Araştırma Makalesi – Original Article

Assessment of Nucleic Acid Extraction Kits for SARS-CoV-2 Surveillance in Wastewater Samples*

Atıksu Örneklerinde SARS-CoV-2 Sürveyansı için Nükleik Asit Ekstraksiyon Kitlerinin Değerlendirilmesi

Ahmet Sait[i], Serol Korkmaz[ii], Ayşe Parmaksız[iii], Bülent Bayraktar[iv], İsmail Aslan[v]

[i]Dr., Pendik Veterinary Control Institute, Virology Lab. https://orcid.org/0000-0001-7658-8793
[ii]Dr., Pendik Veterinary Control Institute, Virology Lab. https://orcid.org/0000-0001-8970-6883
[iii]Dr., Pendik Veterinary Control Institute, Virology Lab. https://orcid.org/0000-0003-1242-7987
[iv] Assoc. Prof. Dr., Bayburt University, Faculty of Health Sciences. https://orcid.org/0000-0002-2335-9089
[v]Dr., University of Health Sciences, Hamidiye Faculty of Pharmacy. https://orcid.org/0000-0001-7075-7103

ABSTRACT

Objective: The aim of this study is to evaluate the effectiveness of three commercial nucleic acid extraction kits (kit A, B and C) in isolating SARS-CoV-2 viral RNA from wastewater samples.

Method: In this study, water samples were collected in March 2021 from three wastewater treatment plants located in different parts of Istanbul, and it was confirmed that they were negative for SARS-CoV-2. Different concentrations of the SARS-CoV-2 virus, previously inactivated at the BSL-3 laboratory of the Pendik Veterinary Control Institute, were added to the wastewater samples. RNA extraction and quantification were performed using commercial nucleic acid extraction kits and and RT-qPCR kit specific to SARS-CoV-2.

Results: At the end of the study, it was determined that kit C yielded the highest total RNA and produced more consistent results, significantly outperforming the other two kits in terms of RNA yield and purity. Statistical analysis revealed significant differences in RNA concentrations ($p < 0.05$) and gene copy numbers ($p < 0.01$) between the kits, and kit C demonstrated superior linearity and reproducibility.

Conclusion: According to the findings, although all three evaluated kits are suitable for detecting SARS-CoV-2 RNA in wastewater samples, kit C provides the most efficient and reliable performance, especially for high-throughput studies. Additionally, this study highlights the importance of selecting appropriate nucleic acid extraction methods for wastewater surveillance, which serves as an early warning system for outbreaks that threaten public health.

Keywords: Nucleic Acid Extraction, SARS-CoV-2, Surveillance, Wastewater.

ÖZ

Amaç: Çalışmanın amacı, atık su numunelerinden SARS-CoV-2 virus RNA'sını izole etmek amacıyla üç ticari nükleik asit ekstraksiyon kitinin (kit A, B ve C) etkinliğini değerlendirmektir.

Yöntem: Çalışmada, 2021 yılı Mart ayında İstanbul ilinde farklı lokasyonlardaki üç atık su arıtma tesisinden su numuneleri toplandı ve SARS-CoV-2 virusu yönünden negatif olduğu teyit edildi. Pendik Veteriner Kontrol Enstitüsü'ndeki BSL-3 laboratuvarında daha önce inaktive edilmiş olan SARS-CoV-2 virusunun farklı konsantrasyonları atık su numunelerine eklendi. Ticari nükleik asit ekstraksiyon kitleri ve SARS-CoV-2 RT-qPCR kiti kullanılarak sırasıyla RNA ekstraksiyonu ve kantitasyonu gerçekleştirildi.

Bulgular: Çalışma sonunda, kit C'nin en yüksek toplam RNA'yı verdiği ve daha tutarlı sonuçlar ürettiği, RNA verimi ve saflığı açısından diğer iki kitten önemli ölçüde daha iyi performans gösterdiği belirlendi. İstatistiksel analiz, kitler arasında RNA konsantrasyonlarında ($p < 0,05$) ve gen kopya sayılarında ($p < 0,01$) önemli farklılıklar olduğunu ortaya koydu. Kit C'nin üstün doğrusallık ve tekrarlanabilirliğe sahip olduğunu gösterdi.

Sonuç: Elde edilen bulgular, değerlendirilen üç ticari kitin de atık su numunelerinde SARS-CoV-2 RNA'sını tespit etmek için uygun olduğunu göstermektedir. Ancak özellikle kit C, yüksek verimli çalışmalar için en etkili ve güvenilir performansı sunmaktadır. Ayrıca bu çalışma, halk sağlığını tehdit eden salgınlar için erken uyarı sistemi işlevi gören atık su gözetiminde, uygun nükleik asit ekstraksiyon yöntemlerinin seçilmesinin önemini vurgulamaktadır.

Anahtar Kelimeler: Atıksu, Nükleik Asit Ekstraksiyonu, SARS-CoV-2, Sürveyans.

Mersin Üniversitesi Tıp Fakültesi Lokman Hekim Tıp Tarihi ve Folklorik Tıp Dergisi 2025;15(1):241-251
DOI: 10.31020/mutftd.1571019
e-ISSN: 1309-8004
Geliş Tarihi – Received: 22 October 2024; Kabul Tarihi - Accepted: 21 November 2024
İletişim - Correspondence Author: Ahmet Sait <saitvet@gmail.com>

Introduction

As of January 31, 2020, the World Health Organization (WHO) designated COVID-19 as a Public Health Emergency of International Concern. Conducting extensive testing, both among individuals with and without symptoms, is essential for mitigating the ongoing pandemic and preventing potential future outbreaks. Several diagnostic testing methods exist to determine whether individuals are infected with COVID-19.[1–3] The polymerase chain reaction (PCR) is a powerful technology increasingly used for diagnosing both infectious and non-infectious diseases.[4–8] Ensuring high yield, purity, and integrity of genomic material is critical for the success of PCR-based studies. Therefore, an efficient genomic material extraction method is a prerequisite for optimal PCR assay performance.[4,9,10] Compared to DNA, RNA is more fragile. PCR assays initiated with low-quality and low-quantity RNA may produce suboptimal results, particularly given the labor-intensive, time-consuming, and expensive nature of these assays.[11–13]

