# Saliva as a Diagnostic Tool for COVID-19: A Proof-of-Concept Study

Kiranmai Durvasula[1], Havva Keskin[1], Julie Baggs[1], Travis Butts[1]
[1]Omega Bio-tek, Inc, Norcross GA 30071

## Introduction

To combat a fast-evolving global pandemic like COVID-19, it is essential to develop rapid and accurate diagnostic tests with a quick turnaround time. In recent times, saliva has emerged as a viable alternate sample source to nasopharyngeal swabs and has been shown to have greater detection sensitivity as well as consistency throughout the course of infection (1). Saliva is a non-invasive collection method that allows donors to collect in the comfort of their home, eliminating the exposure risk to healthcare workers, and reduces the overall burden on swab and personal protective equipment supplies.

DNA Genotek's OMNIgene®•ORAL (OM-505) and ORAcollect®•RNA (OR-100) (Figure 1) collection and stabilization devices are two saliva-based solutions for molecular COVID testing applications that have been proven to inactivate and preserve the SARS-CoV-2 virus. To fully realize the potential of saliva as a diagnostic candidate for COVID, we need reliable and rapid extraction technologies that offer high detection sensitivity. Omega Bio-tek's Mag-Bind® Viral RNA Xpress Kit (M6219-2304) (Figure 2) answers the speed, scale, and high throughput need with its magnetic-bead based approach that can easily be automated on most open-ended liquid handling platforms as well as magnetic processors. This study verifies the use of saliva collected in DNA Genotek devices (OM-505 and OR-100) in conjunction with Omega Bio-tek's chemistry for viral detection. As a proof-of-concept, human coronavirus 229E was spiked into saliva at various copy numbers and viral RNA was then extracted using the Mag-Bind Viral RNA Xpress Kit automated on the Thermo Scientific's KingFisher™ Flex platform to determine the sensitivity of extraction. The high throughput extraction protocol is capable of processing 96 saliva samples in ~90 min including the manual step of transferring sample from tube to 96-well plate. The study results indicate efficient viral RNA recovery and the ability to detect at a virus concentration as low as 0.5 copies/µL with Omega Bio-tek's chemistry.

## Materials and Methods

Saliva was collected in DNA Genotek's OMNIgene•ORAL (OM-505) and ORAcollect•RNA (OR-100) collection and stabilization devices following manufacturer's instructions. Saliva collected in OM-505 and OR-100 devices were pooled separately and screened for the presence of human coronavirus 229E template before the spike-in studies.

Omega Bio-tek's Mag-Bind Viral RNA Xpress Kit was employed for the extraction of viral RNA from the saliva samples stabilized in DNA Genotek devices. The TNA Lysis Buffer included in this kit completely inactivates the SARS-CoV-2 virus ensuring the safety of healthcare workers and lab personnel. The kit chemistry is highly versatile and can be used for the extraction of DNA-based viruses as well.

**Figure 1.** DNA Genotek's OMNIgene•ORAL (OM-505) (Left) and ORAcollect•RNA (OR-100) (Right) saliva collection and stabilization devices.


**Figure 2.** Omega Bio-tek's Mag-Bind Viral RNA Xpress Kit (M6219-2304).

Human coronavirus 229E was chosen for spike-in studies since it is an RNA-based virus like SARS-CoV-2 and belongs to the general coronavirus family. Hence, human coronavirus 229E serves as a good representative of SARS-CoV-2 virus. Heat inactivated human coronavirus was sourced from ZeptoMetrix at a TCID50/mL (TCID50 = Median Tissue Culture Infectious Dose) of $4.17\times10^5$. The viral copy number from the TCID50/mL was estimated as follows. Briefly, as a working estimate the ratio between TCID50/mL to PFU/mL was taken to be 1:0.7 (2) and we assumed roughly 1000 viral copies for each plaque forming unit (PFU) (3,4).

*Necessity of 1 h incubation at 56 °C*

Human coronavirus 229E was spiked at 500 copies/µL into 200 µL preserved saliva in OM-505 and OR-100 devices. The devices were then either incubated at 56 °C for 1 h or not incubated prior to extraction. Each condition for each DNA Genotek device was carried out in triplicate on the same day of collection and termed as baseline condition. The extraction protocol was automated on Thermo Scientific's KingFisher Flex platform with the magnetic bead-based tasks being performed as demanded by the Mag-Bind Viral RNA Xpress Kit. Purified viral RNA was eluted in 100 µL of nuclease-free water. Quantitative reverse transcription

PCR (RT-qPCR) using coronavirus 229E-specific primers and Agilent's Brilliant III 2X SYBR® mix was carried out in triplicate at two template amounts (2 and 4 µL in 20 µL total reaction volume). This would not only determine the necessity of the 1 hour incubation step prior to extraction, but also shed light on the efficiency of downstream RT-qPCR.

