Purification of synthetic SARS-CoV-2 viral RNA from biological samples using the Monarch® Total RNA Miniprep Kit and the Monarch RNA Cleanup Kit

Flexible workflows expand the utility of Monarch RNA Purification Kits

by Barbara Taron, Ph.D., Ashley Luck, Ph.D., Yan Xu, Ph.D., Nicole Nichols, Ph.D., and Eric Cantor, Ph.D., New England Biolabs, Inc.

INTRODUCTION

Many COVID-19 FDA Emergency Use Authorization (EUA) diagnostic tests rely on extraction of RNA from patient samples prior to detection of viral targets in purified RNA using RT-qPCR.The recent overwhelming demand for COVID-19 diagnostic testing materials has led to supply shortages for RNA purification kits and reagents. As a result, testing facilities are in search of alternative RNA purification methods that may replace or plug into existing workflows, including high throughput automated workflows.

New England Biolabs offers two purification solutions for isolation of high-quality RNA, the Monarch Total RNA Miniprep Kit and the Monarch RNA Cleanup Kits. In this technical note, we demonstrate the quantitative purification and recovery of synthetic viral RNA from clinically-relevant samples using these kits. We also evaluate a modified total RNA miniprep workflow whereby initial workflow steps are omitted, decreasing sample processing time. Finally, we describe automated medium and high throughput workflows for processing saliva samples using the Monarch Total RNA Miniprep Kit and Monarch RNA Cleanup Kits, and the Qiagen® QIAcube® or KingFisher™ Flex platforms.

MONARCH TOTAL RNA MINIPREP KIT (NEB #T2010)

The Monarch Total RNA Miniprep Kit is a silica spin column-based RNA purification method for the extraction of high quality total RNA from a wide variety of sample types including cultured mammalian cells, blood, tissue, and tough-to-lyse samples. Although the Monarch Total RNA Miniprep Kit has been used successfully to isolate RNA from clinically-relevant samples including whole blood, plasma, serum, saliva and buccal cells, we have not previously demonstrated the presence of viral RNA in total RNA extracted from these samples.

Purification of synthetic viral RNA from saliva and simulated respiratory secretion samples

Saliva is increasingly becoming a preferred biological sample for viral testing for a number of reasons, including ease of collection, the ability to collect samples at home and reduced exposure at testing sites. We evaluated two workflows for purification of synthetic viral RNA from saliva, the *RNA Extraction from Saliva, Buccal Swabs, and Nasopharyngeal Swabs* Proteinase K Protocol and a shortened version of this workflow, the Quick Protocol. In the Proteinase K Protocol, saliva is initially processed by sequential addition of Monarch DNA/RNA Protection Reagent ("Protection Reagent") and Proteinase K, followed by a room temperature incubation for 30 minutes. Monarch RNA Lysis Buffer is then added to prepare the sample for column purification. In the Quick Protocol, saliva is mixed directly with RNA Lysis Buffer. Additionally, as an alternative to saliva and other potentially infectious clinical samples, a simulated secretion material was evaluated in this study. A simulated respiratory secretion containing epithelial cells, albumin, IgG, IgM, mucin, Na^+, K^+, and Ca^{2+} was prepared as described, except HeLa cells were used in place of A549 cells (1). An aliquot of the simulated secretion (75 µl, containing approximately 150,000 cells) was added to viral transport medium (TM) (4.5 ml) and the resulting sample mixture was used as a surrogate for a clinical sample.

The Proteinase K and Quick Protocols were used to isolate total RNA from saliva and simulated secretion samples containing 10-fold serial dilutions of synthetic SARS-CoV-2 N-gene RNA, with inputs ranging from $5x10^3$ to $5x10^7$ copies per sample. Purified RNA was eluted in 100 µl nuclease-free water to yield 50 to 500,000 copies of viral RNA/µl, and the SARS-CoV-2 N-gene was detected in these samples using RT-qPCR.

In this study, RT-qPCR detection of RNA targets was validated using CDC N2 primers/probe set to detect SARS-CoV-2 N-gene, and β-actin primers/probe set to quantitate human total RNA (Table 1). Using the Luna Universal Probe One-Step RT-qPCR Kit, linear detection of SARS-CoV-2 N-gene and β-actin gene targets was observed in templates containing 5-log dilutions of *in vitro* transcribed N-gene RNA (50 to 500,000 copies) and Jurkat total RNA (10 pg – 100 ng) simultaneously in 2-plex reactions (Figure 1A). Importantly, the detection of N-gene or β-actin gene is similar between 1-plex and 2-plex reactions (Figure 1B and C), validating the use of 2-plex RT-qPCR for simultaneous detection and quantitation of these targets. Additionally, the detection of N-gene in control dilutions (50 to 500,000 copies) is similar using the *in vitro* transcribed N-gene RNA as template alone in water or using the *in vitro* transcribed N-gene RNA in the background of 10 ng total RNA (Figure 1D).