Wastewater-based diagnosis of infectious diseases, such as COVID-19, is a rapidly emerging field because samples can be collected easily and safely.[14–16] Actively monitoring SARS-CoV-2 RNA in wastewater is valuable for identifying critical areas and has proven to be an effective early warning system for potential new outbreaks.[17] Prior to its use in PCR assays, it is essential to verify the specifications of the extracted genomic material. In reverse transcription polymerase chain reaction (RT-PCR) studies, the extraction process must adhere to specific parameters to ensure proper RNA quality. For instance, the final eluate should be free of proteins, genomic DNA, enzyme inhibitors, or any phenol or alcohol carryover, as these could impair RT-PCR processes.[10,18]

RT-PCR reactions heavily depend on purification and clean-up methods. Three widely used RNA extraction methods include organic extraction, silica-membrane-based spin column technology, and paramagnetic particle technology.[10,19,20] These methods have specific advantages and drawbacks. RNA extracted via organic extraction often contains proteins, cellular debris, organic solvents, salts, and ethanol. In contrast, silica column and paramagnetic particle-based RNA extraction techniques are known for their practicality, efficiency, and cost-effectiveness. Additionally, these methods yield intact RNA with minimal contamination from proteins and other biological components.[10,21]

Although several methods are available for RNA isolation and purification, studies comparing extraction methods specifically for wastewater samples are limited.[17,22–24] Selecting the appropriate nucleic acid isolation kit is critical for sample processing and significantly impacts the results obtained. This study aims to provide a thorough evaluation of the effectiveness and efficiency of three frequently employed commercial nucleic acid isolation kits. The assessment includes detecting viral RNA through RT-qPCR analysis and offering a qualitative comparison of the methods.

Materials and Methods

Sampling

Wastewater samples were collected from the influent streams (after grit removal) of three wastewater treatment plants (WWTPs) in Istanbul, Türkiye, in March 2021. The influent samples (n = 3) were obtained as 24-hour composite samples, each comprising 5 liters. The temperature and pH of the samples at the time of collection were 9°C ± 3°C and 7.36 ± 1.52, respectively, and were logged. Within two hours, the samples were transported to the laboratory at 4°C. On the same day, all samples underwent triplicate testing and were confirmed negative for SARS-CoV-2 before use. Wastewater samples verified as negative by RT-qPCR were pooled and stored at 4°C for virus inoculation.

Virus Inoculation into Wastewater

SARS-CoV-2 was cultured and inactivated in the BSL-3 laboratory at Pendik Veterinary Control Institute. The inactive virus stock solution (32 × 10^6 gene copies/µL) was separated from residual cell debris by centrifugation, and the resulting supernatant was stored at –80°C for further analysis. Dilutions of the inactive virus stock solution were prepared in 50 mL of untreated wastewater (6 replicates), with concentrations of 25, 50, 100, 150, and 200 µL/50 mL (equivalent to 4 × 10^6, 8 × 10^6, 16 × 10^6, 24 × 10^6, and 32 × 10^6 GC/µL) in a BSL-2 cabinet.

Filtration and Concentration of the Virus Using Polyethylene Glycol (PEG)

The samples were gently shaken at 4°C and 100 rpm for 30 minutes to facilitate the transfer of attached viruses to the aqueous phase. Microorganisms and large particles were removed from the samples by centrifugation at 7471G for 30 minutes at 4°C. The supernatant (250 mL) was filtered through 0.45 µm and 0.2 µm filters to remove any remaining particles and cell debris. The filtrate was thoroughly mixed with 10% w/v polyethylene glycol 8000 (PEG 8000) by shaking for 1 minute and incubated at 4°C and 100 rpm overnight. After incubation, the mixture was divided into six 50 mL Falcon tubes. Viruses were precipitated by centrifugation at 7471G for 120 minutes at 4°C. The supernatant was carefully removed without disturbing the pellets. The pellets from each Falcon tube were re-suspended with 200 µL of RNA-free water. For total nucleic acid extraction, 1 mL of the virus concentrate was used, with the remaining concentrate stored at -80°C (***Figure 1***).

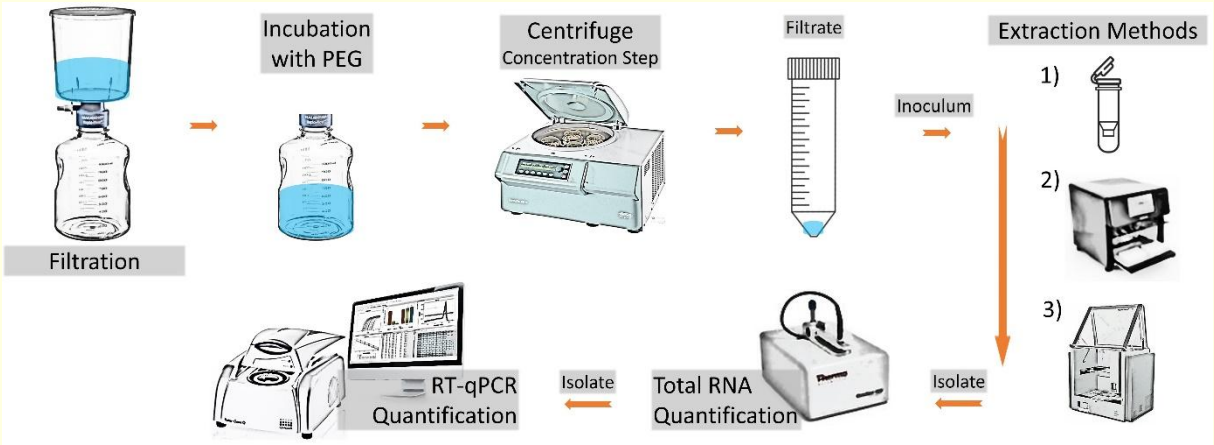

Figure 1. Workflow diagram illustrating all steps from virus concentration to the PCR stage. **1)** Kit A for manual extraction, **2)** Kit B for automated extraction, and **3)** Kit C for automated extraction.

