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LETTER TO THE EDITOR

Comparison of Manual and Automated Nucleic Acid Extraction Methods in Virus Transport Medium

Si-Yu Chen [1], Yung-Che Chou [1], Jian-Wei Huang [1], Tze-Kiong Er [1, 2, 3]

Si-Yu Chen and Yung-Che Chou contributed equally to this work
[1] *Division of Laboratory Medicine, Asia University Hospital, Asia University, Taichung, Taiwan*
[2] *Department of Medical Laboratory Science and Biotechnology, Asia University, Taichung, Taiwan*
[3] *Department of Nursing, Asia University, Taichung, Taiwan*

SUMMARY

Background: **The implementation of an automated nucleic acid extraction system has many advantages over the manual methods. The purpose of this study was to evaluate the validity of two different methods for nucleic acid extraction in virus transport medium.**
Methods: **We collected 20 nasopharyngeal swabs in viral transport medium from the emergency department of the Asia University Hospital for the detection of SARS-CoV-2. The performance of the Maelstrom™ 8 (Taiwan Advanced Nanotech) and the QIAamp Viral RNA Mini Kit (Qiagen) were compared for the extraction of nucleic acid from viral transport medium. The extracts were used for the validation of the RNA extraction procedures. The RNase P target was amplified in a one-step reverse transcription-quantitative PCR (RT-qPCR) reaction, as internal control for the extraction method.**
Results: **In this study, the agreement between the two methods was good and Pearson's correlation coefficient (r) was 0.919 ($p < 0.001$). The mean cycle threshold value of the two methods was 29.1.**
Conclusions: **Overall, the performance values of the Maelstrom™ 8 and the QIAamp Viral RNA Mini Kit were comparable to each other. In summary, the Maelstrom™ 8 provides a standardized procedure, avoidance of sample-to-sample cross contaminations, is easy to use, improves turnaround time and requires less hands-on time as compared to the manual extraction method. The Maelstrom™ 8 is more suitable for clinical laboratories that carry small or medium-sized samples for nucleic acid extraction.**
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Correspondence:
Tze-Kiong Er, PhD
Division of Laboratory Medicine
Asia University Hospital
No. 222, Fuxin Rd.
Wufeng Dist., Taichung City 413
Taiwan
Phone: +886 4-37061668 ext 1297
Email: tzekiong92@gmail.com)
ORCID ID: https://orcid.org/0000-0002-7068-1652

KEY WORDS

nucleic acid extraction, virus transport medium, SARS-CoV-2, and reverse transcription-polymerase chain reaction

LETTER TO THE EDITOR

In late December 2019, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) began to spread rapidly worldwide. The World Health Organization (WHO) reported that more than 130 million cases of COVID-19, including approximately 2.9 million deaths,

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Table 1. Extraction cost per sample for SARS-CoV-2.

Extraction Method		
	QIAamp® Viral RNA Mini Kit	Maelstrom™ 8
Reagents/kit	$ 50.40	$ 29.50
Consumables	$ 5.00	$ 1.70
Technician	$ 5.20	$ 1.40
Total	$ 60.60	$ 32.60

have occurred as of 16 April 2021 [https://covid19.who.int/]. To date, multiple COVID-19 tests have been developed and performed globally. However, there is still high demand for early and accurate detection, as this can reduce the spread and recurrence of SARS-CoV-2. Rapid diagnosis and isolation are key to control the spread of SARS-CoV-2 infections and to avoid cross infection in the hospitals [1].

The technique reverse transcription-quantitative polymerase chain reaction (RT-qPCR) for viral RNA detection is the standard for the detection of the coronavirus SARS-CoV-2 [2-4]. Although the RT-qPCR test is globally the gold standard for detecting SARS-CoV-2, different molecular diagnostic strategies might be adopted for some specific circumstances. Recently, it has been discussed whether RT-qPCR could be considered a gold standard in the diagnosis of COVID-19 [5]. RNA extraction is a key pre-analytical step in the RT-qPCR test. Early detection of infection by SARS-CoV2 depends on the detection of the viral genome using this test [6]. It requires an RNA extraction process to isolate the viral genetic material before its detection.

The Maelstrom™ 8 extraction system uses a magnetic-bead-based system to extract the nucleic acids. This system is designed to be easily used, processing up to eight samples simultaneously. Reagent kits are already prefilled and disposable, making the process user-friendly. Here we demonstrate the Maelstrom™ 8 extraction system for RNA extraction. It takes about 40 minutes to complete eight samples, and is less labor-intensive than manual RNA extraction kits.