MATERIALS

- Monarch Total RNA Miniprep Kit (NEB #T2010)
- Monarch RNA Cleanup Kits (NEB #T2030, #T2040 and #T2050)
- Monarch DNA/RNA Protection Reagent (NEB #T2011)
- Monarch RNA Lysis Buffer (NEB #T2012)
- Luna® Universal Probe One-Step RT-qPCR Kit (NEB #E3006)

TABLE 1: **RT-qPCR primer and probe sequences**

Sequences of primers and probes used to detect the SARS-CoV-2 N-gene and β-actin gene using the Luna Universal Probe One-Step RT-qPCR Kit.

PRIMERS/PROBES	SEQUENCES
CDC N2 primers	TTACAAACATTGGCCGCAAA GCGCGACATTCCGAAGAA
CDC N2 probe	ACA ATTTGCCCCCAGCGCTTCAG
β-actin primers	GGATGCAGAAGGAGATCACTG CGATCCACACGGAGTACTTG
β-actin probe	TGGCACCCAGCACAATGAAGATCA

SARS-CoV-2 N-gene was detected in total RNA from saliva and simulated secretion samples (spiked with synthetic SARS-CoV-2 N-gene RNA and purified using the Proteinase K and Quick Protocols, as described above) with high sensitivity (consistent detection at the lowest input amount tested, 50 copies) and linear quantitation was observed over a broad range of viral RNA titers (50 to 500,000 copies, with 91.1–105.3% and 86.6-93.9% efficiencies for saliva and simulated secretion preps, respectively) (Figure 2). Consistent detection of β-actin mRNA was also observed over this range, demonstrating reliable lysis of cellular material present in saliva and simulated secretion samples. Importantly, no significant differences were observed in viral RNA detection in samples prepared using the Proteinase K or Quick Protocol. Moreover, minimal differences were observed between either saliva protocol (Proteinase K or Quick) compared to the detection of pure N-gene RNA diluted in water. Based on these observations, viral RNA can be quantitatively recovered from saliva and a simulated secretion using the Monarch Total RNA Miniprep Kit; however, the longer, standard Proteinase K protocol is unnecessary. In order to save critical time (approximately 30 minutes) during sample preparation, we recommend shortening this standard protocol by omitting the initial sample processing with Protection Reagent and Proteinase K.

MONARCH RNA CLEANUP KITS

The Monarch RNA Cleanup Kits (NEB #T2030, #T2040 and #T2050) provide silica spin column-based RNA purification, and are available in 3 binding capacities (10 µg, 50 µg and 500 µg) that enable rapid and reliable cleanup and concentration of high-quality RNA, typically from enzymatic reactions. These kits, while not specifically designed to purify RNA from biological samples, utilize similar buffers and workflows common to many RNA extraction kits. We therefore reasoned that viral RNA may be successfully purified from certain biological samples using these kits as well.

FIGURE 1: **Validation of SARS-CoV-2 N-gene and β-actin gene targets using 1-plex and 2-plex RT-qPCR**

Linear quantitation of N-gene and β-actin gene using Luna Universal Probe One-Step RT-qPCR Kit. A. RNA templates containing 5-log dilutions of human total RNA (10 pg – 100 ng) and *in vitro* transcribed N-gene RNA (50 to 500,000 copies) were used to detect β-actin gene and N-gene simultaneously in a 2-plex reaction following recommended conditions. B. The detection of N-gene is similar between 1-plex and 2-plex reactions in terms of C_q values and efficiency (E). C. The detection of β-actin gene is similar between 1-plex and 2-plex reactions in terms of C_q values and efficiency. D. The presence of background human total RNA (10 ng) does not change the detection of N-gene. The 5-log detection of N-gene is similar in the presence or absence of background total RNA in terms of C_q values and efficiency.

