ORIGINAL ARTICLE

# Comparison of Automated and Manual Viral Nucleic Acid Extraction Kits for Covid-19 Detection Using qRT-PCR

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## ABSTRACT

**Introduction:** The emergence of a novel Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has resulted in a pandemic. Rapid and accurate diagnosis method is crucial to reduce the disease burden and to improve early diagnosis approaches to control of the disease. Real time Reverse transcriptase PCR (qRT-PCR) has been identified by the World Health Organization as the most sensitive and specific method of detection. However, the success of this assay relies on the quantity and quality of the extracted viral RNA. **Methods:** Various methods have been developed for nucleic acid extraction however, the methods have not been assessed. RNA extraction was performed from 24 nasopharyngeal swab samples using a manual extraction kit (GF-1) and an automated extraction kit (Genolution). The concentration and purity of the extracted RNA samples were measured, and its performance were tested using qRT-PCR. **Results:** The average concentration and purity of the RNA samples extracted using GF-1 kit was higher compared to Genolution. Similarly, the qRT-PCR assay using the RNA samples extracted using manual extraction was better compared to automated kit. **Conclusion:** Both the manual and automated extraction kits have its advantages and disadvantages in terms of yield and purity. However, with proper optimization, both methods may be used for routine molecular diagnostic of COVID-19 in laboratories.

**Keywords:** Coronavirus, COVID 19, SARS-CoV-2, qRT-PCR, RNA extraction

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## INTRODUCTION

The coronavirus disease 2019 or known as COVID-19 is caused by a novel virus called severe acute respiratory syndrome coronavirus 2 (SARS- CoV-2). The virus first emerged in December 2019 in Wuhan, China and has now spread worldwide posing a serious issue to public health (1). Till date, more than 100 million confirmed cases of COVID-19 were reported, with 2.2 million deaths reported to the World Health Organization (WHO) (2). As the coronavirus pandemic spreads across the world rapidly, there are great effort to identify, delay and stop its spread. As the potential therapies and vaccine candidate are still underway, diagnostic testing becomes an essential tool. Timely and accurate virus testing is a vital prerequisite for the early identification, reporting, isolation and treatment. Real-time technology has considerably expanded and is suitable to detect transcript levels of any genes. It is therefore a technique which assures high sensitivity, specificity and reliability. The WHO recommends the use of real time reverse transcription–polymerase chain reaction (qRT-PCR), as one of the most accurate and sensitive laboratory method for detecting SARS- CoV-2 (3-4). Though the qRT-PCR is a gold standard in diagnosis, there are still limitations. Techniques for nucleic acid extraction could affect the sensitivity of qRT-PCR method. As the quality and the amount of nucleic acid influences any downstream assay, the isolation technique used plays a vital role (5). The extracted RNA needs to be inhibitors free and highly purified to be used in a sensitive and specific method such as qRT-PCR (6). Accurate quantification of the extracted RNA samples is certainly an important step as it is necessary to determine the quality and quantity of the RNA sample being used in the qRT-PCR assay.

The traditional RNA extraction methods from clinical nasopharyngeal swab samples using phenol/chloroform or Guanidine Isothiocyanate are time consuming and are prone to cross contamination among samples. On the other hand, the conventional methods are labor-intensive as it requires multiple extraction steps such as washing and centrifugation. These are not suitable in clinical diagnostic settings where rapid and accurate diagnosis are intensely needed. In addition, false negative results could be obtained due to PCR inhibition or insufficient template, these protocols are therefore not suitable for

high-throughput routines (7). The conventional methods were later replaced by commercially available manual column-based purification kits. The manual column purification technique starts with the use of denaturing agents for cell lysis and denaturation of proteins to release the viral RNA. This is followed by capture of the RNA onto a glass filter membrane spin column using specific buffers provided in the kit (8). Though these commercial kits are modified to overcome the conventional method of extraction, it still holds many drawbacks such as the requirement of large elution volume, the need of bench top centrifuges which can only spin 24 samples at one time and manual pipetting which leads to inconsistent yield.

Recently, several automated extraction platforms were introduced to improve the extraction time and efficiency. Studies comparing the performance of manual and automated extraction systems in pathogen detection have been reported (9-11). RNA isolation by automated are reported to perform better than manual extraction methods which provides high-throughput solutions.

Advancement in biotechnological field has established a novel method of extraction using the magnetic bead or referred as magnetic nanoparticles separation system. The magnetic beads were introduced to solve the scalability concerns with respect to RNA isolation (12). The most beneficial characteristic of the magnetic beads is the ability to strongly immobilise (bind) nucleic acids throughout multiple manipulation steps. The extraction process is also independent of bench top centrifuges and therefore has higher through put. In addition, increasing number of samples in clinical diagnosis increases the need for rapid and efficient method of extraction such as automated platforms (13). Nextractor is a fully automated extraction system from Genolution (South Korea) for rapid extraction of DNA or RNA from a various clinical sample. It provides a high throughput processing with minimum time. However, the comparative performance of Nextractor in viral RNA isolation for qRT-PCR assay to detect infectious diseases has not been previously reported.

