

Implementation of FTA Elute Card for Viral RNA Storage and Cost-Effective Wastewater Surveillance

Qingxin Hui, Yuwei Pan, David Cullen, and Zhugen Yang*

Wastewater-based epidemiology (WBE) has emerged as a significant tool for early infection detection during the COVID-19 pandemic, enabling timely diagnosis and effective control. However, conventional methods for sample transport and storage rely on cold chain logistics, which increase costs and limit accessibility. This study presented a portable and user-friendly strategy for room-temperature storage of RNA in wastewater using FTA Elute cards. Quantitative reverse transcription polymerase chain reaction (RT-qPCR) analysis demonstrated a 15.4% recovery of the original RNA with cards, compared to only 0.5% recovery without cards. Stability assessments under room temperature, -4 °C, and -20 °C confirmed that RNA retained detectability for over one month, with enhanced preservation at lower temperatures. Furthermore, FTA Elute cards exhibited good recovery for SARS-CoV-2, influenza A, and influenza B in spiked wastewater. This work provides a promising solution for RNA preservation and transport in environmental matrices without cold chain dependency.

1. Introduction

The coronavirus disease 2019 (COVID-19) pandemic highlighted the critical need to monitor the spread of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).^[1–3] Wastewater-based epidemiology (WBE) has emerged as a powerful complementary approach to clinical diagnostics, enabling population-level tracking of viral transmission.^[4–6] Most SARS-CoV-2 infections are asymptomatic or present with mild symptoms, making clinical testing often unavailable.^[7] WBE overcomes this limitation by analyzing pooled wastewater samples, providing an early estimate of undetected infections.^[8] Recent studies have confirmed

the presence of SARS-CoV-2 in stool, urine, and wastewater,^[9–11] and research evidence suggests that wastewater viral signals often precede clinical case reports.^[12] Therefore, WBE can serve as an early warning system for epidemics, particularly in resource-limited settings.^[13,14]

Despite the great potential of WBE, its widespread implementation still faces several challenges. Current RNA detection relies on nucleic acid methods, primarily reverse transcription-quantitative polymerase chain reaction (RT-qPCR).^[15–17] Since individual clinical testing is not always economically feasible, WBE offers a cost-effective alternative for community-level monitoring.^[18] However, the process of sample collection, storage, and transport often introduces logistical constraints.^[19]

A key limitation is the need for cold chain logistics to preserve viral RNA integrity.^[20–22] Collecting and transporting large volumes of wastewater under refrigerated conditions increases costs and reduces sampling frequency. Therefore, there is an urgent need for room-temperature storage solutions that can maintain RNA stability without compromising analytical accuracy.

Recent advancements in sample preservation present a promising avenue to address these limitations. Flinders Technology Associates (FTA) cards have gained increasing attention for their potential in RNA stabilization.^[23–25] FTA cards utilize a proprietary chemical matrix, including denaturants and free radical scavengers, that can be impregnated into filter paper to lyse cells, inhibit microbial growth, and immobilize nucleic acids.^[26] The embedded RNA remains stable for long periods even at room temperature.^[27] FTA cards have been successfully adapted for detecting diverse pathogens, including human papillomavirus,^[28] avian influenza virus,^[29] dengue virus,^[30] infectious bronchitis virus,^[31] and foot-and-mouth disease virus.^[32] In addition, the stabilization of RNA remains challenging due to its single-stranded structure and relative instability of ribose sugars compared to deoxyribose sugars.^[33] This challenge is particularly evident in wastewater analysis, where complex matrices and limited preservation techniques exacerbate RNA degradation. It was reported that RNA immobilized on FTA cards could still be detected by RT-qPCR after three weeks of environmental storage.^[34] However, wastewater contains diverse chemical and biological constituents that may interfere with RNA stability.^[35] Therefore, an evaluation of FTA card performance in wastewater

