Viral RNA Extraction: **Norgen vs. Zymo Comparison**

Application Note 95 - *Study B*

Keywords

+ Virus
+ Viral
+ RNA
+ Extraction
+ MagBead
+ SARS-CoV-2
+ COVID-19
+ RT-qPCR
+ E/RdRP genes
+ Assay
+ Silicon Carbide

INTRODUCTION

The control of infectious disease through public health intervention strategies relies heavily on early diagnoses. Thus, the detection of virus titers in biopsies is crucial to infectious disease research and management in both symptomatic and asymptomatic individuals. Traditionally, viral load testing has been used to assess the efficacy of such intervention programs. However, the sensitivity threshold of most real time RT-qPCR assays is limited by the quantity/quality of viral nucleic acid extracted from the individual samples.

One of the most important factors affecting the sensitivity of any RT-qPCR viral assays is the RNA extraction method, particularly in asymptomatic individuals with extremely low viral loads. Thus, choosing the proper RNA extraction technology is a key step for any disease surveillance program designed to monitor viral outbreaks, such as the COVID-19 outbreak caused by the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2).

Studies have shown an association of the SARS-CoV-2 viral load with the severity of the disease as well as with the risk of progression. Additionally, the detection of the minimal viral infection also becomes important as there underlies a greater risk of carrying further infections owing to the test being a false negative. Considering these facts, it becomes of utmost importance for the procedures for the detection of viral RNA, especially the RNA extraction and PCR detection steps, to be of highest accuracy and sensitivity.

Device Quick Links

Click to find out more on Norgen's website.

Saliva/Swab RNA Purification Kit

Saliva/Swab RNA Purification 96-Well Kit

COVID-19 TaqMan RT-PCR Kit (E/RdRP genes)

Norgen's Saliva/Swab RNA Purification Kit has been optimized for the extraction of total RNA, including viral RNA, from fresh or preserved saliva and swab samples. It is based on Norgen's proprietary silicon carbide-based technology (SiC) that boasts several advantages over traditional separation matrices (e.g. silica and phenol-chloroform):

Broad spectrum RNA binding. Traditional silica-based binding methods exhibit a bias towards capturing GC-rich RNA fragments and fragments with a high molecular weight. Silicon carbide technologies, however, demonstrate uniform binding affinity for all RNA species (including small RNAs; 200bp or smaller), offering researchers and clinicians a more complete picture of the sample's true RNA profile. This can have severe implications in molecular diagnostics as bias toward only larger fragments or RNA fragments which are only GC-rich may result in false-negative results.

High sensitivity. Most commercially available diagnostic RT-qPCR kits have a low limit of detection. SiC technology can capture high-quality RNA from samples enabling the detection of as few as 10 viral copies (or 400 copies per milliliter of saliva). This is ideal for the detection of ultra low viral loads particularly in asymptomatic and recovering patient samples. The fact that SiC binds to RNA fragments without size bias is advantageous considering the finding that nucleic acids stored in viral transport media (VTM) can degrade over time as even the fragmented RNAs are bound. With silica-based resin, degraded RNA is not bound.

Carrier RNA-free extraction. A prevalent artificial spike-in, in RNA preparations is poly(A) carrier RNA, present in other commonly used commercial viral RNA extraction kits to help capture ultra low RNA amounts. The presence of carrier RNA in the eluate can severely impact downstream applications like RNAseq, as these carrier RNAs can be incorporated into the cDNA library during the library preparation step. This can significantly reduce sequencing efficiency required for sequencing of targets of interest.

Phenol/chloroform-free extraction. The use of hazardous chemicals such as phenol/chloroform is not amenable to high-throughput processing, as it is quite difficult to automate. Additionally, manual processing of samples can be laborious as it requires extensive care in handling chemical and chlorinated organic waste and managing their disposal. Moreover, the quality of the RNA extracted using phenol/chloroform may not be high enough for sensitive downstream applications. Norgen's RNA extraction methods do not utilize phenol/chloroform or any hazardous organic chemicals, and hence the quality of the extracted RNA will be optimum for any downstream application.