Extraction of Total Nucleic Acids

Total nucleic acid extraction was performed using three different commercial kits: **1)** Kit A used for manual extraction employs the spin column principle. In this method, virus lysis is achieved by incubating the sample with a specialized Lysis/Binding Buffer in the presence of Proteinase K. This process allows nucleic acids to bind specifically to the surface of glass fibers in the presence of a chaotropic salt. The binding reaction occurs rapidly as the organized structure of water molecules is disrupted, facilitating the interaction of nucleic acids with the glass fiber surface. Since this binding process is selective for nucleic acids, a washing step removes salts, proteins, and other impurities, and the purified nucleic acids are eluted in a low-salt buffer or water. **2)** Kit B, designed for automated extraction, utilizes the magnetic particle principle. In this method, samples are lysed in a single step with chaotropic salts and Proteinase K, allowing nucleic acids to bind to the silica surface of magnetic particles. The bound DNA and RNA are then thoroughly washed, air-dried, and finally eluted in an elution buffer to obtain high-quality nucleic acids. **3)**

Kit C, designed for automated extraction, utilizes the magnetic glass particle (MGP) principle. This nucleic acid isolation process is based on the well-established MGP technology. The key steps of the procedure include lysis of the sample material, release of nucleic acids, and denaturation of nucleases. Under chaotropic salt conditions and the high ionic strength of the lysis/binding buffer, nucleic acids bind to the silica surface of the added MGP. The MGP with bound nucleic acids are then magnetically separated from the residual lysed sample. Unbound substances, such as proteins, cell debris, and PCR inhibitors, are removed through multiple washing steps. Finally, the purified nucleic acids are eluted from the MGP. All procedures followed the manufacturer's instructions (***Figure 1***). Two hundred microliters of each sample were used for extraction, and the resulting 100 µL elution was stored at +4°C. Quantifications for all samples were performed on the same day.

Quantitation of Total RNA

Total RNA was measured using a UV/VIS spectrophotometer (ND-1000, NanoDrop, Thermo Fisher Scientific, USA). Before and after each measurement, the upper and lower optical surfaces of the micro-spectrophotometer were cleaned with 2 µL of sterile deionized water, followed by wiping with a Kimwipe (Kimberly-Clark Professional, USA). Nucleic acid samples (six replicates) of 1 µL were used to measure total RNA, with sterile DNAse/RNAse-free water employed for the blank. The absorption ratios of 260/280 were verified to ensure the quality of the measurements.

RT-qPCR Analysis

Detection of SARS-CoV-2 RNA in wastewater samples was performed using a one-step RT-qPCR method with a commercial IVD-certificated kit. The SARS-CoV-2 one-step RT-qPCR diagnostic kit (Ref: KRM-136-002, V2, KrosQuanT, Krosgen Biotechnology, Türkiye) targeted two gene regions (N1 and N2) of the SARS-CoV-2 virus. Primer and probe sequences were based on US-CDC recommendations (US Centers for Disease Control and Prevention, Respiratory Virus Branch, 2020) and are listed in ***Table 1***. Reactions were prepared following the manufacturer's instructions, with a final PCR reaction volume of 20 µL, including 15 µL of Master Mix and 5 µL of extracted RNA. Positive controls and negative controls with distilled water were included in each run. RT-qPCR reactions were performed in six replicates for each sample using the Rotor-Gene Q machine (QIAGEN, Hilden, Germany). PCR conditions were as follows: reverse transcription at 45°C for 10 minutes, denaturation and Taq polymerase activation at 95°C for 2 minutes, followed by 45 cycles at 95°C for 10 seconds and 55°C for 30 seconds (data collection). Fluorescence signals were measured in the FAM channel for viral genes N1 and N2.

Table 1. 2019-Novel Coronavirus (2019-nCoV) Real-time rRT-PCR Panel Primers and Probes

Name	Description	Oligonucleotide Sequence (5'-3')
2019-nCoV_N1-F	Forward Primer	5'-GAC CCC AAA ATC AGC GAA AT-3'
2019-nCoV_N1-R	Reverse Primer	5'-TCT GGT TAC TGC CAG TTG AAT CTG-3'
2019-nCoV_N1-P	Probe	5'-FAM-ACC CCG CAT TAC GTT TGG TGG ACC-BHQ1-3'
2019-nCoV_N2-F	Forward Primer	5'-TTA CAA ACA TTG GCC GCA AA-3'
2019-nCoV_N2-R	Reverse Primer	5'-GCG CGA CAT TCC GAA GAA-3'
2019-nCoV_N2-P	Probe	5'-FAM-ACA ATT TGC CCC CAG CGC TTC AG-BHQ1-3'

Data Analysis

Data analysis, including the calculation of mean, standard deviation, coefficient of variation (CV), and recovery, was conducted using SPSS 21 statistical software (SPSS Inc., Chicago, IL, USA). Spearman's correlation coefficient (two-tailed) was used to determine correlations among recovery rate (%), total RNA (ng/µL), and gene copies (GC). Linear regression analysis assessed associations between the parameters,

and linearity plots were generated using Excel 2013 software (Microsoft, California, USA). Differences in data means were tested for significance at a level of $P < 0.05$.

