Viral RNA Extraction: **Norgen vs. Thermo Fisher Comparison**

Application Note 95 - *Study A*

Keywords

+ Virus
+ Viral
+ RNA
+ Inactivation
+ Buffer
+ Media
+ COVID-19
+ Pathogen
+ Storage
+ Method
+ Protocol

INTRODUCTION

In the wake of the COVID-19 outbreak, the need for accurate and sensitive RNA extraction methods has become increasingly evident. The gold standard for viral detection, RT-qPCR analysis, is limited by the quantity and quality of viral nucleic acid purified from clinical samples. To ensure accurate diagnostic results, the process used for RNA purification must be extremely sensitive in order to capture and purify high-quality RNA from samples with very low viral loads. Additionally, swab-based samples stored in traditional viral transport media may undergo RNA degradation during shipping and storage, further emphasizing the need for a purification method that ensures capture of small fragmented RNA.

Norgen Biotek Corp. has developed an optimized kit for the purification of RNA from fresh or preserved saliva and swab samples, using a patented silicon carbide technology. Unlike silica-based columns or magnetic beads, silicon carbide is able to capture the full range of RNA in a sample, including fragmented RNA and microRNA, without bias towards molecular weight or G-C content. In this application note, we compare Norgen's Saliva/Swab RNA Purification Kit (Cat. 69100) to a leading magnetic-bead based competitor, the MagMAX™ mirVana™ Total RNA Isolation Kit, to assess the yield of human and viral RNA obtained from oral swab samples.

MATERIALS AND METHODS

Sample Collection

Oral swabs were collected from six healthy individuals and placed into Norgen's Total Nucleic Acid Preservation Tubes (Cat. 69200). Tubes were spiked with the E/RdRP/RP Positive Control transcript, provided in Norgen's

Device Quick Links

Click to find out more on Norgen's website.

Total Nucleic Acid Preservation Tubes

Saliva/Swab RNA Purification Kit

COVID-19 Taqman RT-PCR Kit (E/RdRP Genes)

COVID-19 TaqMan RT-PCR Kit (E/RdRP genes) (Cat. TM67200) at a concentration of 0, 4x10^2, 4x10^3, 4x10^4, 4x10^5 and 4x10^6 copies/mL respectively.

RNA Purification

For purification, 250 µL of sample was taken from each tube for Norgen's Saliva/Swab RNA Purification Kit as well as MagMAX™ mirVana™ Total RNA Isolation Kit. For Norgen's Saliva/Swab RNA Purification Kit, the protocol was followed as written for preserved swabs. RNA was eluted in 50 µL of Elution Solution A.

For MagMAX™ mirVana™ Total RNA Isolation Kit, the protocol for blood was followed to extract RNA from the samples. To match the sample input volume of Norgen's kit, for all materials up to the washing step 5 times the volume mentioned in the protocol was taken. RNA was eluted in 50 µL of Elution Buffer.

RT-qPCR

Out of the 50 µL eluted RNA, 5 µL was used as an input for the real-time RT-qPCR amplification of the spiked targets using Norgen's COVID-19 TaqMan RT-PCR Kit (E/RdRP genes) (Cat. TM67200) on CFX96 Touch real-time system (Bio-Rad).

RESULTS AND DISCUSSION

RT-qPCR Assay

Detection of the E/RdRP/RP positive control was performed using Norgen's COVID-19 TaqMan RT-PCR Kit (E/RdRP genes) (Cat. TM67200) as indicated in the methods section. The E gene in RNA extracted using Norgen's silicon carbide-based kit was detectable from as low as 10 viral copies/PCR reaction, while the silica-based MagMAX™ kit was able to detect the E gene starting at 1000 viral copies/PCR reaction. Thus, the MagMAX™ mirVana™ Total RNA Isolation Kit could not effectively extract low copy numbers of the COVID-19 positive control.

Moreover, Norgen's kit detected 4x10^3, 4x10^4, 4x10^5 and 4x10^6 copies/mL positive controls at least 5 cycles earlier as compared to the MagMAX kit. In addition, variation among individual Ct values for a given copy number replicate was greater in samples extracted with the MagMAX™kit.

Both kits had a detection sensitivity up to 4x10^2, copies/mL for RdRp1, RdRp2, and RNaseP genes. However, Norgen's kit allowed for the detection of COVID-19 positive controls at least 4 cycles earlier than the MagMAX™ kit.