*Spike-in Studies*

Ten-fold serial dilutions of the coronavirus 229E, from 500 copies/µL to 0.5 copies/µL per 200 µL input, were spiked into coronavirus 229E-negative saliva matrix. Each dilution was performed in triplicate and carried out in parallel for OM-505 and OR-100 devices independently. 200 µL of the spiked saliva sample at each dilution and for each device was transferred to a 96-well deep well plate. The extraction protocol was carried out on Thermo Scientific's KingFisher Flex platform using Mag-Bind Viral RNA Xpress Kit protocol. Purified viral RNA was eluted in 100 µL of nuclease-free water and 4 µL of the eluate was subjected to RT-qPCR in triplicate with coronavirus 229E-specific primers using Agilent's Brilliant III 2X SYBR mix (total reaction volume = 20 µL) to detect the presence of human coronavirus 229E.

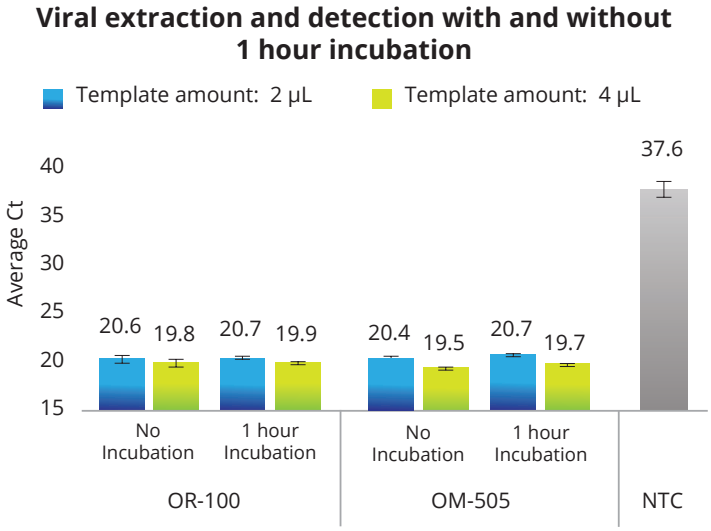


**Figure 3.** 200 µL of preserved saliva in OM-505 and OR-100 was spiked with 500 copies/µL of human coronavirus 229E and subjected to either 1 hour of incubation at 56 °C or not incubated. The average Ct values post RT-qPCR indicate the redundancy of 1 hour incubation for these baseline extractions.

## Results and Discussion

The results of the 1 hour incubation study at 56 °C in the collection devices prior to extraction are as shown in Figure 3. The average Ct value with and without incubation was comparable for both the devices at the 2 and 4 µL template amounts tested (Figure 3). For instance, with a 2 µL template amount, the average Ct value was 20.7 and 20.6 for OR-100, 20.7 and 20.4 for OM-505 with and without incubation, respectively (Figure 3). We found that the 1 hour incubation has no impact on viral RNA recovery at this baseline condition tested and hence, was skipped for the subsequent spike-in study. The average ΔCt between the 2 and 4 µL template amounts was close to theoretical 1 indicating no inhibition during the downstream RT-qPCR analysis.

Figures 4 and 5 show the results of the coronavirus 229E spike-in studies at 10-fold serial dilutions, from 500 copies/µL to 0.5 copies/µL per 200 µL saliva collected in OM-505 and OR-100, respectively. The average Ct values indicate efficient recovery of viral RNA with positive amplification of the target (human coronavirus 229E) at all the spike-in dilutions tested (Figures 4 and 5). The results demonstrate the ability of the extraction chemistry to detect human coronavirus 229E at concentrations as low as 0.5 copies/µL irrespective of the collection device used. Table 1 shows the limit of detection (LOD) reported in the Emergency Use Authorizations (EUAs) issued by FDA that use saliva as the collection matrix. The LOD of SARS-CoV-2 transcript from saliva ranged from 0.4 copies/µL for Rutger's EUA to 10

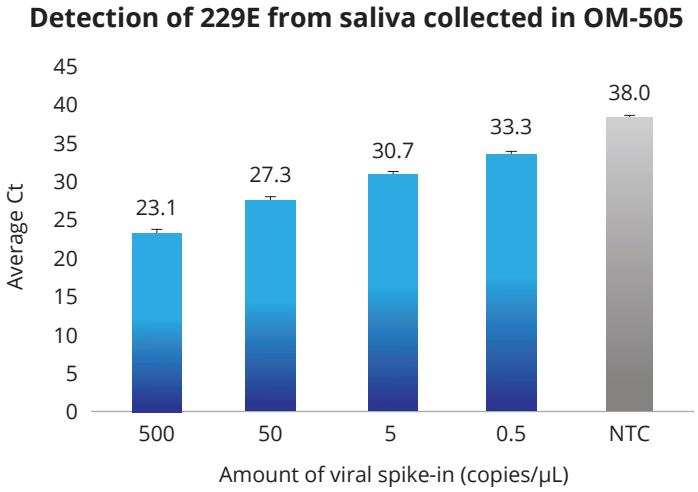


**Figure 4.** Viral RNA was extracted from saliva in OM-505 spiked with human coronavirus 229E at various copy numbers. The results indicate efficient viral RNA recovery and ability to detect at a virus concentration as low as 0.5 copies/µL.

