

Evaluation of Manual, Automated, and Extraction-Free Workflows for SARS-CoV-2 RNA Recovery from Swab Samples

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Abstract

Aim: This study aimed to compare the performance of four RNA recovery approaches—two silica column-based kits, an extraction-free vNAT method, and an automated magnetic bead-based extraction system—for the recovery of SARS-CoV-2 RNA from nasopharyngeal, oropharyngeal, and intubated samples. **Materials and Methods:** Samples collected from 29 patients under biosafety conditions were processed within 12 hours. RNA yield and purity were quantified using A260/280 and A260/230 ratios measured by NanoDrop spectrophotometry. IBM SPSS 21.0 were used for statistical analyses. Normality was assessed with the Shapiro–Wilk test, Wilcoxon signed-rank and Friedman tests were applied. $p < 0.05$ were considered as statistically significant. **Results:** Silica Column–Based Extraction Kit 1 showed low RNA isolation and low purity indices. Silica Column–Based Extraction Kit 2 produced the highest RNA yields but reflected by high A260/280 and low A260/230 ratios. The automated magnetic bead-based extraction method provided the most consistent purity profile with moderate RNA yield; notably, A260/280 ratios were closest to ideal values, although A260/230 ratios remained below optimal levels. **Conclusion:** RNA yield and purity differed significantly among the four workflows. The automated magnetic bead-based isolation method was the most suitable for obtaining high-quality RNA for research applications, whereas the extraction-free vNAT method was appropriate only for rapid RT-qPCR–based diagnostic use.

Keywords: SARS-CoV-2, RNA isolation, nasopharyngeal, oropharyngeal

1. Introduction

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), which emerged in Wuhan, China, in late 2019 and caused a global pandemic called Coronavirus Disease 2019 (COVID-19), is a single-stranded RNA virus belonging to the coronaviridae family [1]. SARS-CoV-2, which has a genome encoding spike (S), envelope (E), membrane (M), nucleocapsid (N), and non-structural proteins, binds to angiotensin-converting enzyme 2 (ACE2) in the host cell via the S protein, and its entry into the host cell is mediated by TMPRSS2, a protease [5]. The expression of ACE2 in respiratory epithelial cells, particularly in the lower respiratory tract, contributes to the tropism of SARS-CoV-2 for lung tissue [6]. Following SARS-CoV-2 infection, the clinical course may remain asymptomatic or present with symptoms such as fever, cough, fatigue, anosmia, and dyspnea within the first few days. In more severe cases,

the disease may progress to acute respiratory distress syndrome (ARDS) and multiorgan failure [7]. It has been reported that the severity and damage of SARS-CoV-2 infection may increase with advanced age, immunodeficiency, cardiovascular disease, and diabetes [8, 9]. As part of the fight against the epidemic, mRNA and inactivated vaccines have been developed and have significantly reduced the rates of serious illness and death [10].

Although serological and antigen tests are used in the diagnosis of SARS-CoV-2, the gold standard method for direct and early detection of infection is real-time reverse transcription polymerase chain reaction (RT-qPCR) [11]. During the SARS-CoV-2 pandemic, nasopharyngeal/oropharyngeal swab samples were transported in viral transport media (VTM) and initially analyzed by RT-qPCR after classical RNA isolation. Later, to expedite the process, direct RT-qPCR protocols without an extraction step were developed [12, 13]. In

our study, we aimed to compare four sample-processing workflows for SARS-CoV-2 from swab specimens in terms of spectrophotometric RNA yield and purity parameters.

2. Materials and Methods

2.1. Sample Collection

Nasopharyngeal (n=12) and oropharyngeal (n=12) swab and intubated (n=5) samples were obtained from 29 patients admitted to Sancaktepe Şehit Professor İlhan Varank Training and Research Hospital (Istanbul, Türkiye). The study protocol was reviewed and approved by the Republic of Türkiye, Ministry of Health, General Directorate of Health Services, COVID-19 Scientific Research Evaluation Commission (Approval form number: 2020-10-30T12_52_33). Ethical approval was obtained from the Clinical Research Ethics Committee of Istanbul Medeniyet University (IMU), Göztepe Prof. Dr. Süleyman Yalçın City Hospital (Date: 18.11.2020; Approval No: 2020/06/56). Informed consent to participate was obtained from all individual participants included in the study. All procedures involving human participants were conducted in accordance with the ethical standards of the institutional and national research committees and with the principles outlined in the Declaration of Helsinki (as revised in 2013).

