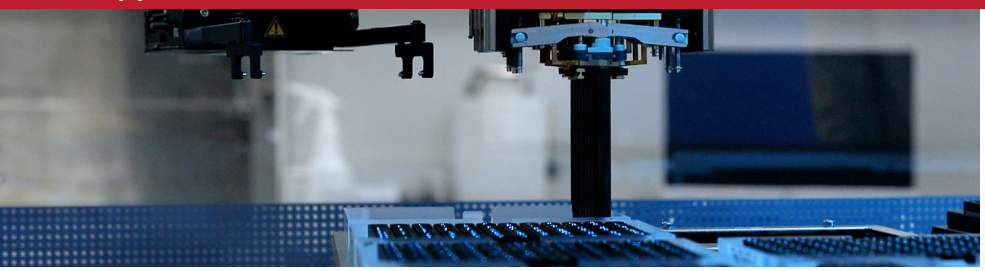
Comparing the Isolation Efficiency of Norgen's Saliva/Swab RNA Purification Kit on Hamilton Vantage and QIAsymphony DSP Virus/Pathogen Kit

Application Note 97



Keywords

+ Automation
+ COVID-19
+ RNA
+ RT-qPCR
+ Extraction
+ microRNA
+ Saliva
+ Silicon Carbide
+ Swab
+ Diagnostics
+ SARS-CoV-2
+ Viral RNA

INTRODUCTION

The COVID-19 pandemic has created a need for high-throughput, sensitive RNA isolation methods from several sample types. Testing labs across the world need access to high quality RNA for RT-qPCR, the gold standard for SARS-CoV-2 detection. However, many automation methods have a reduced yield or isolate RNA with more impurities compared to manual isolation. Isolating even low levels of viral RNA is crucial for early detection of infection and gives public health authorities the data needed to control the spread of the virus. The process used for RNA isolation must be as sensitive as possible to purify even the smallest quantities of RNA in order to detect the virus in samples with low viral loads.

The **Saliva/Swab RNA Purification Kit** (Cat. 69300) from Norgen Biotek has been optimized for automated extraction of total RNA, including viral RNA, from saliva or swab samples. The proprietary silicon carbide technology captures all sizes of RNA, including microRNA. This allows for even small, degraded fragments of the virus to be isolated from samples, further increasing the potential limit of detection.

This kit enables RNA isolation from thousands of samples per day, increasing the total testing capacity of labs without reducing the quality or yield of RNA isolated from samples. This application note compares the isolation of Norgen's Saliva/Swab RNA Purification Kit (Cat. 69300) performed manually and on the automated Hamilton Vantage system with the Qiagen QIAsymphony DSP Virus/Pathogen Kit to determine the isolation efficiency of each.

MATERIALS & METHODS

24 saliva samples were collected and preserved using Norgen's Saliva RNA Collection and Preservation Devices (Cat. 53800). Preserved saliva samples were spiked in triplicate with an RNA transcript in the following dilution series: 6 x 10^7, 6 x 10^6, 6 x 10^5, 6 x 10^4, 6 x 10^3, 6 x 10^2 copies/ml. The remaining 6 samples were used to determine the quality of the endogenous RNA. Each sample was isolated using Norgen's Saliva/Swab RNA Purification Kit manually and on the Hamilton Vantage, and QIAsymphony DSP Virus/Pathogen Kit on the Qiagen QIAsymphony. Each isolation method followed the prescribed protocol without deviation.

From each eluate, 5 µL was used as a template for a real-time RT-qPCR reaction, using Norgen's 2X One-Step RT-PCR Master Mix (Cat. 28113). Reactions were performed in duplicate using the Thermo Fisher QuantStudio™ 7 Pro Real-Time PCR System.

2 µL from each eluate were used to determine the quality of isolated RNA on the Thermo Fisher NanoDrop 2000.

RESULTS

The automated method produces high yields of with good quality.
Figure 1 shows that the automated methods on the Hamilton Vantage and the QIAsymphony had similar yields. These yields were higher than the manual method. Furthermore, the 260/280 ratio indicates the RNA isolated manually and using automation of the Norgen kit produced high quality RNA without impurity. The RNA isolated using the QIAsymphony had a 260/280 ratio significantly higher than 2, which may indicate contamination.

RNA isolated using the automated method yields the lowest LOD
Detection of the spiked RNA transcript was performed as described in the methods section above. All isolation methods provided enough RNA template to amplify in the highest 3 concentrations. Only the automated Norgen method provided enough RNA to amplify at 104 copies/µL (Figure 2, pg 3). In addition, the Norgen kit consistently provided lower Cq values than the other methods.

The eluted RNA can be used for qPCR and is free of inhibitors
Moreover, qPCRs of increasing template concentrations confirm high quality eluate with negligible PCR inhibitors in the samples. For each doubling of the input template (from 2,4,8 µL) there is an approximate decrease of Cq even up to an input of 8 µL (Figure 3, pg 3).

