Journal of Virological Methods 334 (2025) 115116

Contents lists available at ScienceDirect

Journal of Virological Methods

journal homepage: www.elsevier.com/locate/jviromet

Protocols

Phenol-free *in-house* kit for RNA extraction with applicability to SARS-CoV-2 genomic sequencing studies: A contribution to biotechnological sovereignty in Colombia

Bertha Gastelbondo-Pastrana [a,g,h], Luis Flórez [a], Camilo Guzmán [a,i], Karina Torres [a], Evelin Garay [a], Jolaime Ballesteros-Villamizar [a], Rosa Gutierrez [a], Daniel Echeverri-De la Hoz [a], Yésica López [a], Héctor Contreras [a], Germán Arrieta [a,h], Héctor Serrano-Coll [e], Caty Martínez [a], Nérlis Pájaro-Castro [d], Bárbara Arroyo-Salgado [c], Ricardo Rivero-Herrera [a,f], Eliana Hurtado [b], João Pessoa Araújo Jr. [b], Salim Mattar [a,1,*]

[a] *Instituto de Investigaciones Biológicas del Trópico, Universidad de Córdoba, Campus Berastegui, Córdoba, Colombia*
[b] *São Paulo State University (UNESP), Institute of Biotechnology (IBTEC), Botucatu, SP, Brazil*
[c] *Biomedics, Toxicology and Environmental Research Group-BIOTOXAM, University of Cartagena, Cartagena, Colombia*
[d] *Department of Medicine, School of Health Sciences, Medical and Pharmaceutical Sciences Group, University of Sucre, Sincelejo, Sucre CP, 700003, Colombia*
[e] *Instituto Colombiano de Medicina Tropical, Universidad CES, Medellín, Colombia*
[f] *Paul G. Allen School for Global Health, Washington State University, Pullman, WA 99164, United States*
[g] *Grupo de Investigaciones Microbiológicas y Biomédicas de Córdoba-GIMBIC, Universidad de Córdoba, Montería, Colombia*
[h] *Grupo de Salud Pública y Auditoría en Salud, Corporación Universitaria del Caribe-CECAR, Sincelejo, Colombia*
[i] *Grupo de Investigación, Evaluación y Desarrollo de Fármacos y Afines - IDEFARMA, Universidad de Córdoba, Montería, Colombia*

ARTICLE INFO

Keywords:
Molecular Diagnostic Techniques
COVID-19 testing
Innovation
Research and Development
Developing countries
Economic development

ABSTRACT

During the COVID-19 pandemic, reagents for SARS-CoV-2 detection were scarce or sold at high prices, particularly in Latin America. In this study, a significant step towards self-sufficiency was achieved through the development of an in-house extraction kit for detecting SARS-CoV-2 from nasopharyngeal swab samples. The purity and concentration of the RNA extracted using the in-house kit were compared to those obtained using the GeneJET RNA Purification Kit (Thermo-Scientific®) as a reference. The applicability of the RNA extracted using the kit was evaluated using four samples positive for SARS-CoV-2 by NGS sequencing with Illumina®. There were no significant differences between the results obtained with the in-house kit and those obtained with the commercial kit. These findings confirm that the in-house protocol demonstrated satisfactory diagnostic accuracy for detecting the virus in patients with COVID-19. The in-house extraction kit works effectively, providing optimal RNA extraction for genomic characterization and lineage assignment of SARS-CoV-2 within the four positive samples analyzed. This phenol-free kit represents a local design and production achievement, offering an effective solution for RNA extraction and detection and sequencing of SARS-CoV-2 from nasopharyngeal swabs. The data highlight the essential contribution of this study to health and biotechnological sovereignty in Colombia.

1. Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the etiologic agent of coronavirus disease 2019 (COVID-19) (Lamers and Haagmans, 2022). Molecular detection of SARS-CoV-2 using RT-qPCR in respiratory samples is considered the gold standard for routine diagnosis (Burjanivova et al., 2023); Although RT-qPCR is highly sensitive, specific, and fast, the majority of diagnostic laboratories depend on manual extraction methods, which are prone to contamination, consume large amounts of plastic materials (such as microtubes and pipette tips), and

* Corresponding author.
E-mail address: smattar@correo.unicordoba.edu.co (S. Mattar).
[1] ORCID: 0000-0003-0526-463

https://doi.org/10.1016/j.jviromet.2025.115116
Received 5 November 2024; Received in revised form 3 February 2025; Accepted 4 February 2025
Available online 14 February 2025

typically involve the use of toxic organic solvents (Silva et al., 2022). Therefore, its performance can be affected by the efficiency of the viral RNA extraction procedures (Silva et al., 2022).

RNA isolation is essential to ensure the quality of genomic sequencing data (Smela et al., 2022). Although COVID-19 is no longer a health emergency, differential diagnosis of other viruses causing acute respiratory infections is necessary. Differential diagnosis is essential for optimizing the clinical management of patients to avoid clinical complications. However, RNA extraction methods are often limited in clinical laboratories because of the toxicity of the reagents; they usually use dangerous, carcinogenic chemicals such as phenol, chloroform, and formamide, which are difficult to remove from the extracted RNA. In research laboratories, RNA extraction methods are considered the standard for isolating RNA. However, phenol-based extraction techniques have become routine for SARS-CoV-2 detection in clinical laboratories, and the limitations of these clinical methods subsequently affect their performance (Smela et al., 2022). Therefore, the use of phenol in the extraction of nucleic materials is frequent because of its dual hydrophilic and lipophilic properties, allowing it to easily cross cell membranes, denature proteins, and trigger cell death in the biological material under study (Downs and Wills, 2024). Phenol has an additional mechanism of toxicity by uncoupling oxidative phosphorylation, causing hyperthermia and acidosis when exposure is prolonged. Commercial RNA extraction products include these toxic compounds in their kits and protocols (Downs and Wills, 2024).