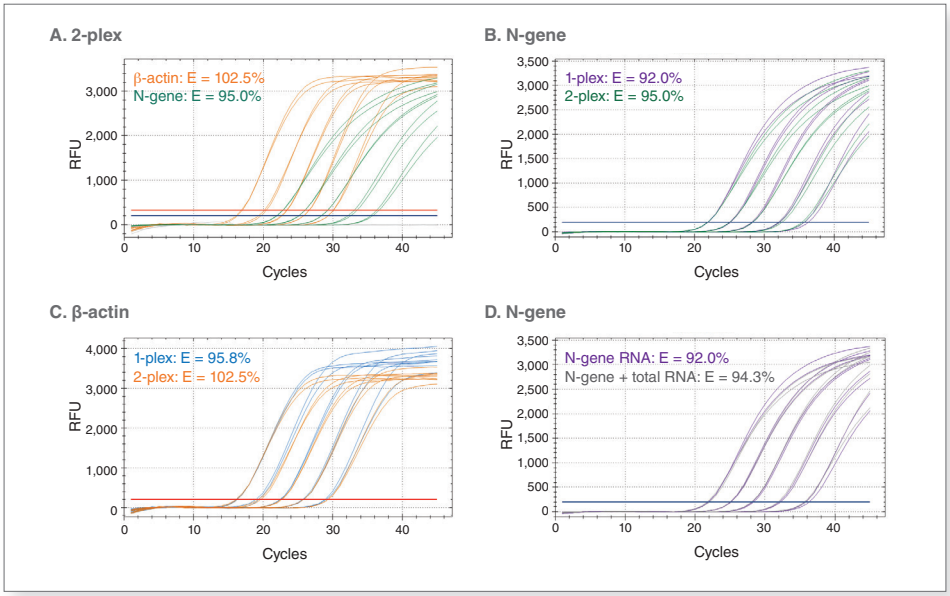
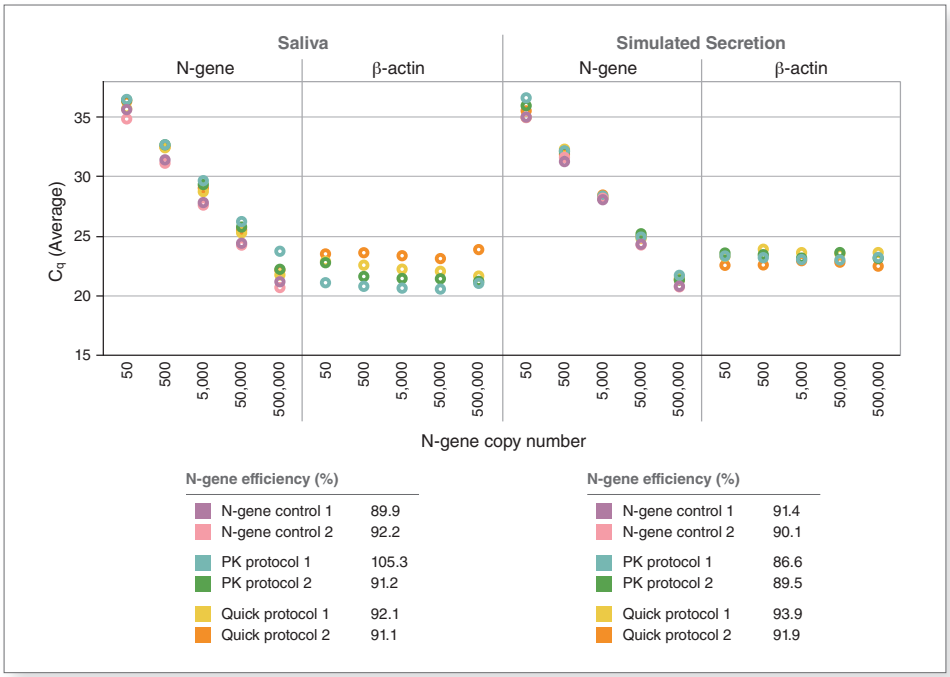


FIGURE 2: **Detection and quantitation of SARS-CoV-2 N-gene RNA from saliva and simulated secretion samples purified using the Monarch Total RNA Miniprep Kit**

Simultaneous quantitation of SARS-CoV-2 N-gene and human β-actin mRNA from total RNA isolated from saliva samples (200 µl) and simulated secretion samples (200 µl, simulated secretion in TM, containing ~6700 HeLa cells) spiked with synthetic SARS-CoV-2 viral RNA, as determined by 2-plex RT-qPCR using the Luna Universal Probe One-Step RT-qPCR Kit. The Monarch Total RNA Miniprep Kit Proteinase K (PK) and Quick saliva protocols were used to isolate total RNA from samples to yield eluates with 50 to 500,000 copies/µl of SARS-CoV-2 viral RNA.



Purification of synthetic viral RNA from a simulated secretion sample and buccal (cheek) swab

A simulated respiratory secretion (containing HeLa cells, albumin, IgG, IgM, mucin, Na^+, K^+ and Ca^{2+}) (1) again served as a proxy for potentially infectious clinical samples. An aliquot of the simulated secretion (50 µl, containing approximately 200,000 cells) was added to transport medium (TM) (3 ml) and the resulting sample mixture was used as a surrogate for a clinical sample. Total RNA was isolated from this simulated secretion/transport media sample (100 µl or approximately 6500 cells) spiked with synthetic SARS-CoV-2 N-gene RNA using the *RNA Purification from Buccal Swabs and Nasopharyngeal Samples Protocol* for the RNA Cleanup Kits in which the simulated sample was mixed with an equal volume of 2X Protection Reagent and processed using the Monarch RNA Cleanup Kit (10 µg, NEB #T2030 or 50 µg, NEB #T2040). Purified RNA samples were eluted in 50 µl of nuclease-free water and the Luna Universal Probe One-Step RT-qPCR Kit was used to detect the presence and titer of the SARS-CoV-2 N-gene. Titers as low as 50 copies/µl were consistently detected in purified eluates and linear quantitation of the SARS-CoV-2 N-gene was observed over the entire dilution series (from 50 to 500,000 copies/µl, efficiencies of 82.3–91.9%) for both Monarch RNA Cleanup Kits tested (Figure 3). Moreover, in the first demonstrated use of the Monarch RNA Cleanup Kits for the lysis and purification of RNA from cells, consistent detection of β-actin was observed over this range.