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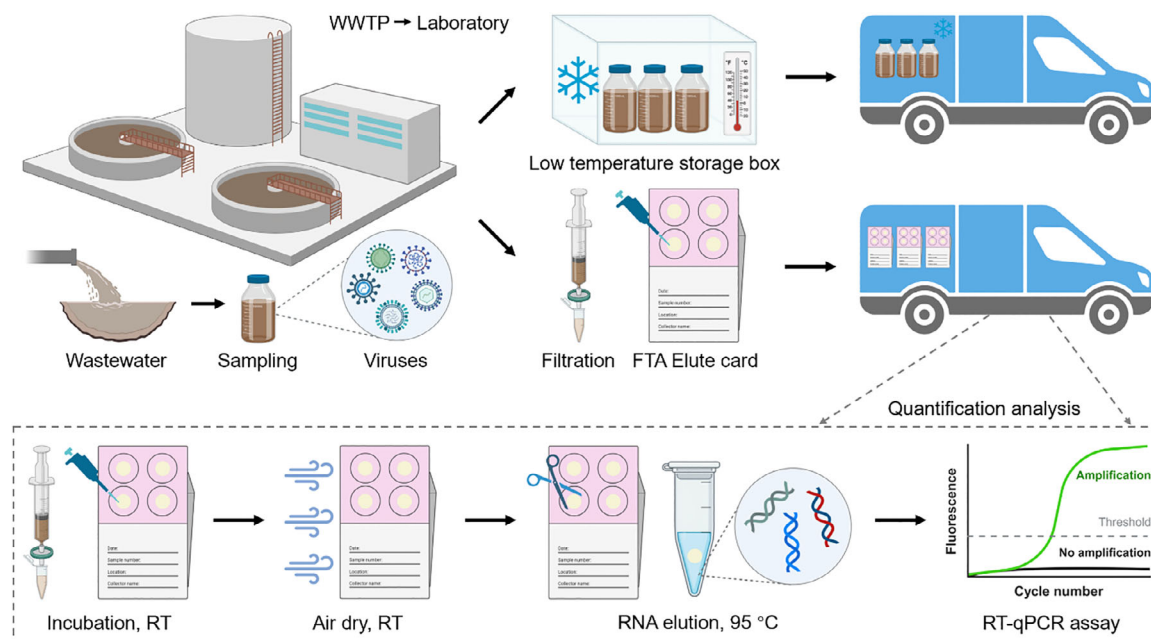


Figure 1. Schematic illustration of conventional and FTA Elute card-based wastewater sample transport methods. Conventional method: Large-volume samples are stored in bottles and transported at low temperatures for sample analysis. FTA Elute card-based method: Small-volume samples are adsorbed onto FTA Elute cards at room temperature, enabling transport without refrigeration prior to sample analysis. For wastewater analysis, wastewater was applied to the card sampling site and incubated at room temperature to facilitate RNA adsorption. The card was air-dried after excess liquid was removed. RNA was subsequently eluted by incubation in buffer at 95 °C and subjected to RT-qPCR analysis. WWTP, wastewater treatment plant; RT, room temperature.

ter is essential to validate their feasibility in real-world applications.

In this work, we presented a portable and user-friendly strategy for room-temperature storage of RNA in wastewater using FTA Elute cards. Following concentration via polyethylene glycol (PEG) precipitation and syringe filtration, wastewater samples were applied to FTA Elute cards. The long-term storage stability of RNA on FTA Elute cards was investigated under three temperature conditions (room temperature, 4 °C, and −20 °C) to determine optimal storage protocols. The recoveries of SARS-CoV-2, influenza A, and influenza B RNA were evaluated from various wastewater samples stored on FTA Elute cards, demonstrating the feasibility for long-term room-temperature storage in wastewater. FTA Elute cards offer a cold chain-independent solution for the preservation, storage, and transport of RNA in environmental matrices, which demonstrates significant potential for monitoring emerging pathogens.

2. Results and Discussion

2.1. Optimization of RNA Elution Method for FTA Elute Card

Molecular diagnosis of viruses presents significant challenges, often requiring cold chain maintenance during sample transport.^[36] FTA Elute cards can inactivate viruses and stabilize nucleic acids, which have been recognized by the World Health Organization (WHO) and the U.S. Centers for Disease Control and Prevention (CDC).^[37,38] This study presented a strategy for room-temperature storage of RNA in wastewater using FTA Elute cards. **Figure 1** illustrates the workflow for wastewater collection,

storage, transport, purification, and nucleic acid detection. FTA Elute cards rely on a proprietary chemical mixture immobilized on filter paper, consisting of denaturants and free radical scavengers, which facilitates the long-term preservation of nucleic acids.^[27] This mixture not only lyses cells and organelles but also inhibits bacterial growth and denatures proteins. A critical step in this process involves isolating nucleic acids from the cards following sample lysis and protein denaturation.