During the pandemic, RNA extraction for the diagnosis of COVID-19 was considered a challenge owing to the shortage of extraction kits, mainly in low-resource countries such as Colombia. This scenario prompted the exploration of alternative extraction protocols to replace commercial kits and eliminate toxic compounds, such as phenol. Additionally, adjustments to the protocol, such as the use of proteinase K treatment followed by thermal shock (98°C for 5 min, then 4°C for 2 min), are considered a means to mitigate supply chain disruptions and high costs driven by the growing demand for molecular tests (Ñique et al., 2021; Ponce-Rojas et al., 2021).

The aforementioned search was performed to avoid future health problems during emergencies (Delgado-Diaz et al., 2022). The extraction of nucleic acids is an essential step in the diagnosis and sequencing of viruses. It is also advantageous in genomic surveillance as it identifies variants of concern (Hernandez et al., 2023; Marchini et al., 2023).

Therefore, complete genomes generated using highly sensitive and specific sequencing methods are required to inform and allow genomic surveillance to provide information for disease management and health decision-making (Marchini et al., 2023). The objective of this study was to develop a simple and low-cost method for RNA extraction from samples of patients with suspected SARS-CoV-2 infection.

2. Material and methods

2.1. Clinical samples and ethical considerations

For the assays, 78 nasopharyngeal swab samples (29 positives and 49 negatives samples) were used. Samples were collected between 2020 and 2022 from the cryopreserved sample bank of the Institute for Biological Research of the Tropics (IIBT) in the Department of Córdoba, Colombia. Samples were selected according to the dates on which different SARS-CoV-2 variants emerged during the pandemic. Patients were registered using an anonymous numerical code and their samples were collected with the authorization of the National Institute of Health of Colombia, which led to the diagnosis of SARS-CoV-2 in the Department of Córdoba, Colombia, during a health emergency. The initial collection of samples was approved by the Ethics Committee of the Institute for Biological Research of the Tropics of the University of Córdoba (0410–2020). The study incorporated procedures, sample handling and conservation, and technical-administrative procedures for health research under the provisions of Resolution 8430 of the Ministry of Health of Colombia in 1993, and the Declaration of Helsinki for ethical and medical research on human subjects. This study was considered to have a minimal risk. Ethical aspects of phylogenetic analysis of sequences was approved by the Ethics Committee by number 33202820.7.1001.5348.

2.2. Manual RNA extraction with the phenol free in-house kit

For the extraction of SARS-CoV-2 RNA using the phenol-free in-house kit, the Chomzynski and Sacchi (1987) protocol (Chomzynski and Sacchi, 1987) was modified (Fig. 1). The protocol, developed for this study, is protected by the National Directorate of Copyrights of the Ministry of the Interior of Colombia under record number 1–2023–111257. It employs guanidine isothiocyanate (GITC) at concentrations ranging from 1 to 6 M as the primary reagent for the lysis buffer preparation. Additionally, adjuvants such as Tris-HCl (30–60 mM), EDTA (10–40 mM), Triton X-100, and bromophenol blue (1–2 % w/v) are incorporated, with bromophenol blue serving as an indicator. The total cost per sample is 40 USD, and the entire protocol can be completed in 1 h and 30 min.

First, 200 μL of *in-house* lysis buffer and 200 μL of nasopharyngeal swab sample were added to a microtube with an equal sample volume to maintain a 1:1 ratio. A brief vortex was then applied to homogenize the mixture. Subsequently, the mixture was incubated at 56°C for 20 min, and 200 μL chloroform was added to separate the aqueous and organic phases. The aqueous phase was collected, transferred to another microtube, briefly mixed in a vortex to homogenize the sample (previously preserved in viral transport medium (VTM)), and centrifuged at 12,000 rpm for 15 min. To precipitate RNA, 400 μL of isopropanol was added and mixed in a vortex for 15 s. The mixture was then centrifuged at 12,000 rpm for 20 min at 4°C. The sample was then centrifuged, and isopropanol was discarded. Ice ethanol was then added, and the sample was homogenized and centrifuged at 12,000 rpm at 4°C for 5 min as a final step. The supernatant obtained was discarded and dried for 10 min to complete the evaporation of ethanol. Finally, the obtained RNA was eluted in 50 μL of nuclease-free water and stored at −70°C until further use.

2.3. Commercial method for RNA extraction

SARS-CoV-2 RNA was extracted using a commercial Thermo Scientific GeneJET RNA Purification Kit #K0731, #K0732 following the manufacturer's instructions without the RNA Cleanup Protocol (Chomzynski and Sacchi, 1987)

2.4. Quantification and evaluation of RNA purity

A NanoDrop™ 2000/2000c spectrophotometer (Thermo Scientific®) was used for both methods. The concentration results were expressed in ng/μL, and for purity, the data obtained were selected using the 260/280 ratio as a parameter to determine the optimal purity between 2.0 and 2.2.