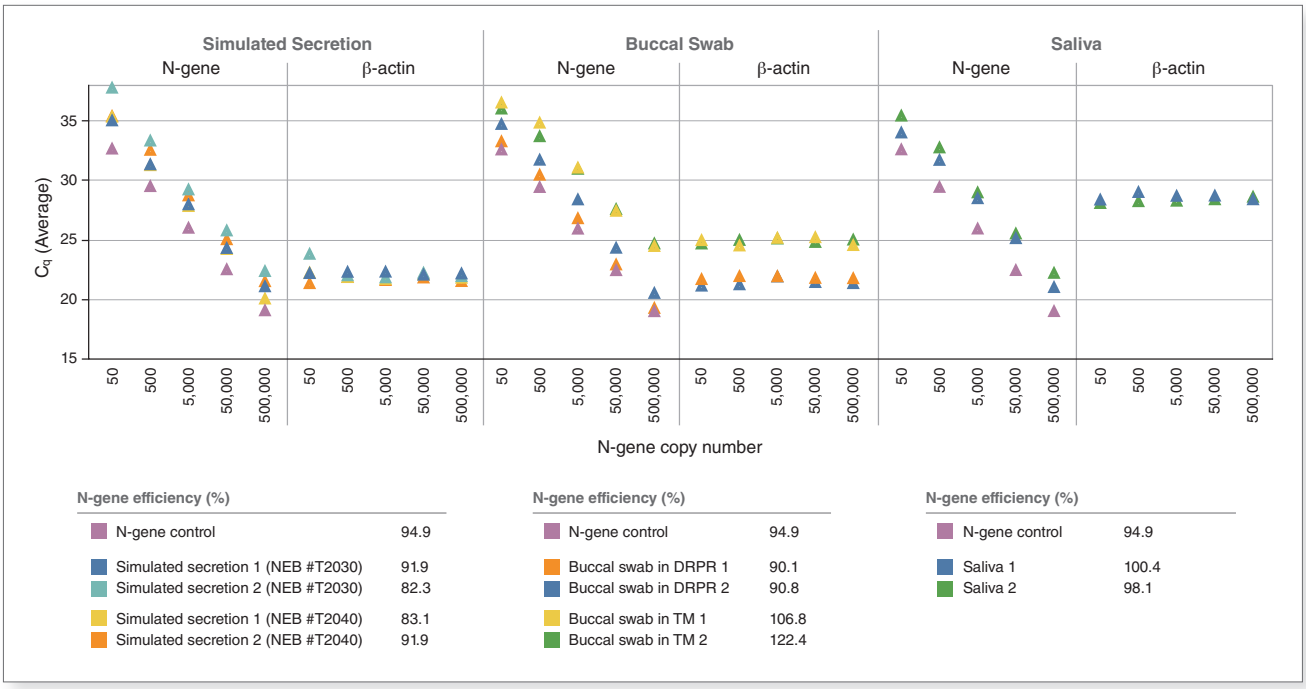
The use of the Monarch RNA Cleanup Kit for the lysis and purification of RNA from cells was further validated using buccal swabs. Buccal swabs in transport media or 1X Protection Reagent (NEB #T2011, sold separately) were spiked with synthetic SARS-CoV-2 N-gene RNA and purified using the modified *RNA Purification from Buccal Swabs and Nasopharyngeal Samples Protocol* for the RNA Cleanup Kits. Similar to the simulated secretion samples, titers as low as 50 copies/µl were detected by RT-qPCR and linear quantitation of the SARS-CoV-2 N-gene was observed over the entire dilution series (from 50 to 500,000 copies/µl, Figure 3) in both TM and 1X Protection Reagent. However, the C_q values of buccal swab samples collected in TM are significantly delayed as compared to buccal swabs collected directly in 1X Protection Reagent and the N-gene controls (Figure 3), presumably due to some RNA degradation potentially occurring during sample preparation. While buccal swab samples placed in TM may still yield accurate results (down to 50 copies), low viral titers may not be captured by this sample collection and preparation method. Therefore, for the most accurate results, we recommend buccal swabs be placed directly in 1X Protection Reagent prior to RNA extraction.