In this study, we collected 20 nasopharyngeal swabs in viral transport medium from the emergency department of the Asia University Hospital for the detection of SARS-CoV-2 using the RT-qPCR test. This study was approved by the Medical Ethics and the Human Clinical trials Committee of China Medical University Hospital (CMUH110-REC3-080). Manual RNA extraction of the swabs in viral transport medium was performed using the QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's manual. The Maelstrom™ 8 was applied for RNA extraction from the same swabs in viral transport medium using the OptiPure Viral Auto Plate (665) following the manufacturer's instructions. The Maelstrom™ 8 is a high-efficiency purification driven by TANBead's patented technology. A maximum of eight samples can be processed during each run. Viral RNA was extracted manually from 140 μL of the sample using the QIAamp Viral RNA Mini Kit and 300 μL of the sample using the Maelstrom™ 8. The extracts were used for the validation of the RNA extraction procedures. The RNase P target was amplified in a one-step RT-qPCR reaction, as internal control for the extraction method. The RT-qPCR was performed using the LightCycler® Multiplex RNA virus Master (Roche) in a Z480 platform (Roche). The RT-qPCR for the RNase P was conducted according to a previous study [7]. Figure 1 showed that the agreement between the two methods was good and Pearson's correlation coefficient (r) was 0.919 ($p < 0.001$). The mean cycle threshold value of the two methods was 29.1.

The cost per samples was calculated for manual and automated extraction, including the reagents/kit, disposables, and technician time. All of the costs are calculated based on list prices (Table 1). The total cost for handing eight samples was greater for the manual method. The required disposables and technician time were more expensive as compared to the manual method. The comparison of hands-on technician time between the manual and automated extraction is 6.9 minutes and 1.9 minutes per sample, respectively.

A recent study showed that the Maelstrom had the advantage of providing increased measured concentration and a tendency towards higher viral recovery and less inhibition in RT-qPCR [8]. On the other hand, a previous study showed that nucleic acid extraction systems using the magnetic particle principle had shorter turnaround time per sample in the system which utilizes the spin column principle [9]. Recently, a study [10] compared the sensitivity as well as sample and reagent contamination of three extraction methods used for viral metagenomics next-generation sequencing. Their findings showed that the eMAG platform (bioMérieux, Marcy-l'Étoile, France) yielded a higher proportion of viral reads, with a limited impact of reagents and sample cross-contamination. However, the highest number of viral reads mapping to bacteriophages in the no-template control was found with the QIAamp, suggesting reagent contamination. Similarly, studies showed that the bioMerieux eMAG is a high-quality extraction platform enabling effective molecular analysis and is mostly suited for medium-sized laboratories [11]. Taken together, automated platforms have several advantages including easy workflow, high throughput, less contamination, and more time saved.

In summary, we evaluated the validity of two different methods for nucleic acid extraction in virus transport medium. Our findings showed that the Maelstrom™ 8 provides a standardized procedure, avoidance of sample-to-sample cross-contaminations, is easy to use, improves turnaround time, and requires less hands-on time as compared to the manual extraction method. The Maelstrom™ 8 is more suitable for clinical laboratories

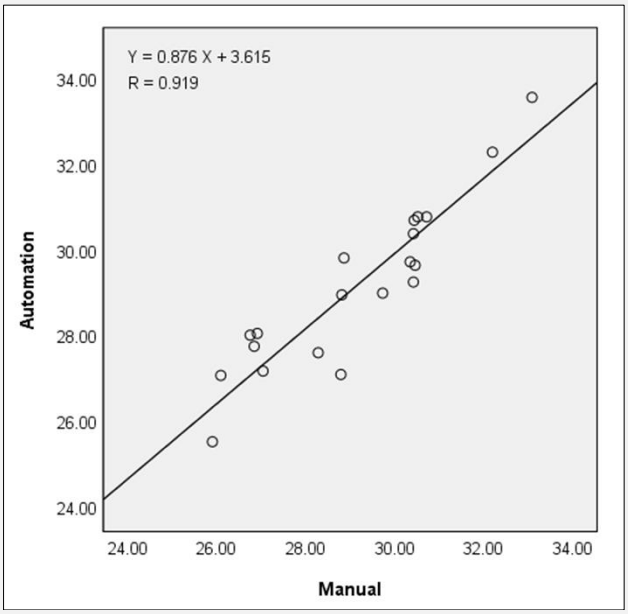

Figure 1. The correlation between automated and manual extraction.

that carry small or medium-sized samples for nucleic acid extraction, especially in this difficult situation.

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Declaration of Interest:
There are no conflicts of interest associated with this paper.

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