OMNIgene®•ORAL

Alternative workflow for viral RNA sample collection and RNA preparation using OMNIgene®•ORAL collection device and prepIT™•Q2A preparation reagents

OMNIgene®•ORAL (OM-505) saliva collection devices combined with prepIT™•Q2A, a rapid and automatable method for nucleic acid preparation, comprise an effective workflow for the safe collection and stabilization of viral RNA while significantly reducing sample-processing time to provide comparable viral detection to standard RNA extraction methods.

Introduction

The emergence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and coronavirus disease 2019 (COVID-19) pandemic has placed a significant burden on medical systems and industry supply chains, triggering an urgent need for alternative methods of sample collection and processing. The most common sample collection method for COVID-19 testing involves taking nasopharyngeal swabs, which must be performed by a trained medical professionals at centralized collection sites. This collection process is not only invasive for the patient, but it also increases the risk of exposure for healthcare professionals and other healthy individuals through interactions at the collection site. Due to the challenges that come with this collection process, groups have turned to self-collected saliva as an alternative sample collection method[1, 2, 3]. The increased demand for testing has also introduced additional challenges to the medical and scientific community. Shortages of nasopharyngeal swabs and viral RNA extraction kits have led to a backlog of samples to be tested, delaying sample diagnosis and highlighting a need for an alternative solution.

OMNIgene•ORAL devices are an all-in-one system for the collection, stabilization and transportation of RNA and DNA from saliva. The kits are designed for at-home self-collection, protecting healthcare professionals and other healthy individuals from exposure to infectious agents. In addition, the OMNIgene•ORAL collection device has been demonstrated to inactivate SARS-CoV-2 in saliva samples spiked with infective virus[4].

prepIT•Q2A is a liquid based nucleic isolation reagent optimized to remove inhibitors found in the OMNIgene•ORAL stabilization solution. The prepIT•Q2A reagent enables a rapid, direct-to-assay workflow that is compatible with automation, allowing laboratories to increase their sample throughput without needing to perform standard extraction methods.

This application note utilizes OMNIgene•ORAL for saliva collection and stabilization of viral RNA and presents prepIT•Q2A as an alternative nucleic acid preparation method prior to viral RNA quantification by RT-qPCR. All reagents used in this workflow are guanadinium-free and compatible with standard decontamination procedures. In addition, minimal reagent volumes are used to reduce waste generation. This workflow addresses the need for a safe and effective sample collection method, while providing a cost effective and time saving solution that allows sensitive detection of viral RNA.

Material and methods

Sample collection and processing

Stability assessment of human coronavirus 229E in OMNIgene•ORAL devices

Saliva from 10 healthy donors were collected in OMNIgene•ORAL stabilization solution. An aliquot was immediately spiked with human coronavirus strain 229E (ATCC® VR740™) at 1 × 107 copies/µL of sample. Baseline samples were prepared by taking 100 µL aliquots of sample, and incubating at 56°C for 1 hour as part of standard pre-processing instructions for the collection device before processing with prepIT•Q2A as described in Table 1. The remainder of each spiked sample was stored at room temperature for 7 days before processing as described for baseline samples.

Table 1. Summary of prepIT•Q2A processing steps

Step	Description
1	Transfer 100 µL of sample to a tube or 96-well plate
2	Incubate at 75°C for 10 minutes
3	Add 10 µL of Reagent AG
4	Add 20 µL of Reagent ST and mix vigorously
5	Incubate undisturbed at room temperature for 15 minutes
6	Transfer 25 µL of upper aqueous phase containing nucleic acids to a new tube or 96-well plate

Hands-on time = 5 minutes
Total time = 30 minutes

prepIT•Q2A inhibitor removal assessment and 229E RNA detection

Saliva from 3 healthy donors were pooled and mixed with OMNIgene•ORAL stabilization solution. The pooled sample was spiked with 229E genomic RNA (ATCC® VR740DQ™) at 74 and 37 copies/µL of sample. In duplicate, 100 µL aliquots of spiked sample were incubating at 56°C for 1 hour, and then processed with prepIT•Q2A as described in Table 1.

Viral particle lysis and RNA recovery

Saliva samples were collected from 3 healthy donors using OMNIgene•ORAL devices. 100 µL aliquots of samples were spiked with intact lentiviral particles expressing 5-HT1A5 at 1000 copies/µL of sample and incubated at 56°C for 1 hour as part of standard pre-processing instructions for the collection device. RNA from spiked samples were prepared using prepIT•Q2A as described in Table 1. In parallel, experimental controls were prepared by extracting RNA using TRI Reagent LS according to the manufacturer's instructions and resuspending samples in 100 µL of nuclease-free water.

DNase digestion

Lentiviral spiked prepIT•Q2A processed samples and TRI Reagent extracted controls were diluted 1:10 with nuclease-free water prior to DNase digestion using Invitrogen Turbo DNase kit.

Viral RNA detection by RT-qPCR

Viral RNA presence was quantified by RT-qPCR with 5 µL of sample input using Promega®'s GoTaq® Probe 1-Step RT-qPCR kit. 229E spiked prepIT•Q2A processed RNA samples were diluted 1:5 with nuclease-free water and detected using TaqMan™ Gene Expression Assay (ID Vi06439671_s1). DNase-digested lentiviral RNA samples were analyzed directly by RT-qPCR using primers and probe targeting a 5-HT1A receptor gene region[5].