Purification of synthetic viral RNA from saliva samples

As previously mentioned, saliva samples are increasingly being used as an alternative to nasopharyngeal samples. Saliva, however, is a more challenging medium from which to extract high quality RNA. In order to purify high quality RNA from saliva using the Monarch RNA Cleanup Kits, some additional modifications were made to the protocol. Due to the viscosity of the sample, we do not recommend purifying RNA from saliva using the smallest capacity Monarch RNA Cleanup Kit (10 µg, NEB #T2030). This silica column is relatively

FIGURE 3: **Detection and quantitation of SARS-CoV-2 N-gene RNA from simulated secretion, buccal swab, and saliva samples, purified using the Monarch RNA Cleanup Kit and Protection Reagent**

Simultaneous quantitation of SARS-CoV-2 N-gene and β-actin mRNA from simulated secretion, buccal swab, and saliva samples as determined by multiplex RT-qPCR using the Luna Universal Probe One-Step RT-qPCR Kit. Quantitation of SARS-CoV-2 N-gene and β-actin mRNA from simulated secretion samples (100 µl of synthetic simulated secretion containing ~6500 HeLa cells in TM), buccal swabs (300 µl) in either TM or 1X Monarch DNA/RNA Protection Reagent (DRPR, #T2011) and saliva samples (100 µl) spiked with synthetic SARS-CoV-2 viral RNA (50 to 500,000 copies) purified using the Monarch RNA Cleanup Kits.



small in diameter, which allows elution of RNA in as little as 6 µl, providing both clean up and concentration of RNA samples. However, even after dilution of a saliva sample with the Monarch RNA Cleanup Binding Buffer (2 volumes) and ethanol (1 volume), and centrifugation at maximum speed (approximately16,000 x *g*) for 5 minutes, the viscosity of the sample combined with the narrow diameter of this column did not allow the entire sample to pass through the silica membrane. For these reasons, we recommend using the Monarch RNA Cleanup Kit (50 µg, NEB #T2040) for the purification of RNA from saliva samples. In order to reduce the viscosity of saliva samples, a number of protocol modifications were made including: 1) the initial saliva sample is diluted 1:1 with 2X Protection Reagent, and 2) each saliva prep using the Monarch RNA Cleanup Kit (50 µg) requires the use of two silica columns. RNA Cleanup Binding Buffer (2 volumes) is added to the sample (saliva diluted with 2X Protection Reagent) and passed through the first silica column. This effectively reduces the viscosity of the sample before ethanol (1 volume) is added to the flow-through of the first column and the sample is bound on a second column.

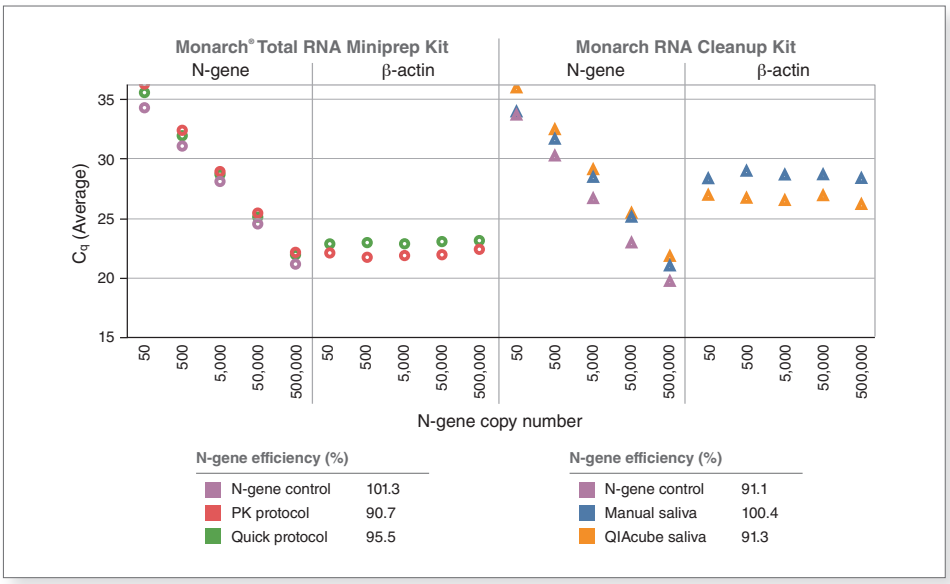
The modified process described above was effectively used to isolate total RNA from saliva samples containing synthetic SARS-CoV-2 N-gene RNA. Using the Luna Universal Probe One-Step RT-qPCR Kit, titers as low as 50 copies/µl were consistently detected in purified eluates and linear quantitation of the SARS-CoV-2 N-gene was observed over the entire dilution series (from 50 to 500,000 copies/µl, with an efficiency of 98.1–100.4%) (Figure 3).

One common protocol modification used to purify potentially low copy number viral RNAs from volatile biological samples is to use a carrier RNA (typically added to the binding buffer of the silica-based purification kit before adding the binding buffer to the sample). We also tested whether the addition of a carrier RNA molecule would be beneficial to preserving low copy number viral RNAs in a more volatile biological sample such as saliva. Interestingly, no significant improvements in the detection or quantitation of low abundance viral RNAs were observed by adding carrier RNA to the modified protocol for the isolation of RNA from saliva (data not shown).