The goal of this study was to evaluate the performance of COVID 19 RT- PCR assay using RNA extracted using two different methods, manual column-based extraction kit, GF-1 (Vivantis) and with automated magnetic beads extractions, Nextractor (Genolution). The analyses included, comparison of extraction time, RNA quantity, RNA quality, reagent costs and qRT-PCR performance with clinical specimens.

## MATERIALS AND METHODS

### Samples

Nasopharyngeal swab samples were obtained from adult patients presented with COVID 19-like symptoms or the person under investigation (PUI). The study was carried out from 24 swab samples in viral transport media (VTM) sent to the Hospital Pengajar Universiti Putra Malaysia by the Ministry of Health, Malaysia from March to May 2020. This study was granted exemption from Ethics Committee of Universiti Putra Malaysia (JKEUPM) review (JKEUPM-2020-428).

### Manual column-based extraction

The column based, GF-1 Viral Nucleic Acid Extraction kit (Vivantis, Malaysia) was used to extract the viral RNA from nasopharyngeal swab samples. The samples were extracted based on the manufacturer's recommendations with some modification. The VTM samples were vortexed for 10 seconds to mix and 190 µL of the sample was transferred to a microcentrifuge tubes containing 50 µL proteinase K, 10 µL of internal control (IC) was added individually into each sample tube and vortexed. This is followed by adding 215 µL of lysis buffer (VL) containing Carrier RNA and the tubes were mixed by vortexing. The samples were then incubated at 72 C for 10 minutes in the heating block. After incubation, 280 µL of molecular grade absolute ethanol was added to each sample and the samples were thoroughly vortexed. The samples were transferred to filter column and were centrifuged at 10,000 Ч g for 1 minute. Then, the columns were washed by spinning at 10,000 Ч g for 1 minute with 500 µL of wash buffer 1 solution. The columns were washed twice using wash buffer 2 for 1 minute at 10,000 Ч g and for 3 minutes at 14,000 Ч g. The flow through were discarded and the column were spun at 14,000 Ч g for 10 minutes. Lastly, the columns were air dried for 5 minutes and the RNA samples were eluted using 50 µL elution buffer. Eluted RNA samples were analysed with a Nanodrop spectrophotometer (Thermo Fischer Scientific, U.S) for sample concentration and purity.

### Automated magnetic beads extraction

The VTM samples were vortexed for 10 seconds to mix and 190 µL of the sample was transferred to the sample wells in the cartridge (1st, 5th, and 9th columns). This is followed by adding 10 µL of IC individually into each well containing sample. The cartridge was then loaded into Nextractor® NX-48S and start extraction. The elution (40 µL) were collected from the elution wells (4th, 8th and 12th columns). Eluted RNA samples were analysed with a Nanodrop spectrophotometer (Thermo Fischer Scientific, U.S) for sample concentration and purity.

### Specific detection of target COVID 19 virus RNA

The qRT-PCR amplification process of the RNA samples obtained through both manual and automated extraction methods was conducted in the CFX 96 thermal cycler (Bio-Rad, USA) using commercialized kit, AllplexTM 2019-nCoV (Seegene, Korea) containing primers for E gene of Sarbecovirus in FAM channel, N gene of SARS-CoV 2 in Cal Red 61, RdRP gene in Quasar 670 and IC in HEX respectively. The PCR reaction mixture (25 µL) comprised of 5x Real-time One-step Buffer (5 µL), 2019-

nCoV MOM (5 µL), Real-time One-step Enzyme (2 µL), RNase-free Water (5 µL) and RNA template (8 µL). The reactions start with reverse transcription at 50 °C for 20 minutes and the amplification cycles were performed as follows: 95°C for 15 minutes, 45 cycles of 94°C for 15 s, 58°C for 30 s.

### Data analysis

Statistical analysis was performed using the SPSS software (version 20.0, SPSS Inc., Chicago, IL, USA). The differences between the different isolation techniques were obtained using paired t-test and a $p<0.05$ was considered to be statistically significant.

## RESULTS

The different RNA extraction method shows an impact on the RNA yield. From the equal volume of the samples (n=24) used for extraction, the quantity of RNA isolated using the manual extraction kit GF-1 was significantly higher compared to Genolution the automated extraction kit (Figure 1). The concentration of RNA extracted using GF-1 was 44.8-114.5-ng/uL (average: 92.26 ±17.16) compared to the yield from Genolution, 2.7- 30.0-ng/uL (average: 16.38 ±6.75).