2.5. Amplification of RNA extracted from SARS-CoV-2 by RT-qPCR

RT-qPCR was used to amplify SARS-CoV-2 RNA. This protocol was based on the detection of the E (envelope) gene and human RNase P gene as an internal RNA extraction control, allowing the detection of the presence of human RNA in the sample. The primer and probe sequences used are listed in Table 1. The molecular detection assay for SARS-CoV-2 was based on the *Charité-Berlin* protocol (Corman et al., 2020). The genes were coupled to HEX and Quasar 670 fluorophores. The Luna Universal Probe One-Step RT-qPCR Kit was used according to the manufacturer's instructions (https://www.neb.com/en/protocols/2016/11/11/luna-universal-probe-one-step-rt-qpcr-kit-protocol-e3006). The interpretation of the results was based on cycle threshold

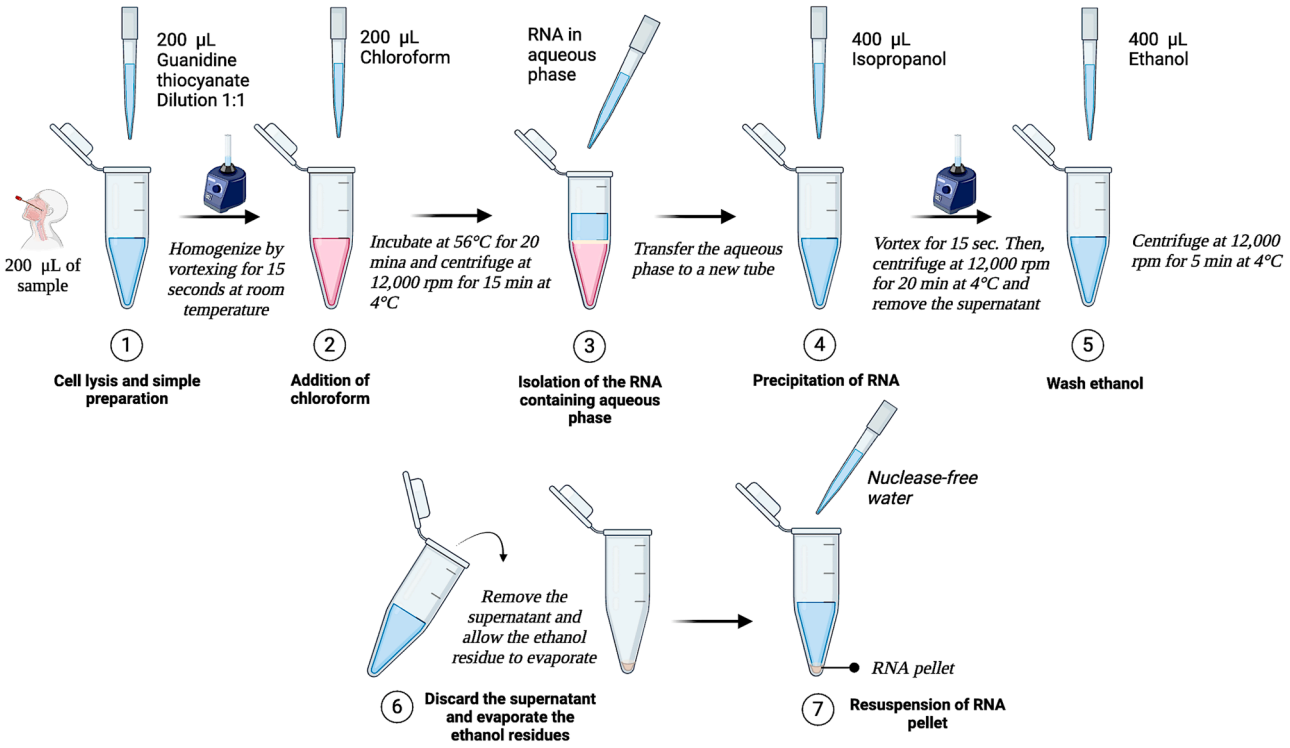

Fig. 1. Summary of the modified protocol for RNA extraction using an in-house kit.

Table 1
Primers and probes for SARS-CoV-2 RT-qPCR (Corman et al., 2020).

Target gene	Name	Oligonucleotide sequence (5′>3′)
E	E_Sarbeco_F1	Forward: ACAGGTACGTTAATAGTTAATAGCGT
	E_Sarbeco_R2	Reverse: ATATTGCAGCAGTACGCACACA
	E-Probe	Probe: FAM-ACACTAGCCATCCTTACTGCGCTTCG-BHQ1
RNAse P	Rnase_P_Fw	Forward: AGATTTGGACCTGCGAGCG
	Rnase_P_Rv	Reverse: GAGCGGCTGTCTCCACAAGT
	RNAaseP	Probe: HEX-TTCTGACCTGAAGGCTCTGCGCG-BHQ1

(Ct) values, which indicate the point at which the fluorescence signal rises above the background noise during the RT-qPCR assay. For the detection of SARS-CoV-2, a sample was considered positive if the Ct value for the E gene and RNAseP (internal control) was <37, whereas samples with a Ct ≥37 were classified as negative.