The Monarch RNA Cleanup Kits are available in 3 binding capacities (10 µg, 50 µg and 500 µg). As described above we do not recommend the use of the 10 µg capacity kit for the purification of RNA from saliva due to physical constraints of such a small column. Moreover, the large capacity of the Monarch RNA Cleanup Kit (500 µg) does not lend itself easily to the preparation of these sample types, which may contain very low copy numbers of viral RNA. The Monarch RNA Cleanup Kit (50 µg) is suitable for purification of RNA from all sample types tested, including buccal swabs, secretions and saliva (following the appropriate protocol).

FIGURE 4: **Detection and quantitation of SARS-CoV-2 N-gene from saliva RNA purified using Monarch RNA purification reagents and columns, and QIAcube automated sample processing**

Simultaneous quantitation of SARS-CoV-2 N-gene and human β-actin gene from total RNA isolated from saliva samples spiked with synthetic SARS-CoV-2 viral RNA using the Monarch Total RNA Miniprep Kit (left panel) or the Monarch RNA Cleanup Kit (right panel) and QIAcube automation, as determined by 2-plex RT-qPCR using the Luna Universal Probe One-Step RT-qPCR Kit. Left panel: Total RNA was purified from 150 µl (PK protocol) or 200 µl (Quick protocol) saliva containing 10-fold serial dilutions of synthetic viral RNA, with inputs ranging from $5x10^3$ to $5x10^7$ copies per prep. Purified RNA was eluted in 100 µl nuclease-free water to yield preps with 50 to 500,000 copies/µl of viral RNA. Right panel: Total RNA was purified from 100 µl saliva spiked with synthetic SARS-CoV-2 viral RNA (50 to 500,000 copies) using either the automated QIAcube protocol or the manual benchtop centrifuge protocol for the Viral RNA Extraction from Saliva Using the Monarch RNA Cleanup Kits.



AUTOMATION OF MONARCH VIRAL RNA EXTRACTION WORKFLOWS

Automated workflows are often used to significantly increase sample prep throughput. Due to RNA purification reagent shortages, some labs need RNA purification methods to replace, or plug into, existing automated workflows. To address this need, we evaluated the compatibility of Monarch Total RNA Miniprep Kit and Monarch RNA Cleanup Kit workflows with automated medium and high throughput sample processing instruments, the Qiagen QIAcube and the KingFisher Flex.

QIAcube automation

The Qiagen QIAcube is a medium throughput, robotic sample prep workstation for processing Qiagen spin columns. Pre-programmed QIAcube protocols align with manual Qiagen extraction kits to process up to 12 samples per run. For RNA purification, RNeasy® spin columns and reagents are used along with specialized QIAcube plastics for automated sample processing. Since Monarch RNA purification and RNeasy Mini kit components and workflows are similar, we reasoned that the Monarch Total RNA Miniprep and Monarch RNA Cleanup kits may be compatible with QIAcube automation.

We evaluated QIAcube automated sample processing using Monarch Total RNA Miniprep Kit and Monarch RNA Cleanup Kit columns and reagents. Saliva samples spiked with synthetic SARS-CoV-2 N-gene RNA were prepared for QIAcube processing using the Monarch Total RNA Miniprep Kit Proteinase K or Quick Saliva Protocol, or the Monarch RNA Cleanup Kit modified workflow for saliva. Monarch Total

RNA Miniprep samples were processed using the QIAcube RNeasy "QIAshredder® DNase digest" protocol and Monarch RNA Cleanup Kit samples were processed using the QIAcube RNeasy "QIAshredder" protocol. Using the Luna Universal Probe One-Step RT-qPCR Kit, viral RNA titers as low as 50 copies/µl were consistently detected in purified RNA and linear quantitation of the SARS-CoV-2 N-gene was observed over the entire dilution series (Figure 4). Moreover, viral RNA recovery was consistent for samples processed on the QIAcube using either the Monarch Total RNA Miniprep Kit Proteinase K or Quick saliva workflows (Figure 4). Additionally, comparable results were observed between saliva samples prepped manually (benchtop centrifugation) and samples prepped on the QIAcube using the Monarch RNA Cleanup Kit (Figure 4).

KingFisher Flex automation

The KingFisher Flex (Thermo Fisher Scientific®) is an automated magnetic particle processing instrument that enables high throughput purification of nucleic acids. KingFisher Flex protocols align with MagMAX® nucleic acid purification kit workflows to process up to 96 samples per run. MagMAX reagents and magnetic beads are used along with KingFisher Flex plates and plastics for sample processing. Although the Monarch Total RNA Miniprep and Monarch RNA Cleanup kits are silica spin column-based purification kits, we reasoned that silica coated magnetic beads may be a suitable replacement for the spin columns, enabling bead-based automated processing.