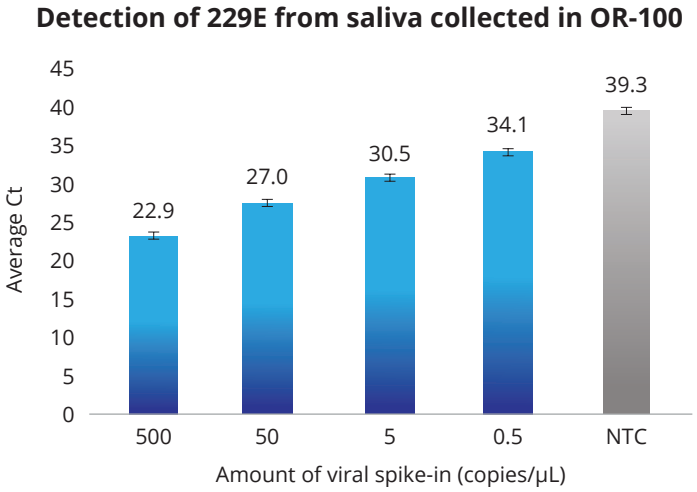


**Figure 5.** Viral RNA was extracted from saliva in OM-505 spiked with human coronavirus 229E at various copy numbers. The results indicate efficient viral RNA recovery and ability to detect at a virus concentration as low as 0.5 copies/µL.

**Table 1.** LODs reported in EUA's issued by FDA using saliva as the collection matrix.

| EUA | Spike-in Source | LOD (copies/µL) | Reference |
|---|---|---|---|
| Rutgers | Exact Diagnostics SARS-CoV-2 | 0.4 | https://www.fda.gov/media/136875/download |
| Phosphorus | Twist synthetic SARS-CoV-2 | 5 | https://www.fda.gov/media/138654/download |
| Yale Saliva Direct | Positive saliva specimen with known SARS-CoV-2 at 3.7E4 copies/µL | 6/12* | https://www.fda.gov/media/141192/download |
| P23 Labs | SerCare Accuplex SARS-CoV-2 | 10 | https://www.fda.gov/media/138297/download |

* Different LOD depending on the downstream RT-qPCR reagents used. NOTE: The EUAs above do not use the Omega Bio-tek extraction kit.

**Table 2.** High throughput capability of Omega Bio-tek's chemistry on various automation platforms.

| Automation Platform | No. of Samples Processed | On-deck Time |
|---|---|---|
| Thermo Scientific KingFisher™ and MagMAX® 96 | 96 | 40 |
| Hamilton Microlab® STAR™ | 384 (96 x 4) | 1 h 45 min |
| Hamilton MagEx STARlet | 96 | 1 h |
| Tecan Fluent® NAP 1080 | 384 (96 x 4) | 1 h 15 min |
| Tecan DreamPrep™ NAP 480 | 96 | 2 h |

NOTE: Omega Bio-tek's chemistry can be easily adapted on other platforms such as Beckman Coulter's Biomek, Opentron OT-2 and Eppendorf epMotion.

copies/µL for the P23 Labs' EUA. Since the spike-in sources and downstream reagents are vastly different for each of the EUAs as well as the current study, it is not appropriate to make direct comparisons. However, the detection sensitivity of 0.5 copies/µL with coronavirus 229E obtained in this study augurs well for Omega Bio-tek's chemistry. It serves as a demonstration of its extraction competency and potential success with other viruses, possibly with high sensitivity of detection.

Table 2 illustrates the capability and versatility of the Omega Bio-tek kit on various automation platforms to answer the need of this hour in terms of speed and throughput. The timings mentioned do not include the sample transfer (tube to 96-well plate) step. On magnetic processors like Thermo Scientific's KingFisher, 96 samples can be processed in 40 min. On a Hamilton Microlab® STAR™, four 96 well plates (i.e. a total of 384 samples) can be processed in 1 h 45 min and on Tecan Fluent® NAP 1080, the same 384 samples can be processed in just 1 h 15 min. The timings achieved using Omega Bio-tek's chemistry are highly competitive and are some of the best on the market. Furthermore, Omega Bio-tek's chemistry is highly versatile and can easily be adaptable to other platforms such as Beckman Coulter's Biomek, Opentrons OT-2, and Eppendorf epMotion®.

## Conclusions

The study reinforces the use of saliva as a diagnostic candidate for COVID-19 detection paving the way for at-home self-collection tests. Here we outline a comprehensive workflow solution – from saliva collection in DNA Genotek devices to extraction of viral RNA using Omega Bio-tek's Mag-Bind Viral RNA Xpress Kit to subsequent detection using RT-PCR reagents of choice. The Mag-Bind Viral RNA Xpress Kit, with its high throughput capability and high sensitivity of detection, can help maximize the testing capacity and offers to be a trusted partner in the fight against COVID-19.

### References

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### Product Information

| Product No. | Description |
|---|---|
| M6219-2304 | Mag-Bind® Viral RNA Xpress Kit (24×96 Preps) |

omega BIO-TEK
400 Pinnacle Way, Suite 450
Norcross, GA 30071
www.omegabiotek.com
info@omegabiotek.com
(800) 832-8896