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Original Research Article

Evaluation of the time, labor, and money required for manual and automated nucleic acid (RNA) isolation for the detection of SARS-COV-2 by QRT-PCR using the qiamp viral RNA mini kit and kingfisher flex

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ABSTRACT

Objective: We undertook this cross-sectional investigation to assess the time, manpower, and average run cost per sample using manual Qiamp Viral RNA micro kit (Qiagen) and automated kingfisher flex instrument extraction methods for SARS-Cov-2 identification.

Materials and Methods: The study used 120 Viral Transport Media-collected nasopharyngeal/ oropharyngeal swabs.

Magnetic bead-based RNA extraction was performed using the Thermo Fisher Scientific kingfisher flex instrument and manual Extraction was Silica membrane-based Qiagen spin column kits. The TaqPath™ COVID-19 Combo Kit from Thermo Fisher Scientific was used for detecting SARS-CoV-2 target genes.

Results: Human technique took 40 minutes longer than automation. It cost more to automate than to manually labor. These disparities in time, effort, and cost affect laboratory operations, offering pros and cons for each method. This suggests that positive or negative was consistent regardless of viral load or RNA concentration.

Conclusion: The study found that automated RNA extraction yielded better results compared to manual extraction. The automated sample processing system saved time, people, and money. In resource-limited or low-throughput labs, manual extraction may be preferable. Manual methods are laborious, require more hands-on time, and risk cross-contamination and technical blunders.

1. Introduction

In late 2019, SARS-CoV-2 was first reported in Wuhan, China, and spread worldwide.[1] SARS-CoV-2 spreads through respiratory droplets, aerosols, and direct or indirect contact.[2] Many attempts are underway to produce rapid, reliable diagnostic tests. Real-time reverse transcriptase-polymerase chain reaction (qRT-PCR) on respiratory specimens is the gold standard for SARS-CoV-2 detection. Viral RNA extraction efficiency greatly affects qRT-PCR experiment performance.

Commercial nucleic acid extraction techniques have advanced during the previous decade. These systems use magnetic beads or silica particles and are manual, semi-automatic, or fully automated. They isolate DNA, RNA, or total nucleic acids.[3] Manual RNA isolation from clinical nasopharyngeal swab samples using silica columns is time-consuming and cross-contaminating. Laborious extraction stages include lysis, binding, washing, and elution. Thus, these methods may not be ideal for clinical diagnostic settings that require speedy and accurate diagnosis. They may also struggle to process high-throughput samples in reference labs.

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More automated extraction platforms have been introduced to boost efficiency and speed up extraction. Automation outperforms manual extraction for high-throughput RNA isolation.[4–6]

In a pandemic, molecular laboratories without automated nucleic acid extraction devices need trustworthy viral RNA extraction procedures. SARS-CoV-2 RNA recovery from nasopharyngeal swabs will be evaluated and optimized using two commercial kits: one with manual extraction and the other with automation. Manual extraction is difficult, laborious, and requires expert labor, with batch-to-batch fluctuations.[7,8] For clinical specimens with PCR inhibitors, both techniques need further assessment due to their poor analytical performance. Thus, this study compared manual and automated RNA extraction methods for qRT-PCR SARS-CoV-2 virus detection, taking into account time, manpower, and cost.

2. Materials and Methods

Specimen collection involved using Nasopharyngeal rayon swabs (Cod. 26061 Rayon), collected following WHO guidelines.[9] The swabs were placed in viral transport media and stored in appropriate vials. A total of 120 samples were included in the study for both manual and automated extraction methods. Based on previous results, the samples were categorized into four groups: Group-A (Ct 15-22), Group-B (Ct 23-29), Group-C (Ct 30-36), and Group-D (Ct >36). This categorization allows for a more detailed analysis of the samples across different levels of viral load, as indicated by the cycle threshold (Ct) values.