2.6. Next-generation sequencing (NGS) of SARS-CoV-2 positive samples by RT-qPCR

The in-house RNA isolation kit was used to extract genetic material from the three clinical samples. SARS-CoV-2 positive samples with Ct <28 for the E gene were prepared for NGS following the Illumina COVIDSeq protocol (Illumina, San Diego, CA, USA). According to the manufacturer's protocol, a pool of libraries was created and purified, starting from the "Anneal RNA" reaction. A TapeStation system (Agilent, Santa Clara, CA, USA) was used to perform quality control and determine the molarity of each library pool. Sequencing was performed using the MiSeq Mid Output v2 kit (300 cycles) on a MiSeq sequencer (Illumina San Diego, CA, USA) programmed to generate 151 bp reads. The DRAGEN COVID Lineage v3.5.8 pipeline was used for sequence analysis, consensus building, and variant determination. Sequences were classified into PANGO lineages using the PangoLEARN v1.13 model database. All genome sequences were deposited in the Global Initiative on Sharing All Influenza Data (GISAID) database.

2.7. Phylogenetic analysis of sequences

The analysis of SARS-CoV-2 sequences was conducted using the sequences reported in GISAID, a dataset containing genomes from the department of Córdoba, Colombia, those obtained from the in-house kit, and the reference sequence NC_045512. Incomplete sequences (< 28,000 bp) and those with low coverage (> 10 % Ns) were removed using RStudio. The dataset, composed of 212 sequences, was subjected to multiple sequence alignment (MSA) using MAFFT (10.1093/molbev/mst010). The alignment was manually edited using UGENE (10.1093/bioinformatics/bts091) software. Phylogenetic reconstruction was performed using Bayesian Evolutionary Analysis Sampling Trees (BEAST). (https://doi.org/10.1186/1471–2148-7–214) under a GTR substitution model with gamma distribution, strict molecular clock, and chain length of 200,000,000 interactions. The effective sample size (ESS) for each parameter was corroborated using Tracer v.1.7.2 (doi:10.1093/sysbio/syy032). Subsequently, a consensus tree was obtained using TreeAnnotator v.1.10.4 (https://doi.org/10.1186/1471–2148-7–214) with a Burnin of 10 % under the maximum credibility tree option, which is visualized in FigTree (FigTree (. ac. UK)). The trees were exported in Newick format and edited in RStudio to add annotation schemes according to the lineage reported in the GISAID.

2.8. Evaluation of the diagnostic accuracy of the in-house kit

Relative sensitivity and specificity analyses were performed to validate the in-house kits. The results obtained for the E gene using the GeneJET® kit from Thermo Fisher Scientific™ were used as reference values.

2.9. Statistical analysis

All data were analyzed using GraphPad Prism v9.0 (GraphPad Software Inc., La Jolla, CA, USA). Statistical significance (p-value) was determined using the nonparametric one-tailed Mann-Whitney *U* test to compare two independent groups. values below 0.05 were considered

statistically significant. RStudio version 4.3.1 was used for sensitivity analysis.

3. Results

3.1. RNA extraction

The *in-house* kit enabled a higher recovery of total RNA than the commercial GeneJET RNA Purification Kit. These results suggest that the *in-house* kit significantly enhanced RNA recovery (Fig. 2). The 260/280 ratio obtained using the *in-house* kit and the GeneJET™ RNA Purification Kit from Thermo Scientific® was analyzed (Fig. 2b). The analysis revealed statistically significant differences ($P < 0.05$) between the two methods.

3.2. Detection of SARS-CoV-2 by RT-qPCR

The results of the *in-house* extraction kit showed that of the 78 nasopharyngeal swab samples evaluated, twenty-five samples were positive for SARS-CoV-2. In contrast, the Thermo Scientific® GeneJET™ RNA Purification Column Kit was used to detect the viral material in 28 samples. Ct values for the E gene showed no statistically significant differences compared to the reference method (Fig. 3A-3B). Regarding the Ct value of RNase P, significant differences were observed between the two extraction methods (Fig. 3C-3D).

3.3. Next-generation sequencing of SARS-CoV-2

Sequencing was performed on the Illumina® platform, and the results identified lineages B.1.625 (1/4), XBB.1.15 (1/4), and BQ.1.1.4 (2/4) (Fig. 4). The base coverage percentages of the samples were 94.12 %, 96.62 %, 98.43 %, and 98.90 %, respectively. Substitutions and deletions in the sequenced samples were determined using the reference genome NC_045512.2 (Wuhan-Hu-1) on the GISAID platform. The sequence access numbers for these samples were EPI_ISL_17073298, EPI_ISL_17886207, EPI_ISL_17886208, and EPI_ISL_17956166, respectively.

3.4. Evaluation of the sensitivity and specificity of the in-house kit

A relative sensitivity and specificity of 95 % were determined using the *in-house* kit for RNA extraction. The kit allowed for the correct identification of 86.21 % of patients with SARS-CoV-2 (Table 2), with a positive predictive value (PPV), which indicates a probability of 89.29 % presenting with the disease (Table 3), and 93.88 % of the patients who did not have SARS-CoV-2 were detected, with a negative predictive value (NPV) of 92.00 %. The results showed that in 6.12 % of the cases, the test presented a single false positive, and in 13.79 % of the cases, it presented a single false negative. Additionally, the ROC curve (Fig. 5) shows that, on average, the test (*in-house* kit) allows the right distinction between positive and negative cases 90 % of the time.