Results

RNA Extraction and Quantification

Three commercial nucleic acid isolation kits were assessed for RNA yield, with all yielding acceptable quantities of RNA. Kit C outperformed the other two kits in RNA yield and quality. Kit C consistently produced higher RNA concentrations and demonstrated superior purity with favorable A260/A280 (around 2.10) and A260/A230 ratios (around 1.97), both of which indicate high-quality RNA with minimal contamination. A ratio greater than 1.8 is considered indicative of low protein contamination, and a higher A260/A230 ratio (>1.8) points to minimal polysaccharide contamination.[25, 26] In terms of yield (ng/µL), kit C produced approximately 2.88 times more RNA than kit B and 39.36 times more than kit A. Kit A exhibited lower efficiency in extracting RNA from wastewater samples (***Table 2, Figure 2***). Nuclease-free water controls did not yield detectable RNA for any kit.

Table 2. RNA Yield in ng/µL

	Inoculated Virus														
	4.000.000 GC/ul			8.000.000 GC/ul			16.000.000 GC/ul			24.000.000 GC/ul			32.000.000 GC/ul		
	Total RNA (ng/µl)		SD	Total RNA (ng/µl)		SD	Total RNA (ng/µl)		SD	Total RNA (ng/µl)		SD	Total RNA (ng/µl)		SD
Kit C	11.127	±	1.298	18.820	±	3.215	30.126	±	3.015	42.688	±	6.894	49.994	±	3.920
Kit B	3.583	±	828	5.664	±	2.285	10.487	±	3.180	16.116	±	2.440	18.779	±	1.619
Kit A	416	±	98	416	±	102	634	±	149	1.763	±	799	2.082	±	300
P value	<0,05			<0,05			<0,05			<0,05			<0,05		

SD: Standard Deviation

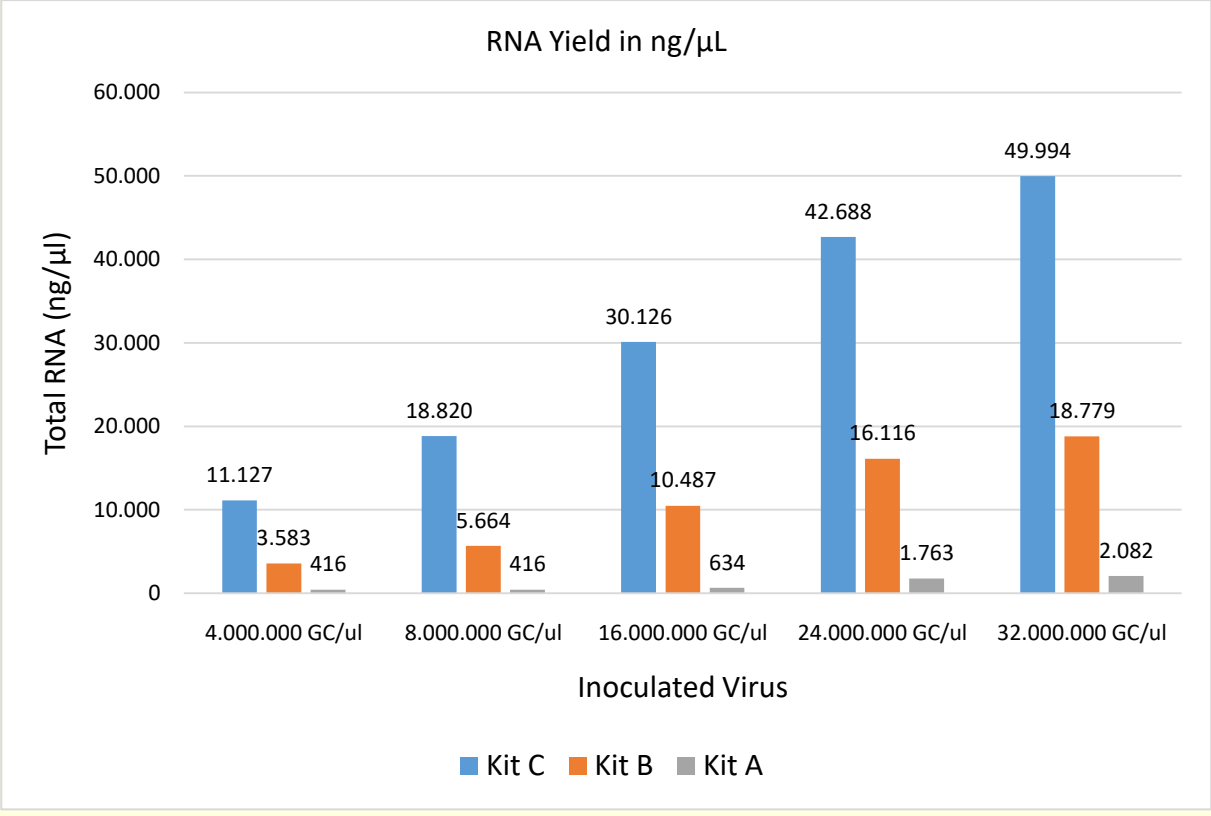

Figure 2. RNA Yield in ng/µL

Statistical Analysis

Statistically significant differences ($p < 0.05$) in total RNA yield were observed among the three commercial kits. The average total RNA concentrations across the three kits were 28.323 ng/µL (range: 11.127–49.994 ng/µL) for kit C, 10.925 ng/µL (range: 3.583–18.779 ng/µL) for kit B, and 1.062 ng/µL (range: 416–2.082 ng/µL) for kit A (Table 2, Figure 2).

RT-qPCR Analysis

The three commercial extraction kits were compared for their ability to extract viral RNA for downstream RT-qPCR analysis. All samples were analyzed in six replicates to increase statistical reliability. Kit C achieved the highest total RNA yield, leading to statistically significant differences in mean gene copies per microliter (GC/µL) for SARS-CoV-2 across the three kits ($p < 0.01$). The average gene copy numbers were 5,874,911 GC/µL for kit C, 2,100,804 GC/µL for kit B, and 203,958 GC/µL for kit A (***Table 3, Figure 3***). No SARS-CoV-2 RNA was detected in the negative controls for any extraction method.