To optimize RNA isolation from FTA Elute cards, SARS-CoV-2 N gene RNA was released by immersing the cards in elution buffer and quantified by RT-qPCR. **Figure 2a** demonstrated the differences in elution efficiency between 500 µL Tris-acetate-EDTA (TAE) buffer and 500 µL nuclease-free water. The lower cycle threshold (C_t) value observed with TAE buffer indicated better RNA elution compared to nuclease-free water. **Figure 2b** further revealed that elution volumes of 500 and 1000 µL produced comparable C_t values, suggesting that increasing elution volume did not enhance RNA recovery. However, when elution volume was reduced to 400 µL, target RNA could no longer be detected. Considering the balance between efficiency and cost-effectiveness, 500 µL TAE buffer was selected for RT-qPCR assay.

2.2. Assessment of Viral RNA Stability on FTA Elute Card

To evaluate the efficacy of FTA Elute cards in stabilizing RNA, three replicates were performed for each storage condition. In **Figure 3a**, an FTA Elute card contained a spiked wastewater sample containing target RNA (N gene), while a micro-

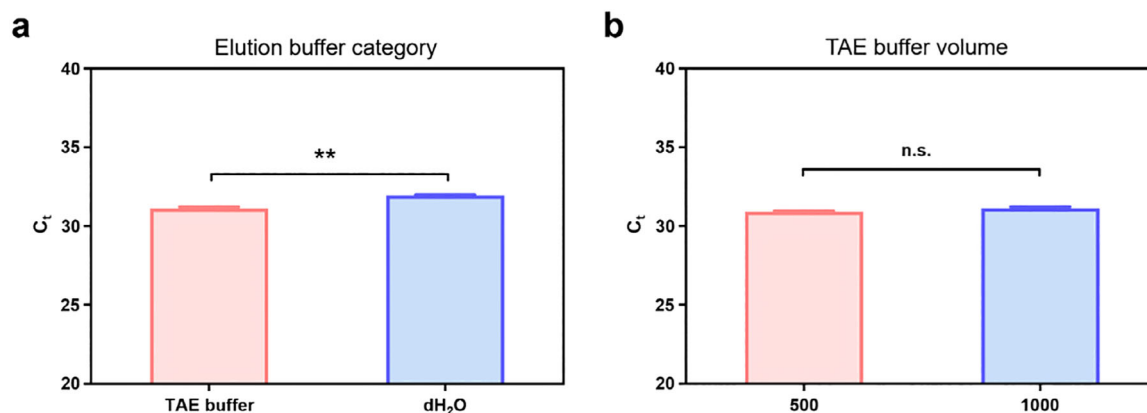


Figure 2. Optimization of RNA elution from FTA Elute cards. a) Comparison of elution efficiencies using TAE buffer versus nuclease-free water. b) Evaluation of elution volume effects (500 µL vs 1000 µL). Error bars represent mean $\pm$ SD of triplicate measurements.