4. Discussion

In 2020, amid the SARS-CoV-2 pandemic, with increasing cases, there was a global shortage and an increase in the price of reagents and supplies used to perform diagnostic tests. The lack of these products prevents the diagnosis of many infected people, and the lack of development of this type of technology exposes dependence on imports, equipment, and supplies (Guzman et al., 2023). Worldwide, many laboratories have been established to rapidly process samples using simpler and more cost-effective RNA extraction methods than commercial RNA extraction kits for nasopharyngeal (NP) swab samples (Graham et al., 2021; Ulloa et al., 2020). Several laboratories in Colombia have developed innovative strategies, including the creation of RNA extraction reagents, to ensure reliable and timely supply (Wu & McGoogan, 2020).

A phenol-free in-house RNA extraction kit was developed, which is considered a good option for detecting and sequencing SARS-CoV-2 in nasopharyngeal swabs. The standardized protocol of this study can be performed reproducibly by other laboratories and countries with limited resources. Guanidium salts have been shown to denature and solubilize proteins, which means that there is no need to add additional steps to RNase inhibitors. However, it is worth noting that different parts of the world experienced significant shortfalls in the availability of reagents during the pandemic (Guzman et al., 2024). For instance, in Europe, guanidine isothiocyanate (GITC) was unavailable for some time. These global challenges highlight the importance of reagent availability as a critical driver in the development of alternative methods. Possebon et al., 2022 (Possebon et al., 2022) also used this method in a comparative study of two magnetic bead-based RNA extraction methods. This method was also used by Amirouche et al., 2021 (Amirouche et al., 2021), who used TRIzol® reagent containing ISTG as a valuable alternative for laboratories lacking commercial kits. RNA extraction, a crucial process for detecting SARS-CoV-2, must be efficient and specific (Nalla et al., 2020; Tesena et al., n.d). Despite the presence of chaotropic substances, manual methods allow for the release of total RNA inside

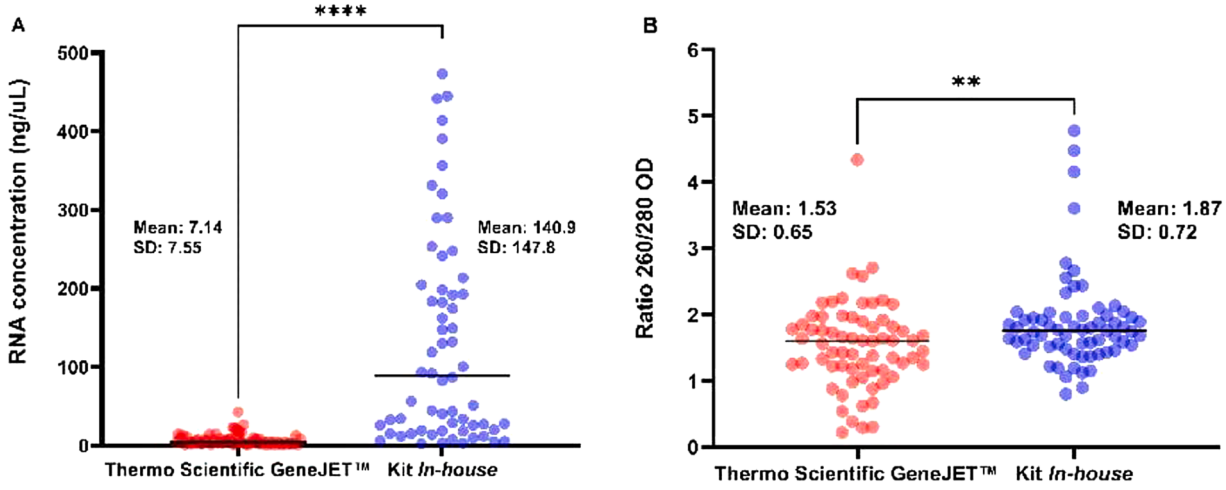

Fig. 2. Total RNA quantified using the GeneJET commercial kit and in-house kit. (A) Concentration of total RNA extracted using the two kits. (B) Purity of total RNA extracted using the two kits. Data obtained from the in-house kit and the reference kit are presented. * ** *Statistically significant ($p < 0.001$). ^{ns}Statistically non-significant. ^{SD}Standard deviation.