2.2. Sample Processing and RNA Preparation

Collected samples were placed in iNADx Viral Transport Kit (SACEM ® Catalog Number: M2001-30), and vNAT™ Transfer Tube (Bio-Speedy ® Catalog Number: BS-NA-513), transported under cold-chain conditions, and stored at +4 °C until processing. All procedures were conducted under biosafety conditions in a laminar flow cabinet with appropriate personal protective equipment (apron, N95 respirator, gloves, and face shield). Because the vNAT solution enabled RNA extraction, it was treated as an independent extraction method and designated as the extraction free group. RNA extraction was performed within 12 hours of sampling from VTM. The RNeasy Mini Kit (Qiagen ® Catalog Number: 74104) as silica column-based extraction kit 1, and Nucleic Acid Purification Kit (SACEM ®) as silica column-based extraction kit 2 were used according to the manufacturer's instructions. Maxwell® RSC system (Promega ® Application Protocol: ER60) ise 300 µL of sample-containing medium was mixed with 300 µL lysis buffer, vortexed, and incubated at 56°C for 20 minutes. The lysed material was loaded into cartridges, 60 µL of elution buffer was dispensed into 0.5 mL elution tubes, and the AS1400 protocol was initiated. The extracted RNA was treated with RQ1 RNase-Free DNase (Promega) and RNA were assessed spectrophotometrically using a NanoDrop™ system. Extracted RNA was stored at -80°C and this group called as the automated magnetic bead-based extraction.

2.3. Statistical analyses

Statistical analyses were conducted using IBM SPSS Statistics v21.0. The distribution of continuous variables was evaluated with the Shapiro–Wilk test. For variables that did not meet normality assumptions, nonparametric methods were applied. Comparisons of total RNA yield and A260/280 and A260/230 ratios obtained from different RNA recovery methods within the same sample groups were performed using the Wilcoxon signed-rank test. The Friedman test was used to compare three or more groups. A p-value <0.05 was considered as statistically significant.

3. Results and Discussion

According to the Shapiro Wilk test, RNA yields obtained using different extraction kits did not follow a normal distribution ($p < 0.001$). Therefore, the Wilcoxon signed rank test was applied, and total RNA quantities were found to differ significantly across all groups ($p < 0.01$). Statistically significant differences were observed in the 260/230 values in all groups except the extraction free group and silica column-based extraction kit 1 ($p < 0.01$). Statistically significant differences were determined in the 260/280 values in all methods except the automated magnetic bead-based extraction and silica column-based extraction kit 1 ($p < 0.01$).

When RNA yields obtained from nasopharyngeal swab samples were compared, the total RNA amounts differed significantly across all extraction groups ($p < 0.01$). The 260/230 ratios showed significant differences among all pairwise comparisons except silica column-based extraction kit 2 vs. silica column-based extraction kit 1, automated magnetic bead-based extraction vs. silica column-based extraction kit 1, and silica column-based extraction kit 2 vs. automated magnetic bead-based extraction ($p < 0.05$). The 260/280 ratios were significantly different among all groups, with the exception of the comparison between automated magnetic bead-based extraction and silica column-based extraction kit 1 ($p < 0.01$).

Comparison of RNA yields from oropharyngeal swab samples revealed significant differences among the four workflows ($p < 0.01$). The 260/230 ratios were significantly different across groups, with the exception of the silica column-based extraction kit 1 vs. extraction free group comparison ($p < 0.05$). The 260/280 ratios differed significantly among all groups ($p < 0.01$).

Comparison of RNA yields from intubated samples showed significant differences among the workflows, except for the comparisons between automated magnetic bead-based extraction and silica column-based extraction kit 2, and between automated magnetic bead-based extraction and the extraction-free group ($p < 0.01$).

The 260/230 ratios differed significantly across groups, with the exception of extraction free group vs. silica column-based extraction kit 1, automated magnetic bead-based extraction vs. silica column-based extraction kit 2, and automated magnetic bead-based extraction vs. extraction free group ($p < 0.05$). The 260/280 ratios were significantly different among all groups except for the comparison between automated magnetic bead-based extraction and silica column-based extraction kit 1 ($p < 0.01$).

When the Friedman test was applied to nasopharyngeal, oropharyngeal, and intubated samples, all RNA yield values were found to be statistically significant across groups ($p < 0.01$). Examination of the 260/280 ratios revealed a significant difference in all groups ($p < 0.01$).

Analysis of the 260/230 ratios showed that all extraction groups, differed significantly from one another ($p < 0.05$).