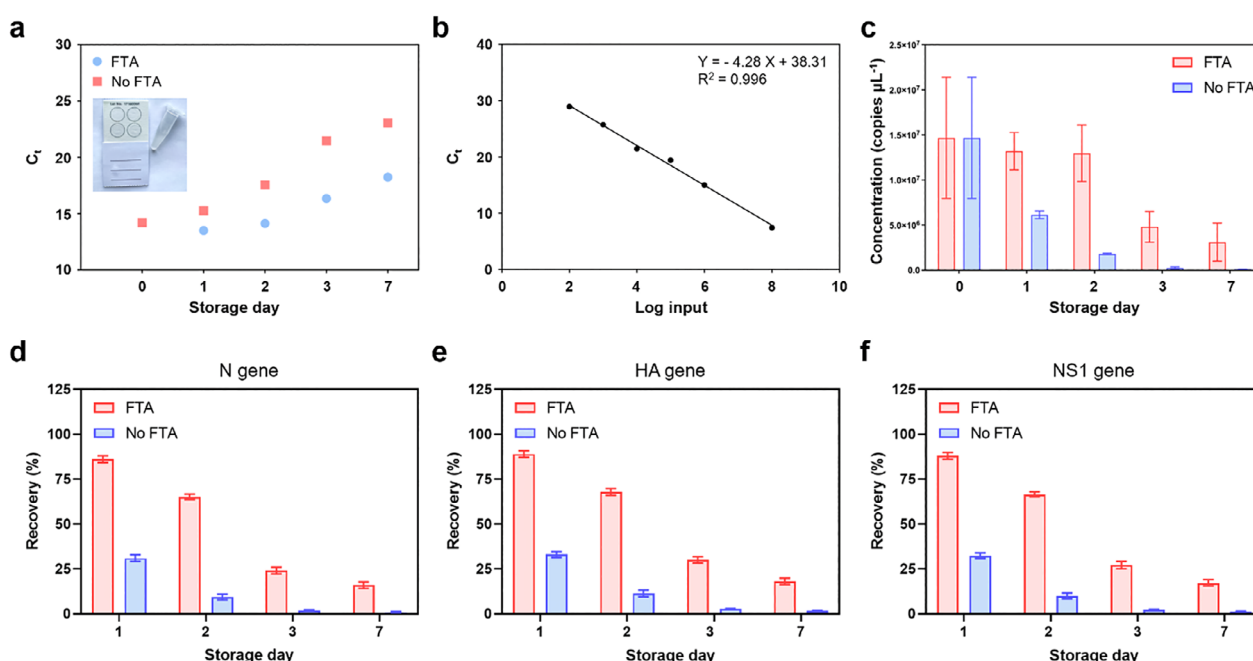


Figure 3. RNA stability and recovery on FTA Elute card. a) Comparative RT-qPCR analysis of RNA stability between FTA Elute cards and non-FTA controls ($n = 3$). The photograph shows samples from an FTA Elute card and a non-FTA control. b) Standard curve for RT-qPCR quantification ($R^2 = 0.996$). c) Comparison of RNA recovery from FTA Elute cards versus controls. d–f) Recovery rates (%) for (d) SARS-CoV-2 N gene, (e) influenza A HA gene, and (f) influenza B NS1 gene ($n = 3$). Error bars represent mean $\pm$ SD.

centrifuge tube contained the same sample. The RNA concentrations were derived from the RT-qPCR standard curve (Figure 3b). The C_t values for both groups increased with storage duration, with a more pronounced rise in the tube-stored samples. Since C_t values were inversely proportional to RNA concentrations, the amount of recoverable RNA decreased over time. However, the rate of RNA degradation varied significantly between different storage media (Figure 3c). RNA recovery was calculated by comparing daily RNA concentrations with initial measurements (Figure 3d). After 24 h at room temperature, less than 33% of target RNA remained detectable in the tube, whereas 85.0% was recovered from the card. By day 7, only 15.4% of target RNA remained on the card,

while target RNA stored in the tube was almost completely degraded.

To validate the broader applicability of FTA Elute cards, similar experiments were performed using influenza A and influenza B RNA. Consistent with the N gene results, RNA recovery from FTA Elute cards was significantly higher than that from RNA stored in tubes. After 7 days, $\approx 2\%$ of target RNA remained detectable on the card, whereas the tube samples yielded negligible recovery. These findings demonstrated the feasibility of FTA Elute cards to enhance RNA stability at room temperature without the need for refrigeration. The superior preservation performance may be attributed to two key factors. First, the proprietary chemical formula on the card may inactivate degradative en-