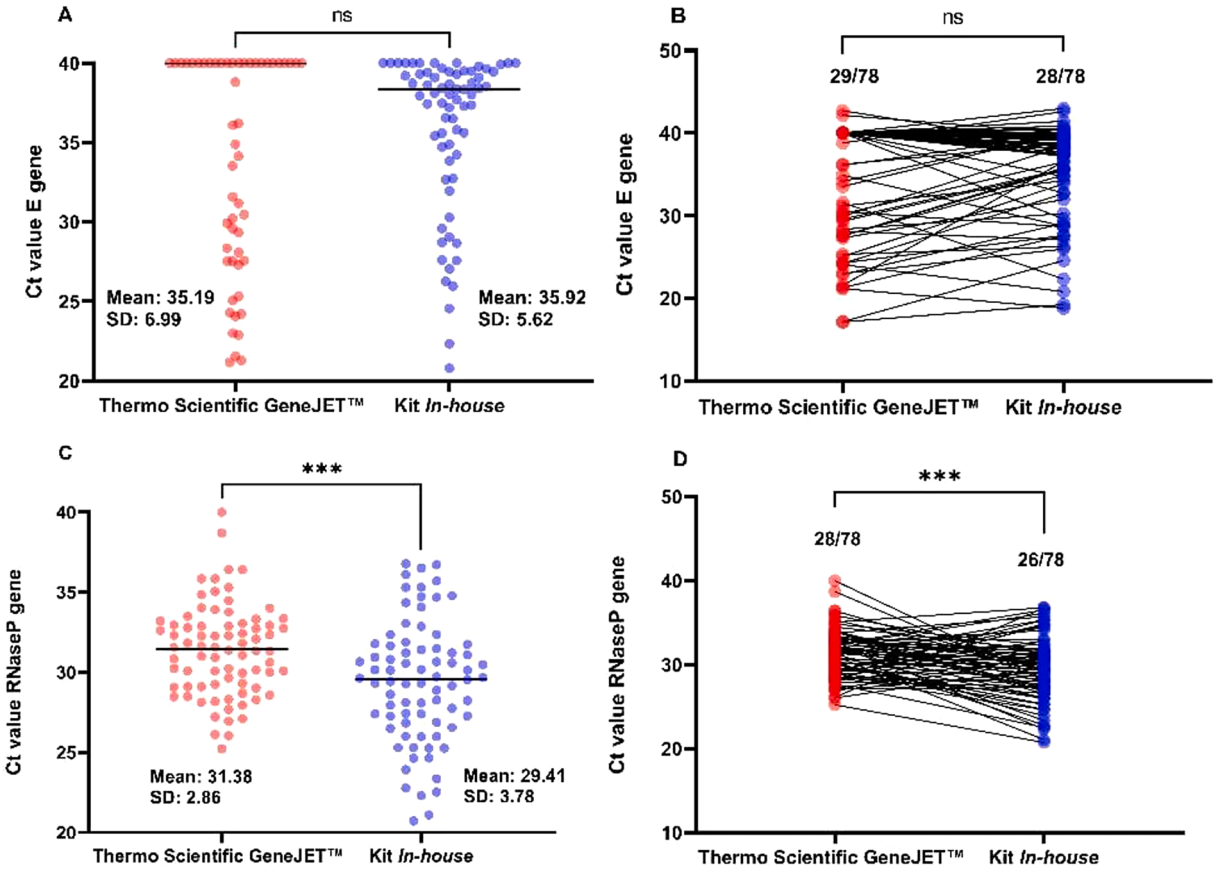

Fig. 3. Evaluation of RNA extracted by RT-qPCR to detect SARS-CoV-2 in nasopharyngeal swab samples. Data obtained from the in-house kit and the reference kit are presented. (A). Quantification of SARS-CoV-2 E gene amplification. (B). Positivity of E gene. (C). Quantification of RNase P gene amplification (internal control). (D). Positivity of RNase P gene.** and ***Statistically significant ($p < 0.001$ and $p < 0.05$, respectively). [ns]Statistically non-significant. [SD]Standard deviation.

cells and the virus nucleocapsid without affecting its integrity under optimal pH conditions, making them useful for virus inactivation (Martinez-Bravo et al., 2020; Park et al., 2022). The lysis buffer used in this study, which was based on GITC, allowed the necessary amounts of RNA to be recovered for subsequent molecular analyses. Therefore, this extraction technique using GITC improves recovery via precipitation with isopropanol (Farrell, 1993).

On the one hand, the 260/280 ratio is a critical step for the quality of the extracted RNA (Palacio Rua et al., 2022). This ratio indicates the purity of the RNA sample, and the optimal ratio was 1.8 – 2.2. A lower ratio suggests the presence of contaminants, such as proteins, phenol, or other substances that strongly absorb at 280 nm or close to 280 nm (Al-Adsani et al., 2022; Green and Sambrook, 2020). The results of this study suggest that both extraction methods produced total RNA of optimal purity despite the low RNA yield typically associated with the GeneJET kit. RNA extraction quality using the *in-house* kit is promising and suggests that it may be appropriate for use in scientific research. The *in-house* kit was efficient for extracting total RNA with optimal quality and relatively low cost compared to the commercial kit. It is important to note that these results were obtained under laboratory conditions, and may vary depending on the experimental conditions and equipment used. This study demonstrates the importance of optimizing RNA extraction protocols for each laboratory and sample type and how an in-house method can provide promising results similar to those of a commercial kit. Other studies have shown that higher amounts of RNA can be obtained using TRIzol® than using silica columns (Palacio Rua et al., 2022). Despite not using purification microcolumns or magnetic beads related to RNA, the standardized protocol for the *in-house* kit allows obtaining total RNA by alcohol precipitation without the presence of contaminants that can inhibit molecular techniques, such as real-time PCR (Abbasi et al., 2023; Khyati et al., 2024). In other words, this protocol is not a single-step single-tube method and requires the use of plastic materials. However, it involves fewer steps and is capable of recovering RNA from nasopharyngeal swabs in the absence of columns. The reagents included in this kit support an environment-friendly process, whereas the use of column extraction kits contributes to increased environmental contamination.