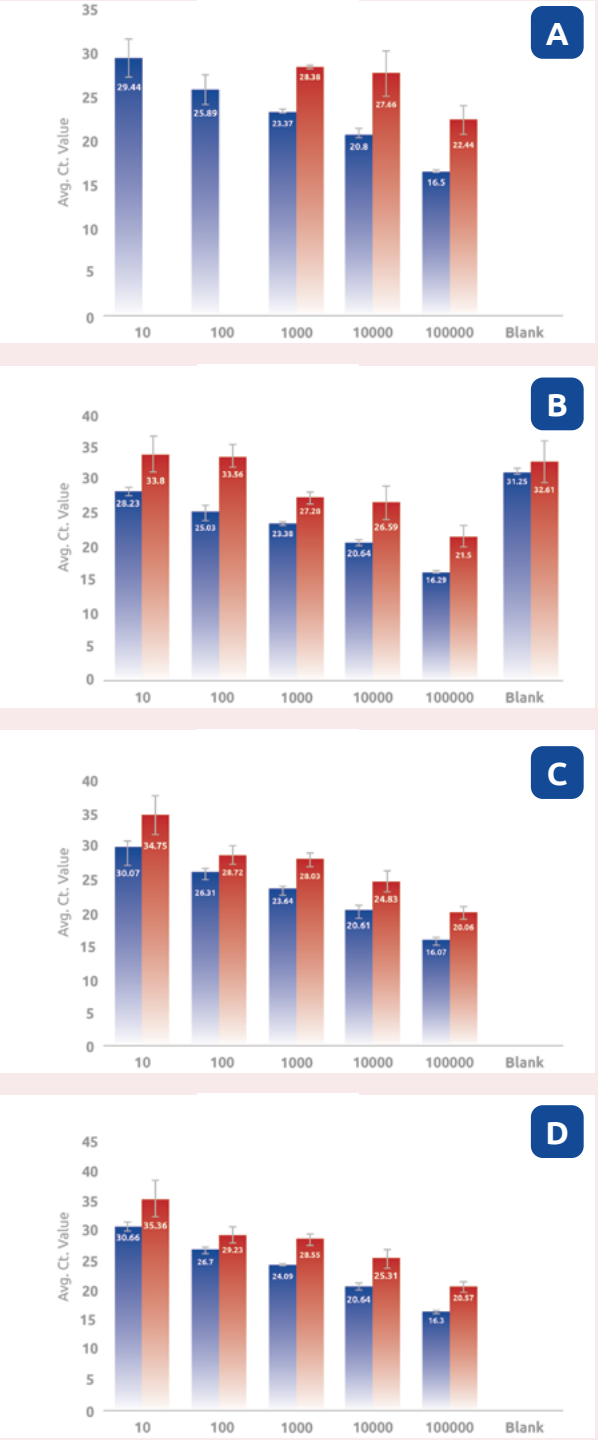

Figure 1. Comparison of Ct values for detection of the COVID-19 TaqMan RT-PCR Kit (E/RdRP genes) positive control via RT-qPCR. Oral swab samples were spiked with E/RdRP/RP positive control transcripts at a concentration of 0, 4x10^2, 4x10^3, 4x10^4, 4x10^5 and 4x10^6 copies/mL respectively. RNA was purified from oral swabs using Norgen's Saliva/Swab RNA Purification Kit and the MagMAX™ mirVana™ Total RNA Isolation Kit. The following genes were amplified: (A) E gene (B) RNaseP gene (C) RdRp1 gene and (D) RdRp2 gene.

CONCLUSIONS

1. Norgen's Saliva/Swab RNA Purification Kit provides extremely high quality RNA allowing for the detection of very low viral loads in comparison to the magnetic bead-based kit.

2. Norgen's kit provides less individual variation in Ct values via RT-qPCR analysis, suggesting more reproducible and consistent RNA extraction and purification than the competitor.

Related Products	Research Use	CE Marked
Total Nucleic Acid Preservation Tubes	69200	Dx69200
Saliva RNA Collection and Preservation Devices	RU5300	53800
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
2019-nCoV TaqMan RT-PCR Kit	TM67100	DxTM67100
COVID-19 TaqMan RT-PCR Kit (N/ORF1ab genes)	TM67300	-

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