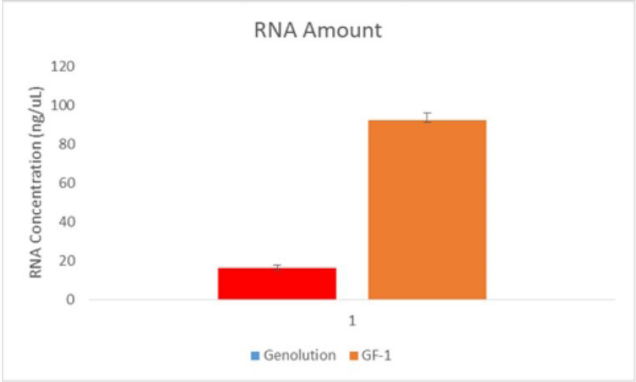


**Figure 1: Mean total amount of RNA extracted using manual (GF-1 kit) and automated (Genolution kit) extraction methods.** Total RNA was measured by Nanodrop spectrophotometer. The RNA concentration was higher with manual extraction (average: 92.26 ±17.16) compared to the yield from automated extraction, (average: 16.38 ±6.75).

### Comparison of RNA quality metrics

Purity of the extracted RNA samples was measured using nano spectrophotometer prior to qRT-PCR. A successful qRT-PCR amplification requires template RNA without contaminants such as proteins. The RNA purity is often tabulate using the A260/280 and A260/230 absorbance ratios. The average A260/280 ratio of RNA samples extracted using GF-1 was comparable (2.99 ±0.08) to Genolution kit (2.94 ±1.5) (Table I). However, the average A260/230 absorbance ratios were significantly different in value for Genolution (1.095 ±1.0) compared to GF-1 (3.15 ±0.16). The amount of time needed for sample processing by automated extraction platform is much shorter compared to manual extraction.

**Table I: Comparison of RNA extraction methods**

| | Extraction method | |
|---|---|---|
| | **Automated (Genolution)** | **Manual (GF-1)** |
| **Time** | 17 minutes | 45 minutes |
| **Samples per run** | 48 samples | 24 samples |
| **A260/A280 (mean ± SD)** | 2.94 (±1.5) | 2.99 (±0.08) |
| **A260/A230 (mean ± SD)** | 1.095 (±1.0) | 3.15 (±0.16) |

### qRT-PCR amplification of SARS CoV-2 genes and Internal Control

Another relevant indicator of RNA quality is the performance of the extracted RNA samples in the downstream applications such as qRT-PCR. Of the 24 samples tested, IC from all the samples extracted using automated kit (Genolution) were amplified, in contrast, 4 samples were not amplified from the RNA samples extracted manually (Figure 2). However, the number of genes detected was higher in manual extraction compared to automated extraction system (Table II). To ensure the viral RNA is efficiently extracted, we compared the internal control (IC) recovery based on the different method of extraction. The differences between IC value obtained using automated and manual extraction were obtained using paired t-test and the p value was 0 ($p<0.05$) therefore the differences is considered to be statistically significant.

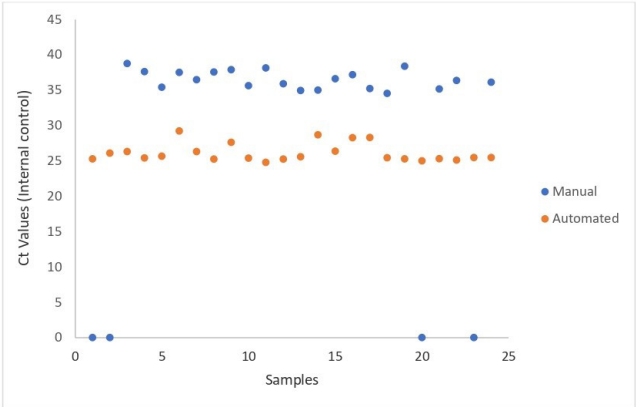


**Figure 2: RT PCR performance comparing the ct values of the internal control from samples (n=24) extracted using manual and automated RNA extraction kits.**

## DISCUSSION

Since early of this year, there has been great challenge diagnose of COVID-19 patients. To prevent and control this pandemic, an early rapid diagnosis is necessary. As such, qRT-PCR is of great attention today in the detection of SARS-CoV-2 (14). As the number of samples to be tested increases tremendously, the need of high