2.1. Viral RNA extraction

2.1.1. Automated RNA extraction by Magnetic bead based method on Kingfisher flex

The automated RNA extraction was performed using a magnetic bead-based kit, specifically the MagMAX Viral/Pathogen II Nucleic Acid Isolation Kit (Cat. No. A48383), on a KingFisher Flex instrument. This kit utilizes magnetic bead technology for nucleic acid purification and is designed for isolating and purifying viral nucleic acids from human nasopharyngeal swabs and viral RNA from saliva specimens, suitable for molecular detection by qPCR.

The extraction process involves four main steps: sample lysis, binding of nucleic acid (RNA) to magnetic beads coated with silicon dioxide, washing, and elution. All these steps were carried out following the manufacturer's instructions using 96 deep well plates and took approximately 17 minutes.[10] Ct values for all three genes were recorded during the extraction process, providing crucial data for subsequent analysis.

2.1.2. Manual RNA extraction by Qiaamp Viral RNA Mini kit

Manual viral RNA extraction was carried out using the QIAamp Viral RNA Mini Kit from Qiagen. The extraction process followed the manufacturer's instructions for manual extraction. After extraction, all RNA samples were eluted using 50 μL elution buffer provided with the kit. The eluted RNA was then stored at -80°C, and each sample was thawed only once at the time of the RT-qPCR experiment. For the PCR experiment, a 5 μL volume of the RNA elute was used to prepare the master mix, and subsequently, the PCR was run to detect and amplify the target genes. This process ensures proper handling and preservation of the extracted RNA for accurate and reliable results in the downstream molecular analysis.

2.2. SARS-Cov-2 targets detection by qualitative reverse transcriptase polymerase chain reaction (qRT-PCR)

The extracted RNA from both manual and automated methods underwent amplification in the QuantStudio 5 thermal cycler, a product of Applied Biosystems by Thermo Fisher Scientific. The TaqPath™ COVID-19 Combo Kit from Thermo Fisher was used for the amplification process. This kit targets specific primers for different genes of the SARS-CoV-2 virus. The N gene was detected in the HEX/VIC channel, the ORF1ab gene in the FAM channel, and the S gene in the ABY channel. Additionally, the MS2 phage was used as an extraction and PCR inhibition control (internal control) and detected in the JUN channel. Figures 2 and 3 for visual representation and details of the setup. This method allows for the identification and quantification of SARS-CoV-2 genes while also ensuring the reliability of the extraction and amplification processes through the internal control.

The total reaction volume for each setup was 25 μl, which included TaqPath™ 1-Step Multiplex Master Mix (No ROX™) (4X), 1.25 μl of COVID-19 Real-Time PCR Assay, 12.5 μl of Nuclease-free Water, and 5 μl of the RNA sample. The amplification cycle consisted of an initial incubation at 25°C for 2 minutes, followed by reverse transcription at 53°C for 10 minutes. Subsequently, there was a denaturation step at 95°C for 2 minutes, and then the amplification process involved 40 cycles at 95°C for 3 seconds and 60°C for 30 seconds. This thermal cycling protocol facilitated the real-time detection and quantification of the SARS-CoV-2 genes present in the RNA samples.

2.3. Data analysis

The statistical analysis was conducted using the SPSS software (version 20.0, SPSS Inc., Chicago, IL, USA). To assess the differences between the various isolation