In contrast, during the SARS-CoV-2 pandemic, one of the most sensitive markers for the diagnosis of COVID-19 was the amplification of the E gene (Rahimian et al., 2023). Therefore, in this study, amplification of the E gene was used to determine the biological activity of the components of the *in-house* kit. As a result, the detection and amplification of E was achieved. It was observed that the Ct values of the E gene were lower for the *in-house* kit, indicating that the kit works effectively in diagnosing patients with COVID-19 (Palacio Rua et al., 2022), results show that the E gene is widely used because it is more sensitive, stable, and has a lower mutation rate. The SARS-CoV-2 E gene is recommended as a first-line diagnostic tool (Abbasi et al., 2023). The present study obtained adequate diagnostic accuracy using *in-house* kits. Based on the sensitivity and specificity results of this kit (Fig. 4), it can be inferred that this phenol-free *in-house* kit can be used for the molecular diagnosis of SARS-CoV-2 with optimal precision. Other studies have reported similar sensitivity and specificity values to those reported in the present study (Khyati et al., 2024; Park et al., 2022).

Interestingly, the assignment of three different SARS-CoV-2 lineages was determined from the samples extracted using an *in-house* kit and then sequenced. This highlights the importance of extracting RNA with this kit after allowing the application of the extracted RNA to genomic sequencing studies. This application shows the wide possibility of this kit providing crucial epidemiological information, referring to genetic

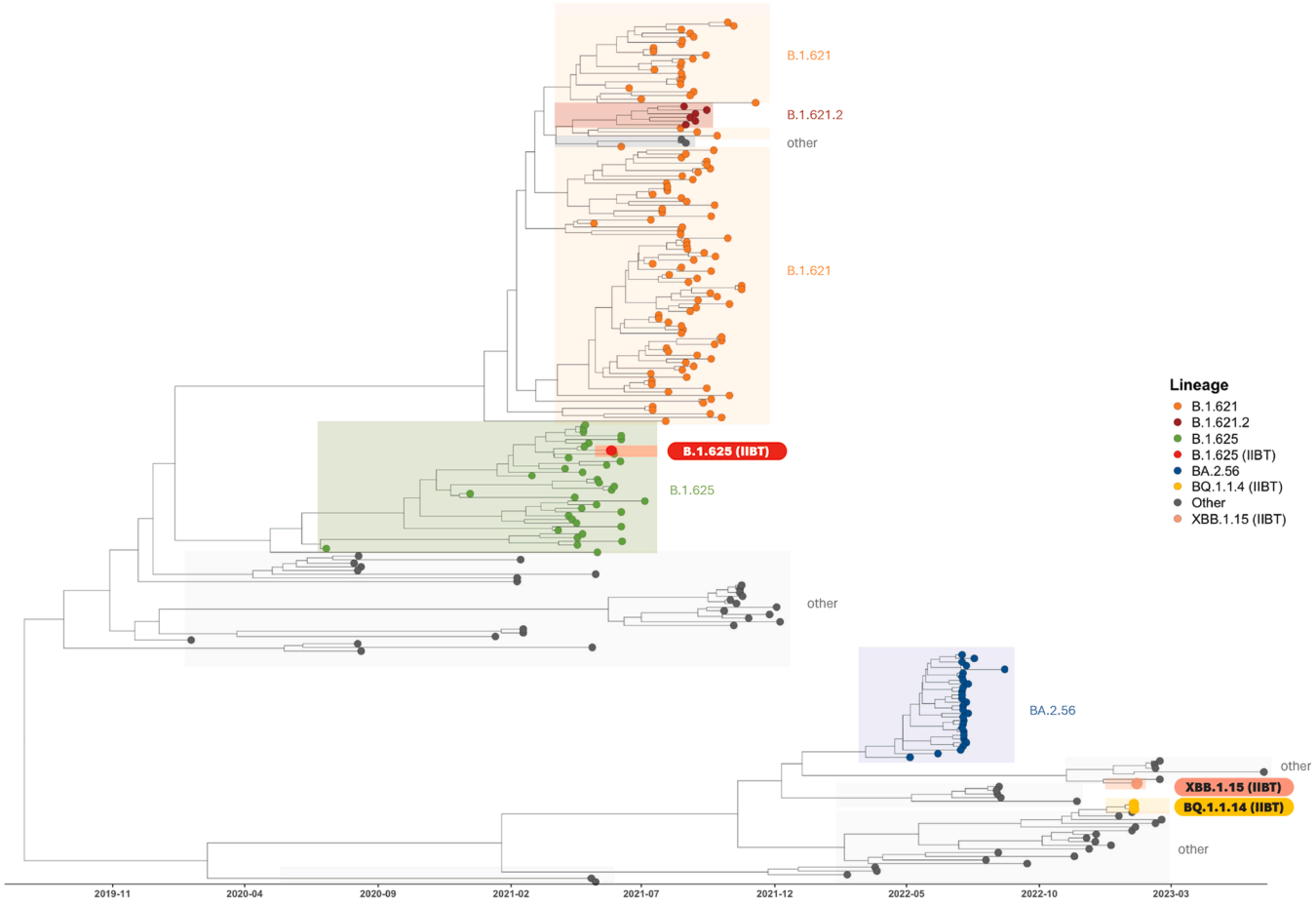

Fig. 4. Phylogenetic relationships between the genomes sequenced by NGS (Illumina ®) from samples extracted using an in-house kit for SARS-CoV-2 RNA extraction. Alignment was performed between the SARS-CoV-2 sequences reported in GISAID from the department of Córdoba-Colombia and the reference sequence NC_045512.