**Table II: SARS CoV-2 genes detection in clinical specimens extracted using automated and manual extraction methods**

| Samples | Allplex™ 2019-nCoV RT-PCR assay results | | | | | |
|---|---|---|---|---|---|---|
| | **Genolution-Automated (Ct value)** | | | **GF-1 Viral Nucleic Acid Extraction Kit-Manual (Ct value)** | | |
| | **E gene** | **RdRP gene** | **N gene** | **E gene** | **RdRP gene** | **N gene** |
| 1 | - | - | - | - | - | - |
| 2 | - | - | - | - | - | - |
| 3 | - | - | - | - | - | - |
| 4 | - | - | - | - | - | - |
| 5 | - | - | - | - | - | - |
| 6 | - | - | - | - | - | - |
| 7 | - | - | - | - | - | - |
| 8 | - | - | - | - | - | - |
| 9 | - | - | - | - | - | - |
| 10 | - | - | - | - | - | - |
| 11 | - | - | 36.33 | 31.26 | 33.25 | 33.02 |
| 12 | - | - | - | 35.05 | - | 37.92 |
| 13 | 30.76 | - | 33.04 | 29.86 | 32.52 | 31.97 |
| 14 | - | - | 35.66 | 34.20 | 39.33 | 35.76 |
| 15 | - | - | - | - | 39.85 | - |
| 16 | - | - | - | - | - | - |
| 17 | - | - | - | 37.55 | - | 36.90 |
| 18 | - | - | 38.68 | - | 39.50 | 36.72 |
| 19 | - | - | - | - | - | - |
| 20 | - | - | - | - | - | - |
| 21 | - | - | 37.39 | 35.67 | 37.42 | 38.69 |
| 22 | - | - | 39.03 | 34.24 | 36.79 | 36.41 |
| 23 | - | - | 37.41 | 38.56 | - | - |
| 24 | - | - | - | - | - | - |

**"-"** Not amplified

throughput RNA extraction method has increased. Yet, the quality and quantity of the nucleic acid extracted should not be compromised in downstream assays such as qRT-PCR. The goal of this study was to compare the performance of COVID 19 qRT-PCR assay with the RNA samples extracted using two different methods, manual (GF-1) and automated (Genolution). The yield and purity of the extracted RNA samples were compared. The Genolution automated platform was chosen for this comparison study with column based manual extraction method because it uses the latest extraction technology with magnetic nanoparticles. Isolation using magnetic beads provides a particularly distinctive ability that allows the binding nucleic acid. The absence of magnetic components in biological samples, makes the separation highly selective (9). The results suggest that the two kits with different extraction method produce RNA samples with comparable purity, amplification and yield. Though the Genolution kit is capable of processing more samples in much shorter time, the yield is relatively lower than manual extraction. This could be due to the multiple optimization steps of manual RNA isolation methods. It is also noted that the RNA yield using manual extraction shows higher variation compared to automated, which proves that automated extraction is more standardized and consistent (15).

The RNA samples extracted using both the Genolution and GF-1 kits produced A260/A280 absorbance ratios indicative of relatively higher than the expected ratio of 2.0 for RNA samples. The variation could be due to the carryover of magnetic beads or contaminant during elution step. However, higher A260/A280 purity ratios do not necessarily indicate problems in extraction and most of the time would not affect the downstream assays. Similarly, another indicator for purity is the A260/A230 ratio, the values for a pure RNA samples would be in the range of 2.0-2.2 (16-17).

In this comparison study, the average A260/A230 ratio obtained from the automated extraction is significantly lower (1.095) but the average value for the manual extraction was higher (3.15). It was therefore observed that the manually extracted RNA samples has the higher purity. Although purity values are an important measurement of sample quality, the greatest indicator is its functionality in the downstream assays. Therefore, to monitor the viral RNA extraction process, the IC is often

used as a control for the sample isolation procedure and is added into the sample during the nucleic acid extraction step. Failure to amplify IC most of the time implies failure in extraction process. A sample is considered negative if the internal control is amplified but the viral genes are not. A specimen is considered invalid when there is no amplification of the internal control, this is to avoid reporting false negative results. In fact, in this study, all the extracted RNA samples were successfully amplified in automated extraction compared to only 20/24 samples were amplified in manual extraction despite the high concentration and purity of viral RNA using the manual extraction method. This could be due to the presence of inhibiting factors such as alcohol in the eluent (18).

Automated nucleic acid extraction has many advantages such as less hands-on time and reduces cross-contamination and technical error (19). With some optimization, they have the potential to improve all the problems posed by manual extraction.

## CONCLUSION

To obtain a good quality RNA from the target sample, different RNA extraction methods or kits have to be carefully examined prior to their use. The establishment of automated extraction is an alternative method to the labor-intensive manual extraction method. The technology allows high throughput of samples with reproducibility and scalability. However, differences in the quality and quantity of RNA extracted via each of the extraction kits indicate that these kits may differ in their ability to yield RNA. Overall, the findings of this study demonstrate that there are practical differences between commercially available RNA extraction kits. This should be taken into account when selecting extraction methods to be used for isolating RNA designated for subsequent downstream processes, analyses or applications.

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