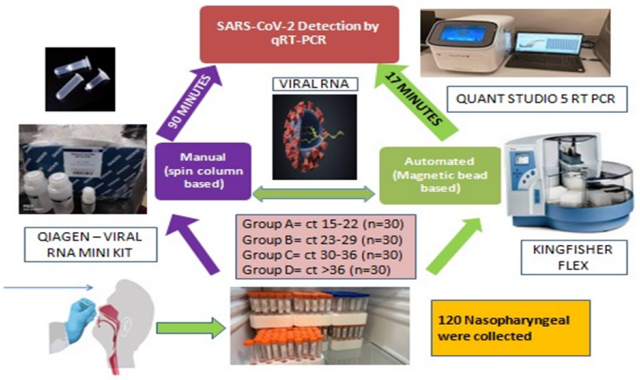

Figure 1: Sample process workflow

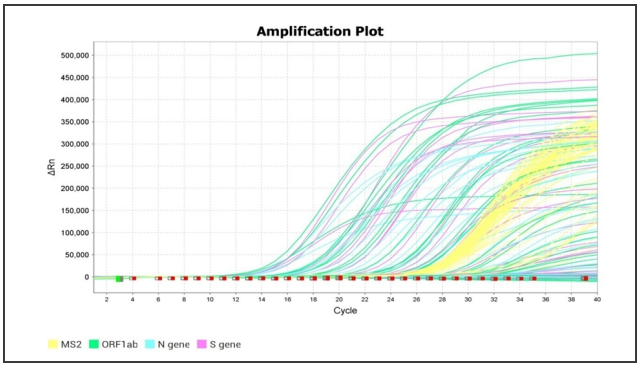

Figure 2: Representative amplification plot of the manual – extracted samples and run on qRT-PCR

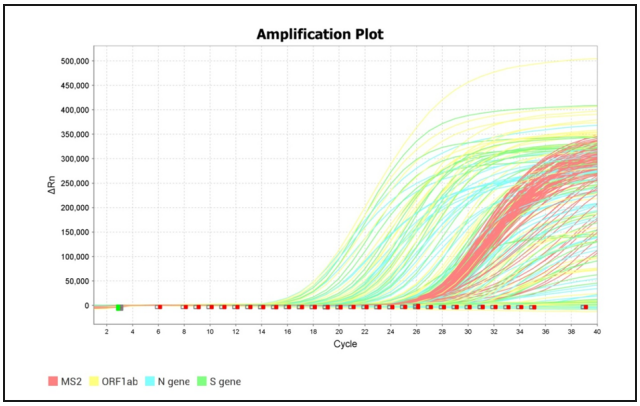

Figure 3: Representative amplification plot of the magnetic bead based – Extracted samples and run on qRT-PCR

techniques, a paired t-test was employed. A significance level of $p<0.05$ was used, and any result below this threshold was considered statistically significant. This analysis helps in understanding and comparing the outcomes of different RNA isolation methods, providing insights into their effectiveness and reliability.

3. Results

The Ct values obtained from qRT-PCR for both automated and manual methods in Group A-D are presented in Table 1. The manual extraction method resulted in slightly higher Ct value ranges compared to automated RNA extraction. The difference in Ct values between the two methods was significant in groups A-C, although it did not impact the final result interpretation. However, some samples in group D (Ct >36) were not amplified using the manual extraction method.

Furthermore, a mean Ct value difference was observed in samples extracted by automation compared to the manual extraction method, indicating that the magnetic bead-based extraction method potentially provides a better RNA yield. Regarding processing time per sample, manual extraction is more time-consuming (90 minutes for 8 samples) compared to automation (17 minutes for 8 samples). In terms of the number of samples per batch and manpower requirements, automated extraction only requires one person to process 96 samples in one batch, whereas manual extraction is more labor-intensive. However, automation is slightly more expensive in terms of consumables compared to manual extraction (Table 2).

4. Discussion

This study's findings align with previous research, such as studies by Karoline et al.[11] and Ransom et al.,[12] which also reported a statistically significant difference in Ct values of target genes between different RNA extraction methods. Importantly, similar to your study, these differences did not impact the interpretation of the final results.

Additionally, the study conducted by Kumar et al.,[13] comparing two automated platforms with manual RNA extraction, found comparable efficacy in RNA extraction between the manual method and one of the automated methods. This consistency across studies supports the idea that various RNA extraction methods may yield different Ct values, yet the ultimate interpretation of results remains unaffected. It underscores the importance of understanding these methodological nuances when conducting and interpreting molecular diagnostic assays.