Virus inactivating buffers. The use of Universal Transport Media (UTM) or Viral Transport Media (VTM) is a common practice in disease screening programs, however, samples stored in these media leave medical laboratory technicians potentially exposed to infectious samples. Thus, RNA extractions that include lytic buffers like Norgen's Lysis Buffer A are ideal for sampling methods that utilize UTMs and VTMs as they render the UTM/VTM samples non-infectious.

The new Saliva/Swab RNA Purification Kit is designed to extract viral nucleic acid from fresh or preserved saliva and swab samples with a range of viral loads. This quick and easy automation-friendly workflow can be used to extract nucleic acid from as few as 20 copies of virus contained in a 400 µL input volume. In the present paper, we demonstrate the use of this kit and compare its sensitivity to Zymo's Quick-DNA/RNA Viral MagBead Kit – which is part of an FDA EUA authorized end-to-end workflow – to extract RNA from both fresh swab samples in a range of copy numbers.

MATERIALS & METHODS

Two and a half milliliters of preserved saliva was collected in six 15 ml falcon tubes and were spiked with SARS-CoV-2 transcript at a concentration of 0, $4x10^2$, $4x10^3$, $4x10^4$, $4x10^5$ and $4x10^6$ copies/mL which is equivalent to 0, 10^1,10^2, 10^3,10^4 and 10^5 copies/PCR reaction. A 250 µL sample was taken from each tube for Norgen's Saliva/Swab RNA Purification Kit (Cat. 69100) as well as Zymo Quick-Viral DNA/RNA Purification Kit.

For Norgen's silicon carbide-based Saliva/Swab RNA Purification Kit (Cat. 69100), 1X PBS (pH 7.4) was then added to the sample, followed by the addition of Lysis Buffer A and ethanol. The lysate was then loaded onto a provided column, and the flow-through was discarded. The column was then washed once with 400 µL of Wash Solution WN and twice with Wash Solution A. The purified RNA

was then eluted with 50µL of Elution Solution A. On the other hand, for Zymo's silica – MagBinding Quick-Viral DNA/RNA kit, 250 µL DNA/RNA Shield Solution was added to 250 µL sample, followed by the addition of 1000 µL Viral DNA/RNA Buffer and 20 µL MagBinding Beads and was mixed for 10 minutes on a rotating mixer. MagBinding beads were separated from the solution using a magnetic stand and the beads were washed with 500µL Wash Solution 1, once with Wash Solution 2 and twice with 95-100% ethanol. The beads were dried for 10 minutes and the RNA was eluted in 50 µL DNase/RNase-Free water.

RT-PCR

Five microliters each of the isolated RNA sample was used as a template for the real-time RT-qPCR reaction using COVID-19 TaqMan RT-PCR Kit (E/RdRP genes) (Cat. 67200), on the CFX96 Touch real-time system (Bio-Rad).

RESULTS & DISCUSSION

The detection of the SARS-CoV-2 transcript was performed using Norgen's COVID-19 TaqMan RT-PCR Kit (E/RdRP genes). The RNA extracted using Norgen's Saliva/Swab RNA Purification Kit (Cat. 69100) was sufficiently sensitive for the detection of the E gene up to a concentration of 400 copies per millilitre (~10 copies/PCR reaction) however the silica-based MagBinding Quick-Viral DNA/RNA kit required 4,000 copies of the E gene per millilitre (~100 copies/PCR reaction) for adequate amplification. Moreover, it was possible to detect target RNA extracted with Norgen's kit 2 cycles earlier than it was using the Zymo kit. This trend was apparent at various concentrations of the positive control (e.g. $4x10^4$, $4x10^5$ and $4x10^6$ copies/mL) except $4x10^3$. In addition, the variation amongst the individual Ct values for a given copy number was higher when RNA was extracted using Zymo's kit (**Figure 1a-1d**). On the other hand, these values were quite precise for the RNA extracted using Norgen's silicon carbide-based Saliva/Swab RNA Purification Kit, demonstrating higher consistency that is ideal for low yield and precious samples.

CONCLUSION

Norgen's silicon carbide-based Saliva/Swab RNA Purification Kit (Cat. 69100) provides a highly sensitive, simple and reliable method for RNA extraction from virus-containing samples. This was demonstrated by successful detection of as few as 10 copies in a PCR reaction. This silicon carbide-based RNA extraction chemistry is superior to its competitors that use silica-based chemistry for viral screening and intervention programs.