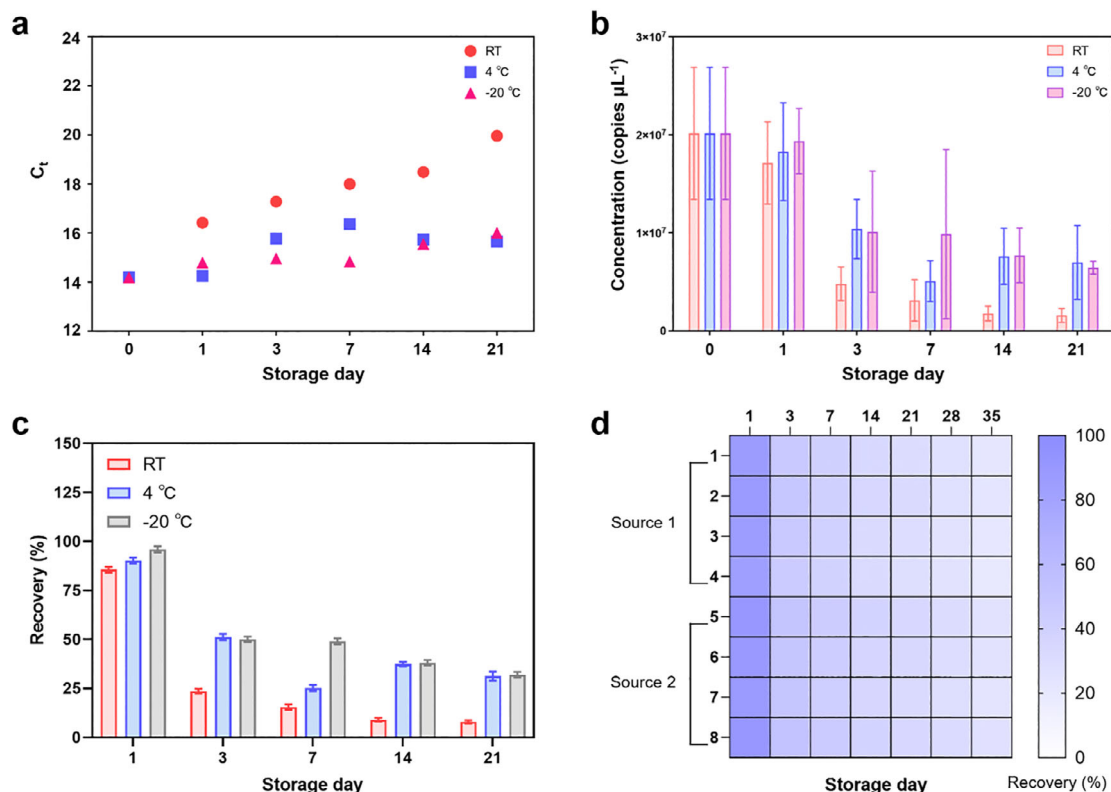


Figure 4. Temperature- and time-dependent stability of RNA on FTA Elute card. a) Comparative RT-qPCR analysis of RNA stability at room temperature, 4 °C, and −20 °C ($n = 3$). b) Comparison of RNA concentrations at room temperature, 4 °C, and −20 °C ($n = 3$). c) Recovery rates (%) of N gene at room temperature, 4 °C, and −20 °C ($n = 3$). d) Recovery rates (%) of N gene in wastewater samples 1–4 (Anglian Water) and wastewater samples 5–8 (Cranfield WWTP) at 4 °C ($n = 3$). Error bars represent mean $\pm$ SD.

zymes in wastewater, thereby preventing RNA degradation. Second, target RNA was immobilized within the dense fiber matrix of the filter paper, which physically protected nucleic acids from degradation.

2.3. Evaluation of FTA Elute Card Stability under Different Storage Conditions

To assess the applicability of FTA Elute cards for RNA storage, stability studies were performed across room temperature (20–25 °C), refrigeration (4 °C), and freezing (−20 °C). The C_t values of all samples increased with storage time, but this trend was most obvious in the room temperature group, reflecting the accelerated RNA degradation at room temperature (Figure 4a). After 3 days, the C_t values in refrigerated and frozen samples stabilized near 15, whereas room temperature samples presented an increase to 20. The C_t values of the 4 °C group increased transiently on day 7 and then decreased on day 14, which may reflect variability in elution efficiency rather than RNA degradation, as this group required longer processing time.

Quantitative analysis of RNA recovery (Figure 4b,c) revealed that FTA Elute cards retained $\approx 50\%$ of N gene RNA after 2 weeks at 4 °C, compared to 15.4%, 8.8%, and 7.8% after 1, 2, and 3 weeks at room temperature, respectively. These findings aligned with prior reports of 0–25% RNA recovery from FTA Elute cards af-

ter 3 weeks.^[34] Superior preservation was demonstrated at lower temperatures, with 34.6% and 32.0% recovery after 3 weeks at 4 °C and −20 °C, respectively, indicating the inhibitory effect of low temperatures on RNase activity.

To evaluate long-term storage, RNA stability was analyzed in diverse wastewater matrices over one month (Figure 4d). Recovery rates of cards stored at 4 °C decreased over time but remained detectable ($\approx 20\%$) even after one month. It was noted that the recovery rates of samples from Anglian Water (municipal wastewater; samples 1–4) were lower than those from Cranfield wastewater treatment plant (WWTP) (community scale; samples 5–8), likely due to the differences in compositional complexity. Municipal wastewater typically contains higher loads of organic interferences and particulate matter, which may compete with RNA for binding sites on FTA Elute cards. In contrast, the simpler matrix of community wastewater may reduce such interference, consistent with studies linking matrix complexity to nucleic acid stability.^[9] This matrix-dependent variation highlighted the need for wastewater matrix validation.

