CoV-2 positive sample group to exhibit positive results in both the N1/N2 gene and RNase P, demonstrating a Cq value less than 40 (Figure 2, Table 1). Additionally, none of the 30 SARS-CoV-2 negative samples demonstrated positive N1/N2 amplification, whilst the internal control RNase P remained positive for all (data for negative results not shown, but available upon request). These results demonstrate 100% agreement between nasopharyngeal swab and saliva-based collection methodologies for this PCR-based SARS-CoV-2 assay.

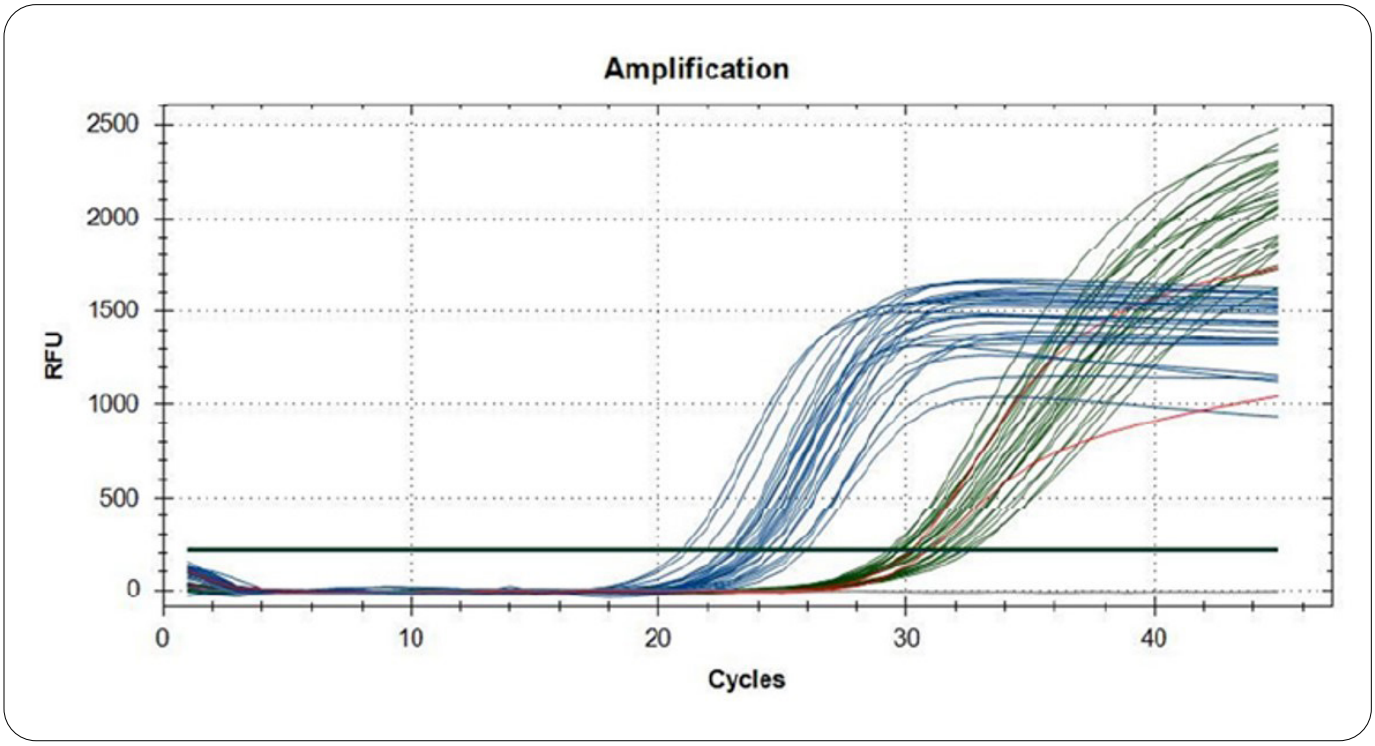

Figure 2: Cq values of saliva samples following RT-qPCR. Red line, positive control. Blue line, SARS-CoV-2 N1/N2 gene. Green line, RNase P gene (internal control). Grey, negative control. All 30 positive samples had Cq values for both N1/N2 and RNase P under 40.

Table 1: Average Cq values and standard deviation from SARS-CoV-2 N1/N2 gene amplification from the SARS-CoV-2 confirmed positive saliva samples. 30 total samples were used in this calculation, all of which had the N1/N2 gene and the PCR internal control positive with RT-qPCR.

	SARS-CoV-2 N1/N2 gene Cq value average	N1/N2 gene Cq value standard deviation
SARS-CoV-2 positive samples	25.16	4.09

Conclusions

Herein, we have demonstrated methodology featuring minimally-invasive sample collection utilizing saliva for PCR-based detection of SARS-CoV-2 that displays 100 % agreement with nasopharyngeal swab sample collection in the same 60 samples tested following the standard PCR-based workflow.

This method utilizes bead-beating homogenization of the saliva followed by standard extraction methods prior to RT-qPCR detection. The homogenization step at the front of this process has proven pivotal in maintaining robust reproducibility in results [2].

References

1. Tang YW, Schmitz JE, Persing DH, Stratton CW. Laboratory Diagnosis of COVID-19: Current Issues and Challenges. J Clin Microbiol. 2020 May 26;58(6):e00512-20. doi: 10.1128/JCM.00512-20. PMID: 32245835; PMCID: PMC7269383

2. Sahajpal NS, Mondal AK, Ananth S, Njau A, Ahluwalia P, Chaubey A, Kota V, Caspary K, Ross TM, Farrell M, Shannon MP, Rojiani AM, Kolhe R. SalivaAll: Clinical validation of a sensitive test for saliva collected in healthcare and community settings with pooling utility for SARS-CoV-2 mass surveillance. medRxiv. 1 September 2020. doi: 10.1101/2020.08.26.20182816

Revvity, Inc.
940 Winter Street
Waltham, MA 02451 USA
www.revvity.com

202019/OM