Results

Stabilization of human coronavirus 229E in OMNIgene•ORAL collection devices

Saliva in OMNIgene•ORAL stabilization solution from 10 healthy donors were spiked with human coronavirus 229E and processed using prepIT•Q2A at baseline and after 7 days of storage at room temperature. No significant change in 229E target signal was observed after 7 days at room temperature as shown in Figure 1. This demonstrates the ability of OMNIgene•ORAL to stabilize 229E viral RNA from saliva samples.

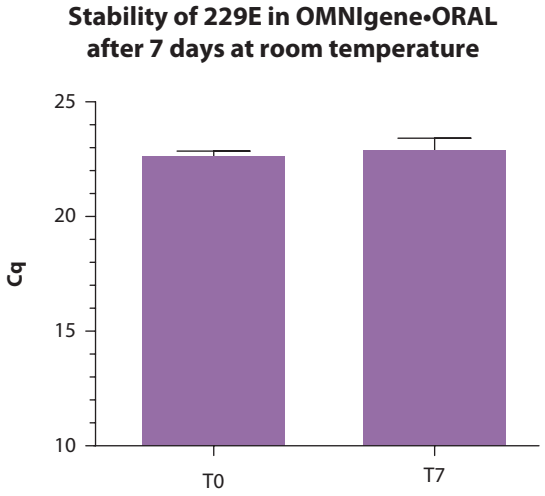

***Figure 1**: Stability of human coronavirus 229E spiked in OMNIgene•ORAL saliva after 7 days of storage at room temperature. Error bars represent the standard deviation of 10 donors.*

prepIT•Q2A reagent allows sensitive detection of 229E viral RNA

The function of prepIT•Q2A reagents is to remove inhibitors found in OMNIgene•ORAL stabilization solution to prepare the sample for downstream assays, bypassing standard extraction procedures. Performance of prepIT•Q2A was evaluated by comparing prepIT•Q2A processed 229E RNA samples against 229E RNA standards as assessed by RT-qPCR. prepIT•Q2A processed samples showed similar detection levels compared to standard RNA at both spike-in levels tested as shown in Figure 2. This indicates that prepIT•Q2A was able to remove chemical inhibitors, allowing efficient viral RNA detection through a molecular assay.

Detection of 229E RNA in OMNIgene•ORAL prepIT•Q2A samples compared to standard

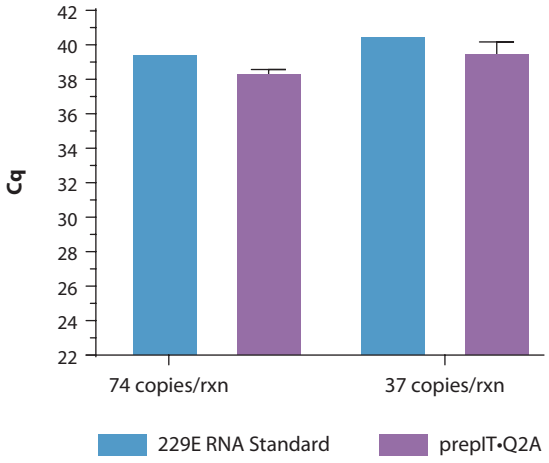

***Figure 2**: 229E RNA detection in OMNIgene•ORAL prepIT•Q2A samples (pooled saliva sample) compared to ATCC RNA standard. Error bars represent duplicate sample preparations.*

Viral RNA recovery from intact lentiviral particles is comparable to control extraction method

To assess recovery of RNA from enveloped viral particles, OMNIgene•ORAL samples were spiked with intact lentiviral particles and nucleic acids were prepared by either prepIT•Q2A reagent or by the control extraction method, TRI Reagent LS. prepIT•Q2A samples gave comparable target detection to TRI Reagent extracted controls by RT-qPCR as shown in Figure 3, indicating similar levels of viral particle lysis and RNA recovery.

Lentiviral RNA recovery from OMNIgene•ORAL using prepIT•Q2A vs. TRI Reagent extraction

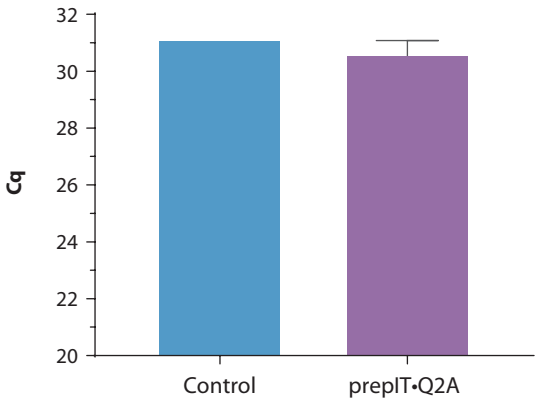

***Figure 3**: Lentiviral RNA recovery in OMNIgene•ORAL with prepIT•Q2A sample preparation vs TRI Reagent Control assessed by RT-qPCR (n = 3).*

Summary

OMNIgene•ORAL saliva collection devices were able to stabilize human coronavirus 229E after storing samples for 7 days at room temperature. After sample collection, prepIT•Q2A nucleic acid preparation reagent can be used to successfully remove chemical inhibitors from the collection device stabilization chemistries allowing direct-to RT-qPCR compatibility, bypassing standard extraction procedures even at low viral titer. It was also demonstrated that this combined workflow allows viral RNA recovery from intact viral particles giving comparable signals to the control TRI Reagent RNA extraction method. The data presented here support the use of OMNIgene•ORAL devices with prepIT•Q2A reagent as a more efficient workflow for saliva-based viral RNA sample collection, stabilization, and preparation for downstream assays.

References

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- Toll-free (North America): 1.866.813.6354, option 6
- All other countries: +1.613.723.5757, option 6
- Email: support@dnagenotek.com