The relatively high standard deviations observed in some groups likely reflect heterogeneity among clinical specimens, including differences in sample type, cellular content, viral burden, and matrix-related effects; therefore, these findings should be interpreted with appropriate caution. This issue was particularly relevant for the intubated subgroup, where the limited sample size may also have amplified variability. Figure 1 shows the case-based measurement values according to RNA recovery workflows.

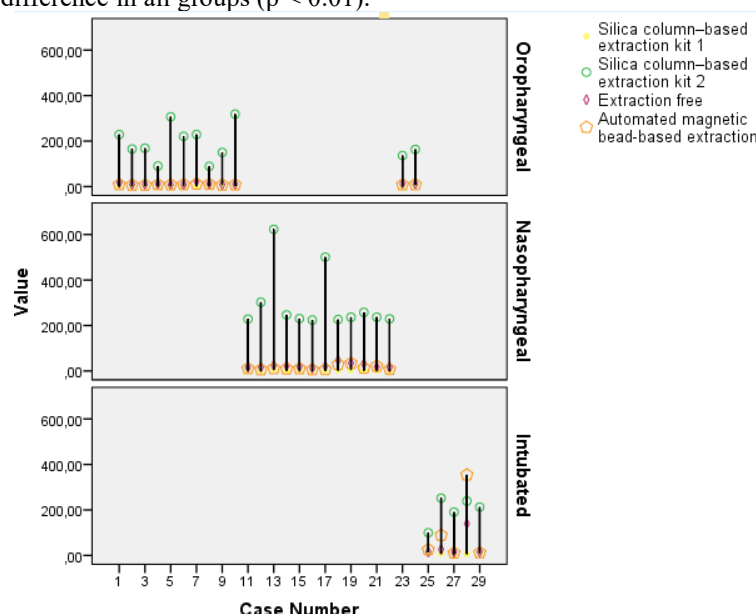


Figure 1. Comparison of case-based measurement values according to RNA recovery workflows. The case-by-case distribution of measurement values obtained using four different nucleic acid extraction approaches in oropharyngeal, nasopharyngeal, and intubated patient samples is shown. Case number is on the x-axis, and measurement value is on the y-axis.

Table 1. Comparison of RNA Yield and Purity Ratios (A260/230 and A260/280) Across Manual, Automated, and Extraction-Free Workflows

Extraction-Free Workflows										
Isolation kit/method	N	Quantity (ng)			260/230			260/280		
		Min	Max	Median, IQR	Min	Max	Median, IQR	Min	Max	Median, IQR
Total										
Silica column-based extraction kit 1	29	0.3	13.3	1.8, 3.85**	0.01	0.11	0.03, 0.04**	1.56	7.39	1.8, 0.39**
Silica column-based extraction kit 2	29	89.1	623	228.7, 82.2**	0.39	3.14	1.83, 1.29**	2.04	3.63	3.43, 0.25**
Extraction free	29	8.3	139.7	16, 10.8**	-2.58	6.01	-0.24, 1.3**	0.66	1.14	0.82, 0.16**

Automated magnetic bead-based extraction	29	6.1	354.3	9.3, 4.80**	0.65	2.11	1.11, 0.17**	1.6	2.04	1.82, 0.2**
Nasopharyngeal										
Silica column–based extraction kit 1	12	0.3	5.2	1.6, 1.42**	0.01	0.08	0.025, 0.01*	1.6	7.39	1.83, 0.44**
Silica column–based extraction kit 2	12	224.1	623	236.6, 62.43**	0.69	3.14	2, 0.8*	3.23	3.63	3.485, 0.14**
Extraction free	12	13	47.9	20.45, 14.85**	-2.58	6.01	0.375, 1.69*	0.77	1.11	0.87, 0.2**
Automated magnetic bead-based extraction	12	6.1	34.4	10.15, 12.42**	0.97	1.71	1.155, 0.18*	1.64	1.99	1.795, 0.24**
Oropharyngeal										
Silica column–based extraction kit 1	12	0.4	6.8	1.4, 0.98**	0.01	0.11	0.025, 0.04*	1.58	2.25	1.85, 0.52**
Silica column–based extraction kit 2	12	89.1	318.3	167.05, 89.07**	0.71	3.14	1.69, 1.76*	2.04	3.5	3.4, 0.31**
Extraction free	12	8.3	22.5	11, 3.78	-0.76	1.09	-0.39, 0.29*	0.66	0.97	0.755, 0.15**
Automated magnetic bead-based extraction	12	6.4	10.9	8.15, 1.45	0.65	1.18	1.025, 0.11*	1.6	2.04	1.86, 0.14**
Intubated										
Silica column–based extraction kit 1	5	4.9	13.3	8.4, 6.15**	0.04	0.1	0.07, 0.05*	1.56	1.91	1.66, 0.2**
Silica column–based extraction kit 2	5	99.8	251.9	212.6, 100.25**	0.39	1.75	0.97, 1.08*	2.97	3.48	3.31, 0.42**
Extraction free	5	15.1	139.7	16.5, 68.1**	0.46	2.01	1.04, 1.01*	0.75	1.14	0.82, 0.25**
Automated magnetic bead-based extraction	5	10.7	354.3	24.9, 210.3**	1	2.11	1.57, 1.01*	1.65	1.84	1.79, 0.18**