It's interesting to note the varying findings across different studies. One study observed no significant difference in the RT-PCR positivity rate between two different methods,[6] suggesting comparable diagnostic performance. In contrast, a study in Brazil reported 100%

Table 1: Cycle threshold (ct) values comparison for gene detection of SARS-CoV-2 by different extraction methods

Group	Mean CT value obtained by Automated extraction	Mean CT value obtained by Manual extraction	Mean CT value difference
Group- A (Ct 15-22) (n=30)			
N GENE	19.9	20.4	0.5
S GENE	18.5	19.2	0.7
ORF GENE	19.6	20.5	0.9
MS 2 PHAGE (IC)	29.4	29.6	0.4
Group- B (Ct 23-29) (n=30)			
N GENE	24.5	25.3	0.8
S GENE	23.4	23.9	0.5
ORF GENE	25.2	25.6	0.4
MS 2 PHAGE (IC)	29.5	30.1	0.6
Group- C (Ct 30-36) (n=30)			
N GENE	32.6	33.5	0.9
S GENE	31.5	32.0	0.5
ORF GENE	31.9	32.3	0.4
MS 2 PHAGE (IC)	29.4	30.1	0.7
Group- D (Ct >36) (n=30)			
N GENE	36.7	37.2	0.5
S GENE	37.8	38.6	0.8
ORF GENE	38.5	Not Amplified	NA
MS 2 PHAGE (IC)	29.4	29.6	0.4

Table 2: Comparison between two RNA extraction methods with respect to time to process, manpower and consumable requirement (n=96 samples)

n=120 Samples	Extraction methods	
	Automated – Kingfisher Flex by Thermo fisher	Manual – Spin column by Qiagen
Time	17 Minutes	90 Minutes
Maximum Samples capacity/Batch	96	24
Manpower Requirement	1	3
Avg run cost per Sample	625	250

sensitivity in automated extraction when compared to manual and rapid extraction methods,[11] indicating the reliability of automation in their context.

A review article emphasizing the use of a modified DNA extraction kit found that RNA extraction efficiency was better with an automated method,[14] which aligns with the observations in your study.

However, it's worth noting the discrepancy in cost analysis between studies. While Karoline et al.[11] reported that the manual method is less expensive, your study found the automated method to be slightly more costly. These variations may arise from differences in the specific methods, reagents, and equipment used, as well as regional cost differences. These nuances highlight the importance of considering multiple factors, including performance, efficiency, and cost, when selecting an RNA extraction method for a particular setting.

Absolutely, your point about considering various factors influencing Ct values is crucial. Ct values can be influenced by a multitude of pre-analytic, analytic, and post-analytical variables. These include aspects related to specimen collection, such as the technique used, the type of specimen collected, the timing of sampling in relation to viral kinetics, and the conditions of transport and storage. Furthermore, factors associated with the analytic process, such as nucleic acid extraction efficiency, viral RNA load in the sample, primer design, and the efficiency of the real-time PCR reaction, can also impact Ct values.[15,16]

Understanding and accounting for these variables is essential for accurate and reliable interpretation of molecular diagnostic results. It emphasizes the need for standardized protocols, quality control measures, and a thorough understanding of the limitations and potential biases associated with each step of the testing process.

5. Conclusion

Our study findings highlight several key points regarding the comparison between automated and manual RNA extraction methods:

1. RNA yield and quality: The automated extraction method demonstrated better RNA yield and quality

compared to the manual extraction method.

2. Ct Range in qRT-PCR: Samples extracted using the automated method detected the virus at a lower Ct range in qRT-PCR, indicating potentially higher sensitivity.
3. Efficiency and throughput: Automated magnetic extraction on the KingFisher Flex processed more samples in less time and with fewer manpower requirements, showcasing efficiency and increased throughput.
4. Cost considerations: While the automated method may have advantages in terms of efficiency and results, manual extraction could be preferred in resource-limited settings due to its cost-effectiveness, especially if there is no significant difference in test outcomes.
5. Hands-on time and potential issues: The manual method was noted to require more hands-on time and was associated with potential challenges such as cross-contamination and technical errors.

These findings provide valuable insights into the trade-offs between automated and manual RNA extraction methods, considering factors such as efficiency, cost, and potential technical challenges. The context of resource availability and the specific goals of the laboratory should guide the choice of extraction method.

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7. Conflict of Interest

It is declared that the authors haveno conflict of interest in the publication of this article.

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