Table 2
Results of the 2 × 2 matrix for COVID-19 diagnosis and SARS-CoV-2 E gene detection using an in-house kit.

Assay kit *in-house*	COVID-19 (+)	COVID-19 (-)	Total
Gene E (+)	25	3	**28**
Gene E (-)	4	46	**50**
Total	**29**	**49**	**78**

Table 3
Sensitivity and specificity of the in-house assay kit for the detection of SARS-CoV-2.

Assay kit *in-house*			Accuracy		
Evaluation	Results (%)	IC95	Mean	x	n
Sensitivity	86.21	0.6834–0.9611	0.8621	25	**29**
Specificity	93.88	0.8313–0.9872	0.9388	46	**49**
PPV	89.29	0.7177–0.9773	0.8929	25	**28**
NPV	92.00	0.8077–0.9778	0.9200	46	**50**
False positives	6.12	**IC95**: Statistical confidence index 95 %			
False negatives	13.79				

changes in the virus, which include phenotypic aspects, as demonstrated in other genomic surveillance studies (Capriotti et al., 2024). After all, using commercial kits for RNA extraction is expensive; in particular, column-based kits are only considered economically suitable for processing some samples (Kalikiri et al., 2023). In other words, the *in-house* kit is a low-cost RNA extraction method for the detection of infectious

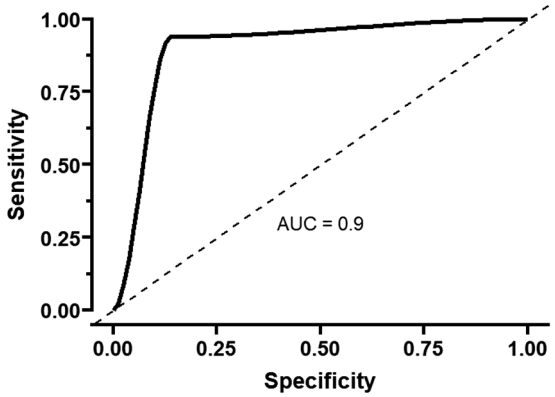

Fig. 5. Relative sensitivity and specificity analysis area under the receiver operating characteristic curve (AUC-ROC) of the in-house extraction kit.

agents. The results obtained in the design and evaluation of the *in-house* kit not only contribute to the knowledge of an appropriate method for RNA purification under laboratory conditions but also provide a cost-effective solution for detecting SARS-CoV-2.

The data from this study support the notion that generation of this biotechnological product could contribute to biotechnological independence and health sovereignty in Colombia. In addition, controlling the limitations related to the importation processes of this type of reagent for molecular diagnosis could help contain the spread of biological

agents at the local or national level. Thus, the high spread of the virus during the pandemic has caused a shortage of supplies and, in turn, the need to increase the local availability of reagents for the molecular diagnosis of SARS-CoV-2. The development of an *in-house* RNA extraction kit is a step towards overcoming these challenges and achieving biotechnological independence.

In conclusion, the *in-house* extraction method was shown to be efficient in obtaining purified RNA, as well as optimal genetic material for molecular diagnostic testing and sequencing of SARS-CoV-2 from nasopharyngeal swabs. The molecular detection of SARS-CoV-2 was demonstrated using a safe and low-cost method compared with commercially available methods. These results are critical for strengthening health and biotechnological sovereignty in Colombia, and highlight the efficiency and reliability of the phenol-free *in-house* kit.

CRediT authorship contribution statement

Torres Karina: Writing – original draft, Methodology, Investigation. **Guzmán Camilo:** Investigation, Conceptualization. **Ballesteros-Villamizar Jolaime:** Methodology, Investigation. **Garay Evelin:** Data curation. **Flórez Luis:** Methodology. **Gastelbondo-Pastrana Bertha:** Writing – original draft. **Serrano-Coll Héctor:** Writing – review & editing, Methodology, Conceptualization. **Arrieta Germán:** Visualization, Validation, Supervision. **Pájaro-Castro Nérlis:** Validation, Software, Methodology. **Martínez Caty:** Formal analysis, Conceptualization. **Mattar Salim:** Supervision. **De la Hoz Daniel Echeverri:** Visualization, Validation, Software, Formal analysis, Data curation. **Araújo Jr João Pessoa:** Writing – review & editing, Validation, Supervision. **Gutierrez Rosa:** Visualization, Methodology, Investigation. **Contreras Héctor:** Methodology, Investigation. **López Yésica:** Data curation, Conceptualization. **Rivero-Herrera Ricardo:** Writing – review & editing, Visualization, Validation. **Arroyo-Salgado Bárbara:** Validation, Supervision. **Hurtado Eliana:** Writing – original draft, Visualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

Acknowledgments

The authors thank to Ministerio de Ciencia, Tecnología e Innovación, Colombia (MINCIENCIAS). Research project financied: “Aportes a la independencia y seguridad biotecnológica a través del desarrollo de herramientas diagnósticas como iniciativa de I+D para atender problemáticas asociadas a SARS-CoV-2 y otros agentes biológicos de alto riesgo en la región Caribe” code 84730 – CT631–2021. The Instituto de Investigaciones Biológicas del Trópico (Universidad de Córdoba) and Institute of Biotechnology (IBTEC-UNESP) (Botucatu, Brazil) for their support.

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