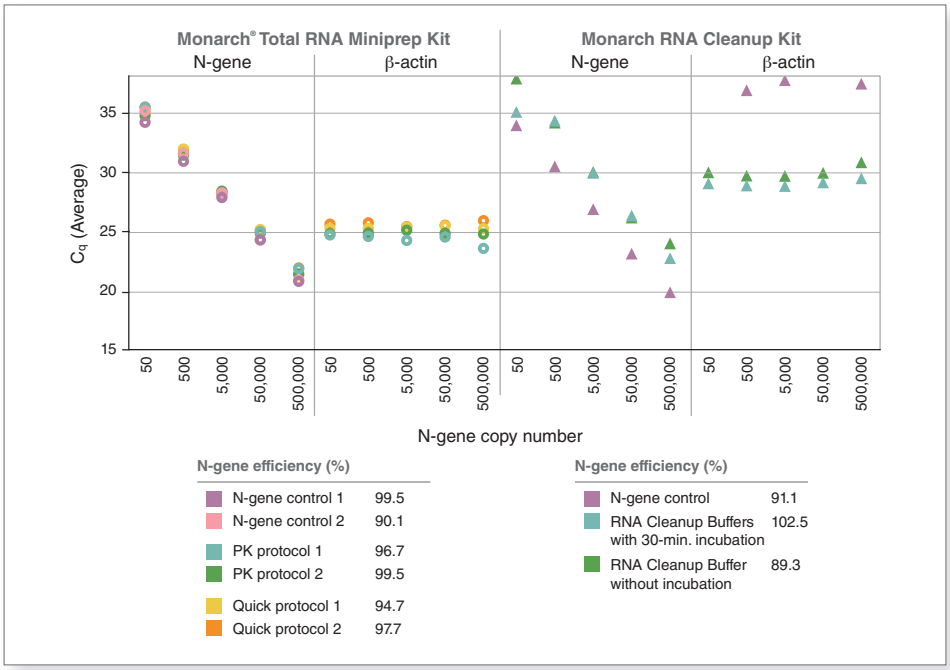
We evaluated KingFisher Flex automated sample processing using Monarch RNA purification kit reagents and Dynabeads® MyOne™ Silane magnetic beads. Using the Monarch Total RNA Miniprep Kit Proteinase K or Quick Protocol, saliva samples spiked with synthetic viral RNA were prepared as described previously; however, additional sample preparation steps were performed prior to bead-based processing. Specifically, after addition of RNA Lysis Buffer (NEB #T2012), an equal volume of ethanol was added to samples, followed by 20 µl Dynabeads MyOne Silane magnetic beads. Samples were then transferred to individual wells in a KingFisher 96 deep well plate and agitated (1000 rpm for 30 minutes at room temperature). The 96 deep well sample plate was then loaded onto the KingFisher Flex instrument and processed using a modified "MagMAX Pathogen High Volume" protocol. In this modified protocol, the fourth wash step was eliminated to align with the Monarch Total RNA Miniprep workflow, which has three wash steps.

Saliva samples spiked with synthetic viral RNA were also prepared using the Monarch RNA Cleanup Kit modified workflow, again with additional sample preparation steps required for bead-based processing. After addition of RNA Cleanup Binding Buffer, an equal volume of ethanol was added to samples followed by 20 µl Dynabeads MyOne Silane magnetic beads. Samples were then transferred to individual wells in a KingFisher 96 deep well plate and agitated (1000 rpm for 30 minutes at room temperature). The 96 deep well sample plate was loaded onto the KingFisher Flex instrument and processed using a modified "MagMAX Pathogen High Volume" protocol with two wash steps, to align with the Monarch RNA Cleanup Kit workflow.

Once again, the SARS-CoV-2 N-gene was consistently detected with high sensitivity and linear quantitation was observed over a broad range of viral RNA titers (50 to 500,000 copies) from saliva samples purified using either the Proteinase K or Quick workflow and Monarch Total RNA Miniprep Kit reagents, or the modified Monarch RNA Cleanup Kit workflow and reagents (Figure 5). Since the recommended 30-minute room temperature RNA to bead-binding step significantly increases the amount of time to process samples using this method, we also tested the Monarch RNA Cleanup Kit workflow without the 30-minute bead-binding step. While viral RNA as low as 50 copies was detected using either protocol (+/– 30-minute bead-binding step), it does appear that the 30-minute incubation may be beneficial and enable RNA binding to the beads, especially in low viral load cases (<500 copies)/50 µl saliva sample (Figure 5).

FIGURE 5: **Detection and quantitation of SARS-CoV-2 N-gene from saliva RNA purified using Monarch RNA purification reagents and KingFisher Flex automation**

Simultaneous quantitation of SARS-CoV-2 N-gene and human β-actin gene from total RNA isolated from saliva samples spiked with synthetic SARS-CoV-2 viral RNA using the Monarch Total RNA Miniprep Kit (left panel) or the Monarch RNA Cleanup Kit (right panel) and the KingFisher Flex, as determined by 2-plex RT-qPCR using the Luna Universal Probe One-Step RT-qPCR Kit. Left panel: Total RNA was purified from 100 µl (PK protocol) or 125 µl saliva (Quick protocol) containing 10-fold serial dilutions of synthetic viral RNA, with inputs ranging from $5x10^3$ to $5x10^7$ copies per prep. Purified RNA was eluted in 100 µl nuclease-free water to yield preps with 50 to 500,000 copies/µl of viral RNA. Right panel: Total RNA was purified from saliva samples (50 µl) spiked with synthetic SARS-CoV-2 viral RNA (50 to 500,000 copies) using the KingFisher Flex (automated protocol) with and without the recommended 30-minute, 25°C incubation to facilitate RNA binding to the beads.