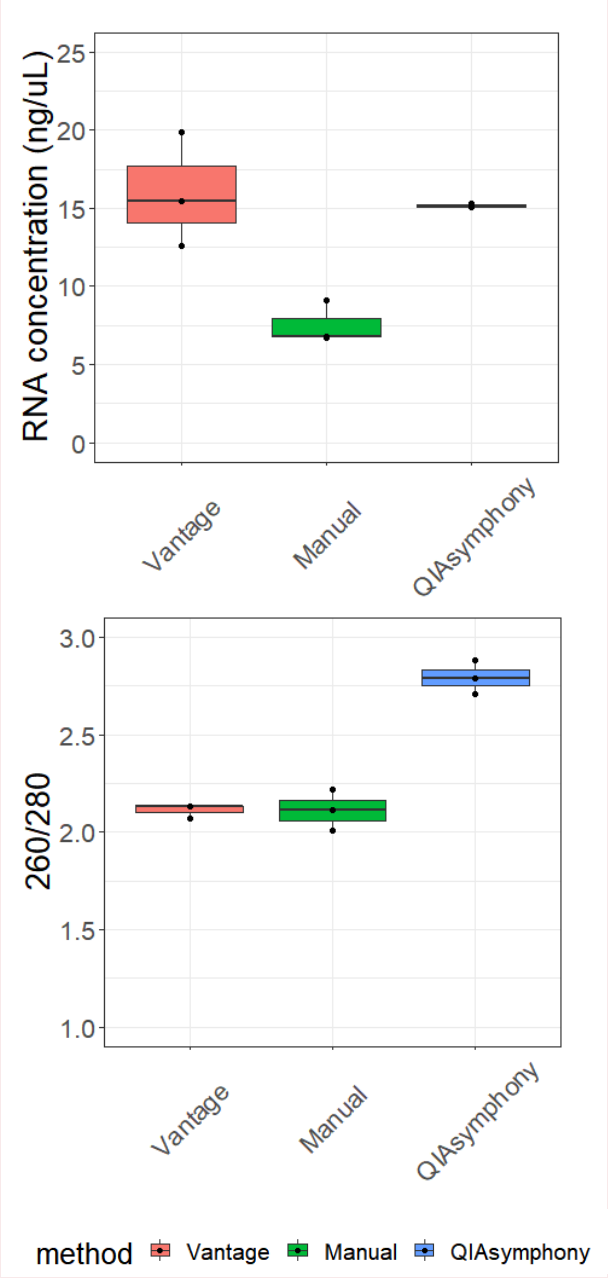


Figure 1. RNA yield and quantity for 6x10^6 spiked samples as determined by nanodrop.

CONCLUSION

The automated extraction of RNA using Norgen's silicon carbide technology produces a higher yield of RNA than the manual method and a similar yield to the QIAsymphony method. However, the eluate is of higher quality as indicated by lower Cq values in RT-qPCR, a 260/280 ratio around 2 and decreasing Cq with increasing eluate input up to 8 µL. The eluate from the QIAsymphony method has an abnormally high 260/280 ratio that may indicate some contaminant that absorbs close to 260 nm, possibly guanidine isothiocyanate. This reduction in RT-qPCR sensitivity can lead to a greater number of false-negative results and a higher limit of detection.

Using Norgen's Saliva/Swab RNA Purification Kit (Cat. 69300) on the automated Hamilton Vantage provides high-quality RNA that will provide a lower limit of detection for a diagnostic workflow than the QIAsymphony DSP Pathogen/Virus Kit. This is especially important for the detection of low viral loads.

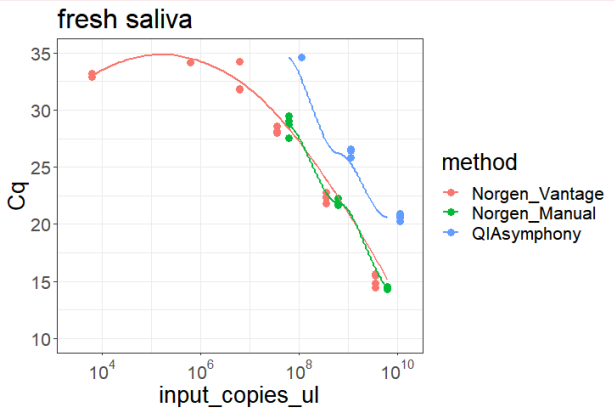


Figure 2. Detection of spiked RNA through RT-qPCR Cq values of a dilution series of spiked saliva samples using 3 isolation methods.

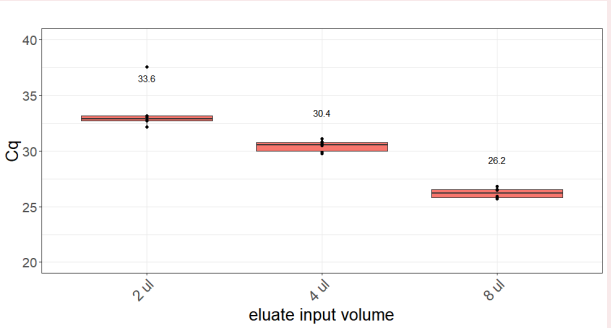


Figure 3. The RNA isolated using the Hamilton Vantage is free of inhibitors as illustrated by increasing Cqs when the eluate input is doubled.

Cat No.	Related Products	Size
53800	Saliva RNA Collection and Preservation Devices Dx CE	50 Devices
RU53800	Saliva RNA Collection and Preservation Devices	50 Devices
Dx69300	Saliva/Swab RNA Purification 96-well Kit Dx CE	2 x 96-well plates
69300	Saliva/Swab RNA Purification 96-well Kit	2 x 96-well plates
Dx69100	Saliva/Swab RNA Purification Kit Dx CE	50 Preps
69100	Saliva/Swab RNA Purification Kit	50 Preps
72000	Saliva/Swab RNA Purification 96-Well Automation Accessories Kit	1 Kit
28113 28114 28115	2X One-Step RT-PCR Master Mix	100 Reactions 200 Reactions 500 Reactions

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