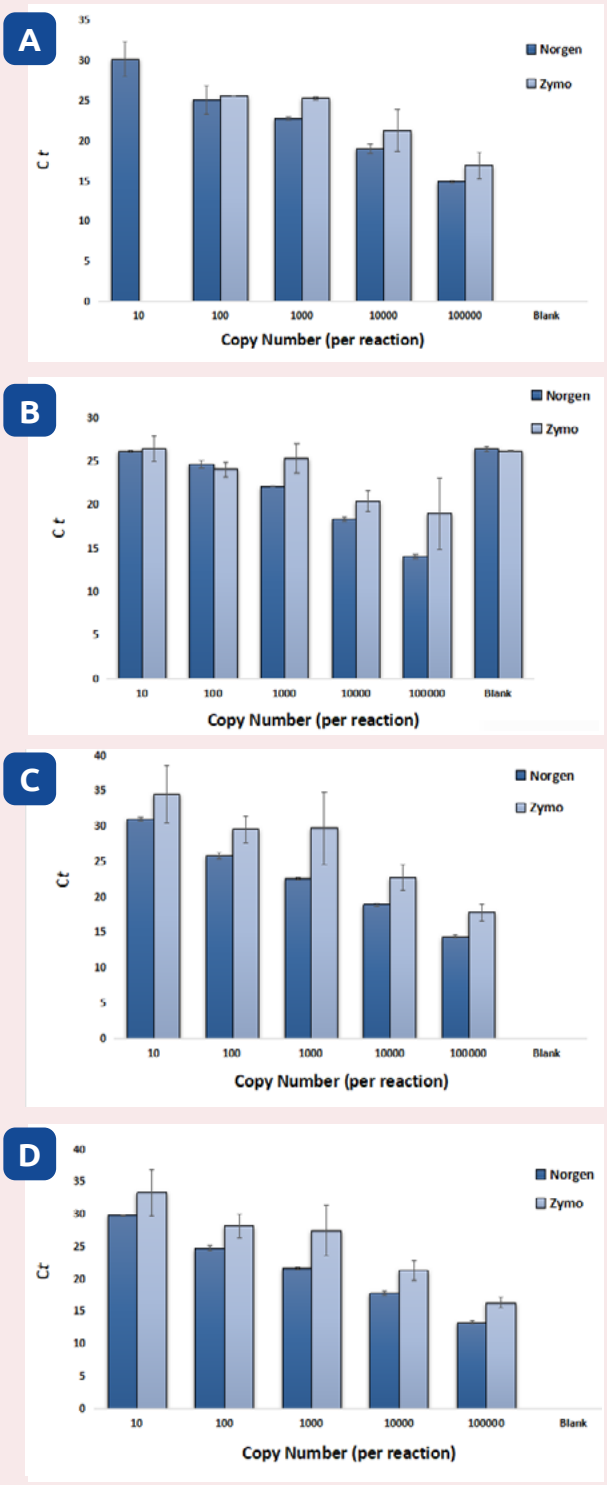

Figure 1. Detection of COVID-19 positive control using COVID-19 TaqMan RT-PCR Kit (E/RdRP genes) (Cat. TM67200) from purified RNA samples. RNA was isolated from saliva spiked with COVID-19 transcript using Norgen's Saliva/Swab RNA Purification kit or Zymo's Quick-DNA/RNA Viral MagBead kit. Five microliters which were equivalent to 10^1, 10^2, 10^3, 10^4, 10^5 copies of transcript were used as a template in an RT-qPCR reaction for the detection of WHO-based COVID-19 transcript. The following genes were amplified: (A) E gene (B) RNaseP gene (C) RdRp1 gene and (D) RdRp2 gene.

Related Products	Research Use	CE Marked
Saliva/Swab RNA Purification Kit	69100	Dx69100
Saliva/Swab RNA Purification 96-Well Kit	69300	Dx69300
COVID-19 TaqMan RT-PCR Kit (E/RdRP genes)	TM67200	DxTM67200

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