CONCLUSION

We confirmed Monarch Total RNA Miniprep Kit (NEB #T2010) and Monarch RNA Cleanup Kit (NEB #T2030, #T2040, #T2050) protocols can be successfully modified and performed for the purification of synthetic viral RNA from clinically related biological samples, including saliva and buccal swabs. Using the Luna Universal Probe One-Step RT-qPCR Kit (NEB #E3006), we demonstrated sensitive and quantitative recovery of synthetic viral RNAs purified by these methods. We consistently detected titers as low as 50 copies/µl (the lowest input tested) in RNA eluates and observed linear, quantitative recovery of the SARS-CoV-2 N-gene in purified RNA over a 5-log range (50 to 500,000 copies/µl).

In this technical note, we also describe flexible workflows that expand the utility of the Monarch RNA purification kits. Using the Monarch Total RNA Miniprep Kit, we demonstrated that a shortened workflow for RNA purification from saliva decreases manual sample processing time (by approximately 30 minutes) and is equivalent to the standard saliva Proteinase K workflow. Additionally, using the Monarch RNA Cleanup Kits, we demonstrated for the first time that RNA can be isolated from cells (HeLa and buccal) and saliva via modified workflows, enabling purification of synthetic viral RNA from these samples. Finally, we demonstrated that the Monarch Total RNA Miniprep Kit and Monarch RNA Cleanup Kit saliva workflows are compatible with automated medium and high throughput sample processing platforms (QIAcube and KingFisher Flex).

Reference

1. Bose, M. et al. (2016) *PLoS ONE* 11(11): e0166800. doi:10.1371/journal.pone.0166800

HIGHLIGHTS

- Monarch Total RNA Miniprep and Monarch RNA Cleanup Kits successfully purify synthetic SARS-CoV-2 viral RNA from clinically-relevant biological samples.
- Using RT-qPCR, we observe linear, quantitative recovery of the SARS-CoV-2 N-gene from purified RNA over a 5-log range (50 to 500,000 copies).
- Flexible workflows expand the utility of Monarch RNA purification kits:
  - A streamlined Monarch Total RNA Miniprep Kit saliva protocol shortens sample processing time by ~30 minutes.
  - Modified protocols for the Monarch RNA Cleanup Kits enable quick extraction of viral RNA from saliva, buccal swabs and a simulated respiratory secretion, with the addition of DNA/RNA Protection Reagent (NEB #T2011)
  - Monarch RNA purification workflows can be automated on the QIAcube and KingFisher Flex platforms with only minor protocol modifications.

Access these and other related protocols at **www.neb.com/covid19**

Visit **www.neb.com/Monarch** to see all products available for nucleic acid extraction

VIRAL INACTIVATION BY MONARCH RNA BUFFERS

NEB has worked with a third-party research organization to examine the ability of some of the Monarch buffers to inactivate live SARS-CoV-2 virus. We reasoned that significant collection and handling of virus occurs outside of clinical settings. We sought to evaluate the effectiveness of Monarch DNA/RNA Protection Reagent and Monarch RNA Lysis Buffer for inactivation of SARS-CoV-2 in the context of simulated secreta in transport media or with infected cells. To validate chemical inactivation, two limited studies were performed. In the first, intact virus was spiked into a simulated secretion/transport medium mixture and mixed with either Protection Reagent or RNA Lysis Buffer. The buffer treated material was passed through two blind serial passages on naïve cultured cells and the resulting cell culture supernatant was used to test for viable virus in a plaque titration assay. These studies found that each of the aforementioned buffers completely inactivated up to 5 x 10^5 pfu/mL of SARS-CoV-2 virus in the simulated secretion in transport media.

In a separate but related study, SARS-CoV-2 infected Vero cells were used as a source of the virus to examine the ability of each buffer to inactivate and eliminate replication competent virus. To determine if viable virus was present after treatment with buffer, treated test material was subjected to two blind serial passages on naïve cultured cells and the resulting cell culture supernatant was used to test for viable virus in a plaque assay. Under these conditions, complete inactivation was observed of up to 1 x 10^7 pfu/mL of SARS-CoV-2 virus in a cell culture slurry.

Taken together, these results suggest that when used as directed in the protocols, the methods provide for successful inactivation of SARS-CoV-2 under the conditions tested. These kits are sold as RUO (research use only). Users should always adhere to the safety guidelines of their institution.

New England Biolabs, Inc., 240 County Road, Ipswich, MA 01938-2723 Telephone: (978) 927-5054 Toll Free: (USA Orders) 1-800-632-5227 (USA Tech) 1-800-632-7799 Fax: (978) 921-1350 e-mail: info@neb.com