Table 3. Gene Copies per Microliter (GC/µL)

	Inoculated Virus																			
	4.000.000 GC/ul				8.000.000 GC/ul				16.000.000 GC/ul				24.000.000 GC/ul				32.000.000 GC/ul			
	Recovered Virus, GC/ul		SD	%RC	Recovered Virus, GC/ul		SD	%RC	Recovered Virus, GC/ul		SD	%RC	Recovered Virus, GC/ul		SD	%RC			SD	%RC
Kit C	2.139.597	±	249.305	53	3.618.856	±	617.964	45	5.793.191	±	579.431	36	8.209.002	±	1.325.489	34	9.613.912	±	753.563	30
Kit B	688.745	±	158.876	17	1.088.960	±	439.165	14	2.016.355	±	611.311	13	3.098.873	±	468.889	13	3.611.091	±	311.088	11
Kit A	79.768	±	18.508	2	79.681	±	19.331	1	121.577	±	28.349	1	338.764	±	153.293	1	400.052	±	57.491	1
P value	< 0,01				< 0,014				< 0,01				< 0,01				< 0,01			

GC: Gene Copy, SD: Standard Deviation, %RC: % Recovered Copy

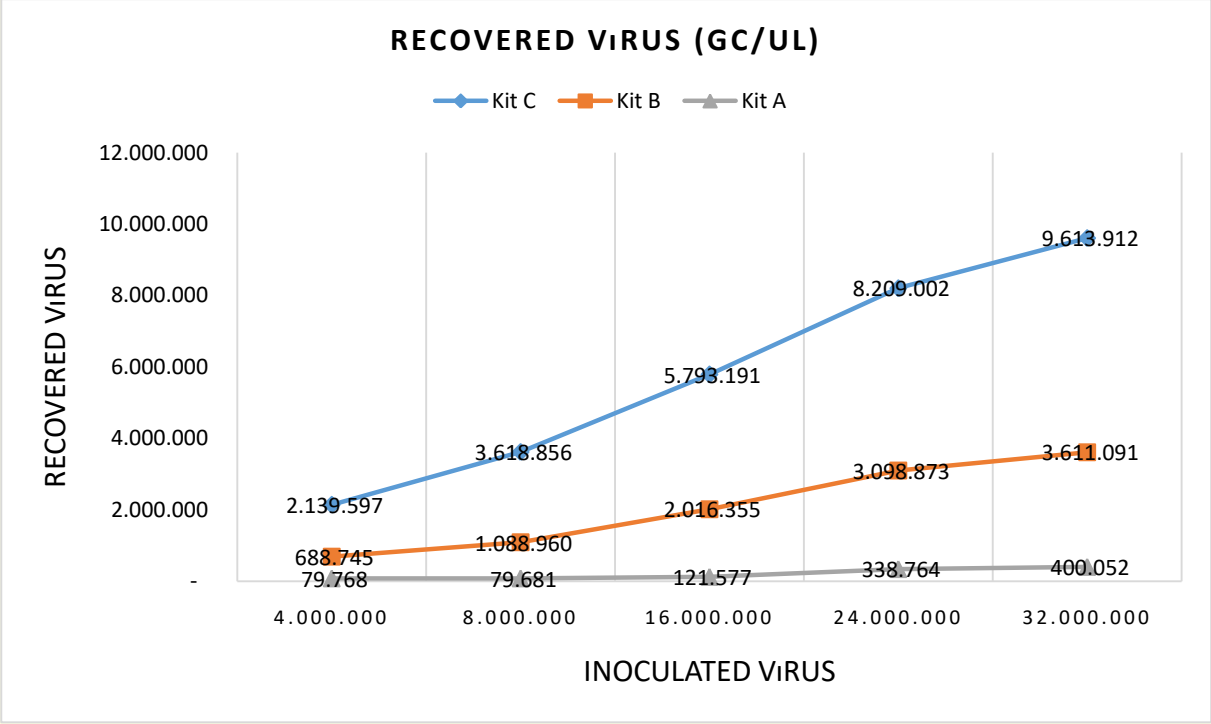

Figure 3. Gene Copies per Microliter (GC/µL). GC: Gene copy

Coefficient of Variation (CV) and Statistical Evaluation

The coefficient of variation (CV) was calculated for each extraction kit, with lower CV values indicating more consistent results. Kit C demonstrated the lowest CV across all viral concentrations, with an overall CV of 12.5%, suggesting a more homogeneous distribution of results. Lower CV values indicate that the measurements within the group are closer to each other, indicating reliability and reproducibility. In contrast, kit B exhibited an overall CV of 23.5%, and kit A had a CV of 26.1%, indicating greater variability in RNA yield and thus less consistent performance. These results highlight that kit C provides more stable and reproducible results, especially in high-throughput scenarios (***Table 4, Figure 4***).

Table 4. Coefficient of Variation (CV) Values and Linearity

	Coefficiency of variance (CV), %						Linearity		
Recovered Virus, GC/ul	4000000 GC/ul	8000000 GC/ul	16000000 GC/ul	24000000 GC/ul	32000000 GC/ul	Overal CV	R2	LOD	LOQ
Kit C	11,65	17,08	10,00	16,15	7,84	12,54	0,98	7.908.663,67	96.533.327,01
Kit B	23,07	40,33	30,32	15,13	8,61	23,49	0,99	11.522.501,46	106.649.784,39
Kit A	23,20	24,26	23,32	45,25	14,37	26,08	0,93	14.538.456,31	125.075.370,17

R^2: Determination Coefficient, LOD: Limit of Detection, LOQ: Limit of Quantification