N, Number; Min, Minimum; Max, Maximum; SD, Standard deviation; *, p<0.05, **, p<0.01

The diagnosis of viral infections by culture depends on the collection of appropriate clinical specimens and the use of transport media capable of preserving viral integrity against environmental degradation. Sucrose-based and broth-based viral transport media are commonly employed, and multiple studies have demonstrated that many viruses remain stable in these media for up to three days. In most circumstances, specimens can be maintained at +4 °C during transport, thereby reducing or eliminating the requirement for a strict cold-chain system [14]. Viral transport media sterilized by autoclaving, filtration, or gamma irradiation have been shown to preserve SARS-CoV-2 infectivity, with samples stored either at room temperature or at +4 °C exhibiting comparable infectious viral titers over a 24-hour period [15]. Although previous work indicates that specimens transported under cold-chain conditions or kept at ambient temperature provide similar viral stability within the first 24 hours, our study implemented cold-chain transport, storage at +4 °C, and completion of RNA extraction within the first 12 hours to minimize degradation risk, given the inherent instability of single-stranded RNA.

Swabs used for collecting samples are flexible plastic swabs with Dacron, rayon, or polyester fiber tips. These swabs, including cotton-tipped and wooden swabs treated with calcium alginate, may contain substances that inhibit some viruses and molecular tests (such as PCR), and are therefore not recommended for use in the diagnosis of viral infections [16]. For virological examinations, a sterile swab with a plastic handle is used to collect samples by vigorously rubbing the swab against the tonsils and posterior pharynx and placing it in a tube containing viral transport medium (TC, SB, HSGM). For SARS-CoV-2, it is recommended to collect first an oropharyngeal swab and then a nasopharyngeal swab with the same swab and place it in VTM [17].

The Bio-Speedy® vNAT™ Transfer Tube (Catalog Number: BS-NA-513), used in Turkey, was used as a buffer for the rapid detection of SARS-CoV-2. Nasopharyngeal and oropharyngeal swab samples are transferred to the vNAT Transfer Tube containing 2 mL of formalin. Within five minutes, viral and bacterial pathogens in the sample are inactivated, and this fluid can be used in quantitative PCR (qPCR) reactions without requiring any additional processing. While polyethyleneimine-coated tetradecyl dimethyl benzyl ammonium chloride-based nanoparticles (NP) and Tween-20 mediate the destruction of the SARS-CoV-2 membrane and nucleocapsid structure and the release of the genome, guanidinium thiocyanate prevents the degradation of nucleic acids by inhibiting RNase and DNase, and helps preserve the integrity of the SARS-CoV-2 genome with sodium azide (NaN₃) [18]. The RNeasy Mini Kit (®Qiagen), widely used for RNA isolation, releases RNA, and purifies it through a silica membrane. The resulting high-purity RNA is suitable for

downstream RT-qPCR analyses [19]. The Nucleic Acid Purification Kit (®SACEM) which, like the other brand, consists of lysis, RNA binding, washing, and elution steps, enables RNA isolation from samples. Promega® Maxwell Automated RNA Extraction, on the other hand, provides automated, machine-assisted RNA isolation [20].