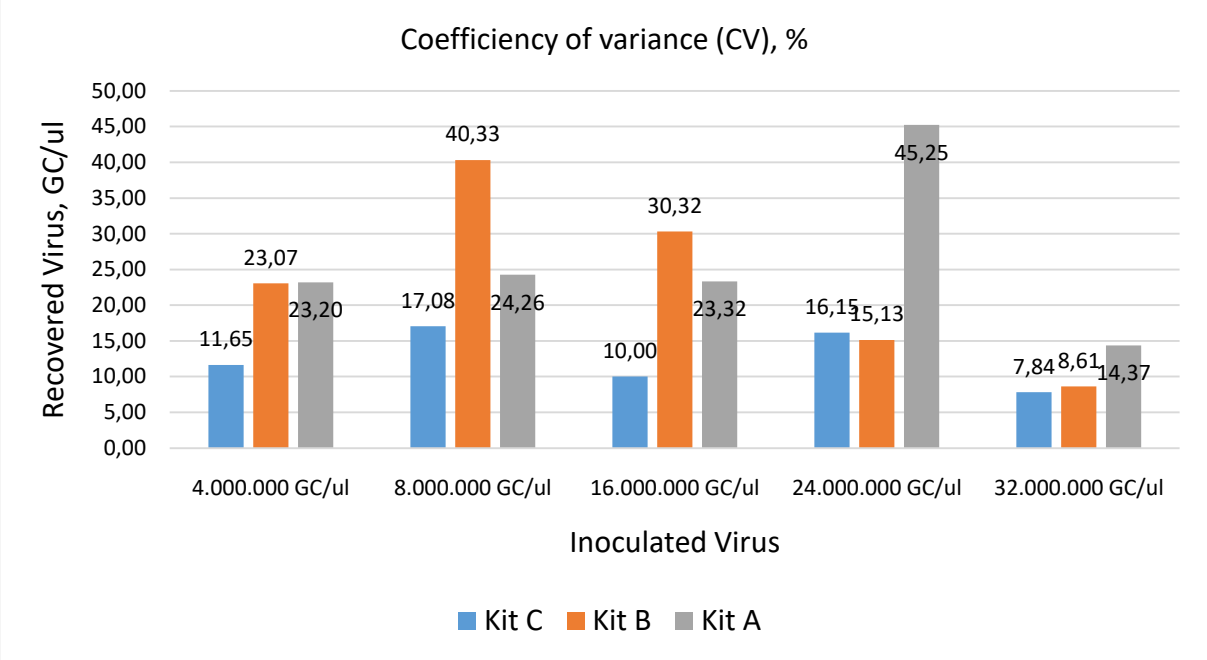

Figure 4. Coefficient of Variation (CV) Values and Linearity. GC: Gene copy

Lower CV values indicate that the measurements are more homogeneous and close to the standard deviation, suggesting better reliability of the data and consistency of the method. A higher CV, as seen with kit B and kit A, suggests more variability within the data, which could affect the consistency of downstream applications.

Linear Regression Analysis

A linear regression analysis revealed a stronger correlation between RNA concentrations and CT values for kit C (R^2 = 0.356) compared to kit B (R^2 = 0.03) and kit A (R^2 = 0.03). The maximum/minimum CT values for kit C were 15.04/17.85, while kit B and kit A exhibited higher variability in their CT values, with maximum/minimum values of 16.56/19.71 and 20.04/23.28, respectively.

Discussion

Wastewater-based epidemiology (WBE) has emerged as a powerful tool for public health authorities to monitor epidemics such as SARS-CoV-2. WBE analyzes viral load in wastewater samples, providing insight into community-level infections regardless of individuals' symptoms, testing, or reporting. As a result, WBE serves as an early warning system for COVID-19 and other outbreaks.[22,23,27,28] However, several factors can affect WBE results, including water temperature, dilution due to precipitation, the presence of PCR inhibitors, and sampling design. Despite these challenges, wastewater testing remains a cost-effective and practical approach for long-term monitoring of COVID-19 epidemiology.[27,29–34] This underlines the need for sensitive and cost-effective workflows for detecting SARS-CoV-2 in wastewater, essential for tracking pandemic dynamics within communities. RT-qPCR remains a vital component of this strategy, where the accuracy of results hinges on high-quality, DNA-free, intact RNA.[4,22,35,36]

In this study, all tested protocols successfully extracted high-quality RNA from wastewater, although the quantities of RNA varied among the kits. The presence of genomic DNA (gDNA) was observed during total nucleic acid extraction with all kits, highlighting the need for additional DNAse-I treatment to remove gDNA contamination, which aligns with findings from previous studies on TRIzol-based extractions.[35,37] Quality control of the RNA involved a variety of techniques, including spectrophotometry and assessing RNA integrity. To ensure suitability for downstream applications such as RT-qPCR, the RNA should ideally have an A260/280 ratio of 2.0±0.1, indicating minimal protein contaminatio. Our results demonstrated that all three kits produced RNA of sufficient quality, consistent with previous studies that used similar protocols.[38–40]

Kit B and kit C offer the advantage of automated workflows, which minimize human error and increase reproducibility. In contrast, kit A requires manual handling, making it more labor-intensive and prone to variability depending on user expertise. Our findings revealed significant differences in RNA yield across the three kits, with kit C consistently outperforming kit B and kit A. On average, kit C yielded 2.9 and 33.5 times more RNA (ng/µL) than kit B and kit A, respectively. Additionally, kit B produced 11.3 times more RNA than kit A.

It is important to note that while the Thermo NanoDrop measures total RNA concentration (predominantly ribosomal RNA), RT-qPCR quantifies mRNA exclusively. Our linear regression analysis demonstrated a stronger correlation between RNA concentration and gene copy number for kit C compared to the other two kits. Specifically, Ct values decreased steadily as RNA concentration increased with kit C, indicating higher sensitivity and efficiency in RNA extraction. In contrast, no such correlation was observed with kit B or kit A, which suggests that these kits may be less effective for RT-qPCR analysis.

The yield discrepancy for kit A indicates its limitations, especially when dealing with limited wastewater samples. In such cases, users may need to consider alternative methods that offer higher RNA recovery. Overall, kit C emerged as the most effective kit, both in terms of RNA quantity and quality, making it the optimal choice for high-throughput settings and applications requiring consistent RNA yields.

The main reasons why kit C provides higher RNA yield compared to kit B and kit A may be the operating principle of the device, the stages of the extraction process, and the magnetic bead-based extraction technology. Automated systems minimize human error and provide more consistent and efficient results. Both the kit C and the kit B use magnetic bead-based extraction technology. This technology allows for more efficient binding and purification of nucleic acids, while the yield may be lower in the kit A with spin column technology. This demonstrates the superiority of both automated extraction kits over the manual extraction kit. Although both kits use magnetic bead-based extraction technology, there may be several

important reasons why the kit C provides higher RNA yield. The washing and incubation steps of kit C are more extensive and take longer compared to kit B, which may allow for more efficient RNA purification. Another difference between the two kits is that the device for kit C applies heat during incubation, whereas the device for kit B does not apply heat at any stage. In addition, the device for kit C uses new pipette tips at each step by changing pipette tips during operation, while the device for kit B completes the entire process with a single rod cover. More washing and incubation steps, heat application during incubation and pipette tip changes can remove potential contaminants and isolate RNA with higher purity. The differences between the technologies and protocols used, especially the differences in the number of washes and incubation times, heat application and pipette tip changes, can be decisive factors on RNA yield and purity, while at the same time, they can be expressed as advantages of kit C over kit B.

Each kit investigated in this study comes with distinct advantages and limitations. While kit C excelled in RNA yield and consistency, its automated nature may make it less accessible in resource-limited settings. Kit A, though less efficient, may still find utility in smaller labs where manual workflows are more feasible. Kit B strikes a balance between automation and performance, though its lower RNA yield compared to kit C suggests it may not be ideal for all applications. Nonetheless, the selection of the appropriate extraction kit should consider the specific needs of the laboratory, the nature of the samples, and the required throughput.

Conclusions

This study demonstrated that RNA extraction from wastewater samples can vary significantly depending on the extraction kit employed. Kit C consistently surpassed both kit B and kit A in terms of RNA yield and purity. Its superior performance can be attributed to several key features, including extended washing and incubation steps, the application of heat during incubation, and automated pipette tip changes, all of which enhance RNA quality and minimize contamination. These results underscore the importance of selecting an RNA extraction kit that aligns with the specific requirements of the sample type and experimental demands. Kit C, with its efficiency and reliability, stands out as the optimal choice for large-scale SARS-CoV-2 wastewater surveillance.

Acknowledgements

The authors declared no potential conflicts of interest. The authors declared no receipt of the financial support for the research.

Author Contributions

Ahmet Sait: Concept, design, supervision, data collection, analyses and interpretation, literature search, writing the article, critical review, references and materials

Serol Korkmaz: Design, supervision, data collection, analyses and interpretation, literature search, writing the article, critical review, references and materials.

Ayşe Parmaksız: Concept, design, supervision, data collection, literature search, critical review, references and materials.

Bülent Bayraktar: Concept, design, supervision, data collection, critical review.

İsmail Aslan: Concept, design, data collection, analyses and interpretation, critical review.