The COVID-19 pandemic, which disrupted laboratory supply chains and material production, leading to sudden shutdowns, prompted rapid adaptation and the development of diagnostic kits that enable early diagnosis. Because the quantity of epithelial cells and viral particles collected from nasopharyngeal and oropharyngeal swabs is limited, placing these swabs into the VTM sample causes significant dilution, leading to a significant decrease in SARS-CoV-2 concentration in the samples and low RNA/DNA yields. In our study, the highest RNA concentrations were obtained using Silica Column-Based Extraction Kit 2. This was followed by the extraction free workflow, automated magnetic bead-based extraction, and Silica Column-Based Extraction Kit 1. Evaluation of RNA purity (260/280 ratio) observed that automated magnetic bead-based extraction gave purity values closest to the ideal ratio in all sample groups, followed by Silica Column-Based Extraction Kit 1. The expected value of the 260/230 ratio, which provides information about purification and contamination, is approximately 2.0–2.2; however, no extraction kit in our study achieved values in this range. Silica Column-Based Extraction Kit 2 yielded 260/230 ratios closest to the ideal range; however, values below 2 suggest the presence of salts, phenol, or other organic contaminants. Furthermore, the elevated A260/280 ratios observed in this group should be interpreted cautiously, as they may reflect possible impurities, spectrophotometric bias, or limitations of the measurement conditions rather than unequivocally indicating RNA degradation. The relatively high RNA concentrations obtained despite the limited presence of cells in the swab samples suggest that VTM components may not have been sufficiently removed during extraction. In conclusion, while Silica Column-Based Extraction Kit 2 can perform effectively for RNA isolation from blood, our findings indicate that it does not provide sufficient RNA purification for the isolation of SARS-CoV-2 from swab samples containing VTM.

In Silica Column-Based Extraction Kit 1, RNA concentrations (ng/μL) were significantly lower, with 260/280 ratios ranging from 1.7 to 2.3. Low absorbance values at both 260 nm and 230 nm support the presence of excess salt and phenolic residues. For the automated magnetic bead-based extraction method, 260/280 ratios were generally within the acceptable range (1.75–1.84) indicating minimal protein contamination, while 260/230 values between 0.99 and 1.56 indicated salt or phenol contamination. RNA concentrations were highest in intubated samples and lowest in oropharyngeal swabs,

which is an expected result, supporting the conclusion that automated magnetic bead-based extraction performs best overall for SARS-CoV-2. Although the extraction free method yielded relatively high RNA concentrations, the A260/A280 and A260/A230 ratios were significantly suboptimal, given the composition of the extraction chemical. While this rapid method is particularly suitable for diagnostic applications because it does not interfere with qPCR amplification, it is unlikely to be considered a suitable isolation strategy for research applications requiring high-purity RNA.

4. Conclusion

Our findings are consistent with recent evidence demonstrating that VTM has a significant impact on RNA isolation and RT-qPCR sensitivity due to extraction reagents [21]. Studies comparing manual and automated extraction systems have reported that automated magnetic bead-based systems provide more consistent RNA isolation and better RT-qPCR performance, particularly in low viral load samples [22, 23]. These findings are consistent with our results showing purer concentration values in automated magnetic bead-based extraction compared to silica column kits. In contrast, extraction free approaches, which are advantageous for rapid diagnosis, have been shown in several studies to result in lower RNA integrity and lower analytical sensitivity [24, 25]. This is in line with our findings that vNAT facilitates rapid diagnosis but may not provide suitable RNA for studies requiring good-quality viral RNA. Current literature supports the idea that automated extraction platforms provide more reliable SARS-CoV-2 RNA isolation from VTM-containing swab specimens, while rapid extraction-free systems remain clinically useful.

This study has several limitations that RNA quality assessment relied exclusively on spectrophotometric measurements and RNA Integrity Number (RIN) or RNA Quality Number (RQN) generated by microfluidic electrophoresis systems were not obtained. This is limiting the accuracy of purity and degradation assessments. Furthermore, no downstream functional validation was performed such as RT-qPCR amplification efficiency or cycle threshold (Ct) values for each extraction method. Consequently, direct comparison of vNAT, which is an extraction free inactivation buffer rather than a true isolation kit, with silica column-based or automated magnetic bead-based extraction procedures may bias conclusions regarding extraction efficiency. Lastly, the limited sample size—particularly for intubated specimens—may have reduced statistical power and increased variability in the measured parameters. Therefore, future studies should also evaluate RIN/RQN values, RT-qPCR performance for each extraction method, including cycle threshold values, amplification efficiency.

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Author's Contributions

Burcu Çaykara Peran: Drafted and wrote the manuscript, performed the experiment and result analysis.

Ethics

Ethical approval was obtained from the Clinical Research Ethics Committee of Istanbul Medeniyet University (IMU), Göztepe Prof. Dr. Süleyman Yalçın City Hospital (Date: 18.11.2020; Approval No: 2020/06/56). Informed consent to participate was obtained from all individual participants included in the study. All procedures involving human participants were conducted in accordance with the ethical standards of the institutional and national research committees and with the principles outlined in the Declaration of Helsinki (as revised in 2013).

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