References

1. Gandhi M, Yokoe DS, Havlir DV. Asymptomatic Transmission, the Achilles' Heel of Current Strategies to Control Covid-19. N Engl J Med 2020;382(22):2158-2160. doi:10.1056/NEJMe2009758.
2. Jee Y. WHO International Health Regulations Emergency Committee for the COVID-19 outbreak. Epidemiol Health 2020;42:e2020013. doi:10.4178/epih.e2020013.
3. Moghadas SM, et al. The implications of silent transmission for the control of COVID-19 outbreaks. Proc Natl Acad Sci U S A 2020;117(30):17513-17515. doi:10.1073/pnas.2008373117
4. Deng MY, et al. Comparison of six RNA extraction methods for the detection of classical swine fever virus by real-time and conventional reverse transcription-PCR. J Vet Diagn Invest 2005;17(6):574-578. doi:10.1177/104063870501700609
5. Paton DJ, et al. Classical swine fever virus: a ring test to evaluate RT-PCR detection methods. Vet Microbiol 2000;73(2-3):159-174. doi:10.1016/s0378-1135(00)00142-5.
6. Paton DJ, et al. Classical swine fever virus: a second ring test to evaluate RT-PCR detection methods. Vet Microbiol 2000;77(1-2):71-81. doi:10.1016/s0378-1135(00)00264-9.
7. Risatti GR, et al. Rapid detection of classical swine fever virus by a portable real-time reverse transcriptase PCR assay. J Clin Microbiol 2003;41(1):500-505. doi:10.1128/JCM.41.1.500-505.2003.
8. van Rijn PA, et al. Detection of economically important viruses in boar semen by quantitative RealTime PCR technology. J Virol Methods 2004;120(2):151-160. doi:10.1016/j.jviromet.2004.04.014
9. Tan SC, Yiap BC. DNA, RNA, and protein extraction: the past and the present [published correction appears in J Biomed Biotechnol 2013;2013:628968]. J Biomed Biotechnol. 2009;2009:574398. doi:10.1155/2009/574398
10. Tavares L, Alves PM, Ferreira RB, Santos CN. Comparison of different methods for DNA-free RNA isolation from SK-N-MC neuroblastoma. BMC Res Notes 2011;4.
11. Imbeaud S, et al. Towards standardization of RNA quality assessment using user-independent classifiers of microcapillary electrophoresis traces. Nucleic Acids Res 2005;33(6):e56. doi:10.1093/nar/gni054
12. Kurar E, et al. Comparison of Five Different RNA Isolation Methods from Equine Endometrium for Gene Transcription Analysis. Kafkas Univ Vet Fak Derg 2010;16 (5): 851-855. doi:10.9775/kvfd.2010.1829
13. Raeymaekers L. Quantitative PCR: theoretical considerations with practical implications. Anal Biochem 1993;214(2):582-585. doi:10.1006/abio.1993.1542.
14. Bélec L, Brogan TV. Real-time PCR-based testing of saliva for cytomegalovirus at birth. Expert Rev Anti Infect Ther 2011;9(12):1119-1124. doi:10.1586/eri.11.130.
15. Maron JL, Johnson KL. Comparative performance analyses of commercially available products for salivary collection and nucleic acid processing in the newborn. Biotech Histochem 2015;90(8):581-586. doi:10.3109/10520295.2015.1048289.
16. Parisi MR, et al. Cross-sectional study of community serostatus to highlight undiagnosed HIV infections with oral fluid HIV-1/2 rapid test in non-conventional settings. New Microbiol 2013;36(2):121-132.
17. O'Brien M, et al. A comparison of four commercially available RNA extraction kits for wastewater surveillance of SARS-CoV-2 in a college population. Sci Total Environ 2021;801:149595. doi:10.1016/j.scitotenv.2021.149595.
18. Bustin SA, Nolan T. Pitfalls of quantitative real-time reverse-transcription polymerase chain reaction. J Biomol Tech 2004;15(3):155-166.
19. Rossen L, et al. Inhibition of PCR by components of food samples, microbial diagnostic assays and DNA-extraction solutions. Int J Food Microbiol 1992;17(1):37-45. doi:10.1016/0168-1605(92)90017-w
20. Wilson IG. Inhibition and facilitation of nucleic acid amplification. Appl Environ Microbiol 1997;63(10):3741-3751. doi:10.1128/aem.63.10.3741-3751.1997.
21. Esser, K, et al. Nucleic Acid-free Matrix: Regeneration of DNA Binding Columns. BioTechniques 2005;39(2):270–271. doi.org/10.2144/05392AF01
22. Lucansky V, et al. Comparison of the methods for isolation and detection of SARS-CoV-2 RNA in municipal wastewater. Front Public Health 2023;11:1116636. doi:10.3389/fpubh.2023.1116636.
23. Mazumder P, et al. Sewage surveillance for SARS-CoV-2: Molecular detection, quantification, and normalization factors. Curr Opin Environ Sci Health 2022;28:100363. doi:10.1016/j.coesh.2022.100363.
24. Sait A, et al. Investigation of the recovery efficiency of CeUF method through RT-qPCR quantification of inactivated SARS-CoV-2 in untreated wastewater. Desalin Water Treat 2022;262:54-59.
25. Chirgwin JM, et al. Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. Biochemistry 1979;18(24):5294-5299. doi:10.1021/bi00591a005.
26. Glisin V, Crkvenjakov R, Byus C. Ribonucleic acid isolated by cesium chloride centrifugation. Biochemistry 1974;13(12):2633-2637. doi:10.1021/bi00709a025.
27. Medema G, et al. Presence of SARS-Coronavirus-2 RNA in Sewage and Correlation with Reported COVID-19 Prevalence in the Early Stage of the Epidemic in The Netherlands. Environ Sci Technol Lett 2020;7(7):511-516. doi:10.1021/acs.estlett.0c00357.

28. Heijnen L, et al. Droplet digital RT-PCR to detect SARS-CoV-2 signature mutations of variants of concern in wastewater. Sci Total Environ 2021;799:149456. doi:10.1016/j.scitotenv.2021.149456.
29. de Freitas Bueno R, et al. Wastewater-based epidemiology: A Brazilian SARS-COV-2 surveillance experience. J Environ Chem Eng 2022;10(5):108298. doi:10.1016/j.jece.2022.108298.
30. Hillary LS, et al. Wastewater and public health: the potential of wastewater surveillance for monitoring COVID-19. Curr Opin Environ Sci Health 2020;17:14-20.
31. Kocamemi BA, et al. Nationwide SARS-CoV-2 surveillance study for sewage and sludges of wastewater treatment plants in Turkey. medRxiv 2020:2020-11.
32. Kocamemi BA, et al. Routine SARS-CoV-2 wastewater surveillance results in Turkey to follow Covid-19 outbreak. medRxiv 2020:2020-12.
33. Kocamemi BA, et al. SARS-CoV-2 detection in Istanbul wastewater treatment plant sludges. MedRxiv 2020:2020-05.
34. Randazzo W, et al. SARS-CoV-2 RNA in wastewater anticipated COVID-19 occurrence in a low prevalence area. Water Res 2020;181:115942. doi:10.1016/j.watres.2020.115942.
35. Mannhalter C, Koizar D, Mitterbauer G. Evaluation of RNA isolation methods and reference genes for RT-PCR analyses of rare target RNA. Clin Chem Lab Med 2000;38(2):171-177. doi:10.1515/CCLM.2000.026.
36. O'Connell J. The basics of RT-PCR. Some practical considerations. Methods Mol Biol 2002;193:19-25. doi:10.1385/1-59259-283-X:019.
37. Phongsisay V, Perera VN, Fry BN. Evaluation of eight RNA isolation methods for transcriptional analysis in Campylobacter jejuni. J Microbiol Methods 2007;68(2):427-429. doi:10.1016/j.mimet.2006.09.002.
38. Farrell RE. RNA Methodologies (Third Edition) A Laboratory Guide for Isolation and Characterization. Farrell RE Editor(s). RNA Isolation Strategies. Academic Press; 2005. pp:67-113.
39. Madabusi LV, Latham GJ, Andruss BF. RNA extraction for arrays. Methods Enzymol 2006;411:1-14. doi:10.1016/S0076-6879(06)11001-0.
40. Schroeder A, et al. The RIN: an RNA integrity number for assigning integrity values to RNA measurements. BMC Mol Biol 2006;7:3. doi:10.1186/1471-2199-7-3