2.4. Evaluation of FTA Elute Card for RNA Detection in Wastewater

Actual wastewater samples were treated with FTA Elute cards and analyzed by RT-qPCR assay to evaluate the feasibility of FTA

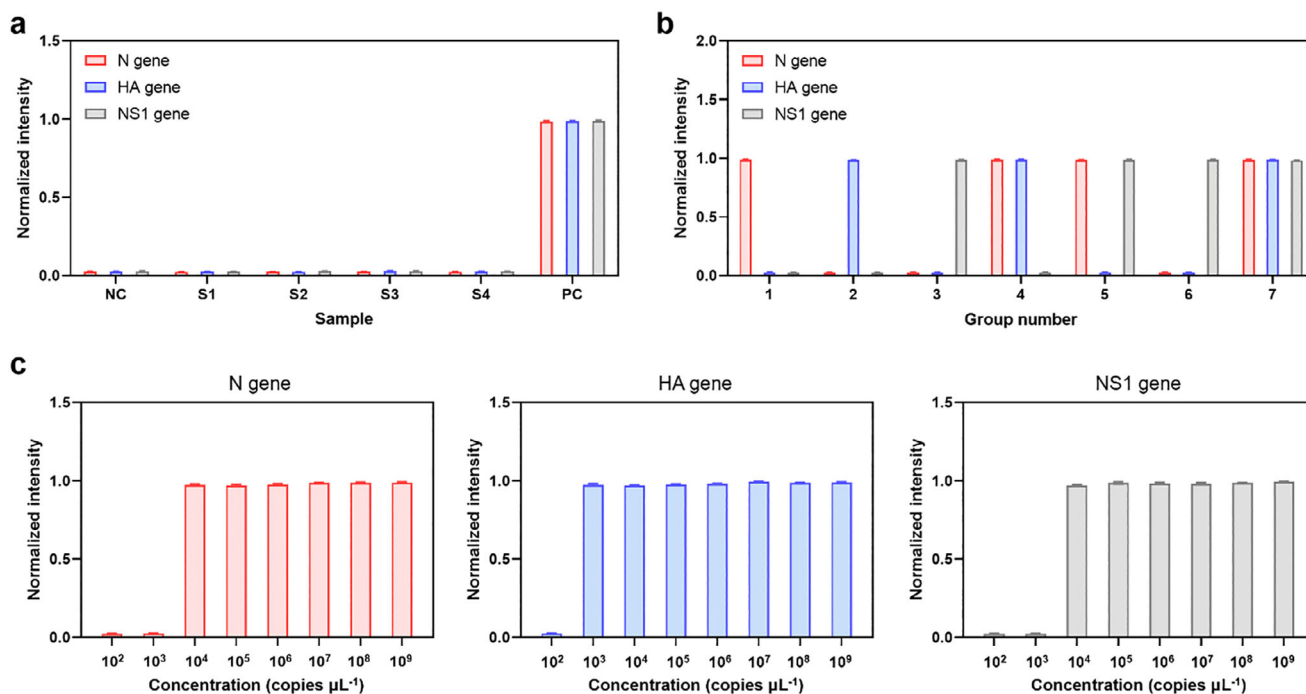


Figure 5. Performance evaluation of FTA Elute card-based RNA detection in wastewater samples. a) RT-qPCR analysis of RNA recovered from FTA Elute cards, including a negative control (NC), four samples (S1–S4), and a positive control (PC). b) Multiplex detection capability demonstrated using spiked wastewater samples containing various combinations of N, HA, and NS1 RNA targets: 1, N RNA; 2, HA RNA; 3, NS1 RNA; 4, N + HA RNA; 5, N + NS1 RNA; 6, HA + NS1 RNA; 7, N + HA + NS1 RNA. c) RT-qPCR analysis of N, HA, and NS1 RNA targets recovered from FTA Elute cards in spiked wastewater samples across six orders of magnitude (10^3 – 10^9 copies μL^{-1}).

Elute cards. It was noted that no N, HA, or NS1 RNA targets were detected in any samples (Figure 5a). The positive control confirmed the functionality of the assay through the amplification curve. This discrepancy likely stemmed from the low viral RNA concentration in wastewater (60 copies μL^{-1}), which was several orders of magnitude lower than the spiked samples (10^9 copies μL^{-1}). Additional RNA losses during loading and elution further exacerbated the challenge of wastewater analysis. Experiments using spiked wastewater samples containing combinations of N, HA, and NS1 RNA targets demonstrated the multiplex detection capability of FTA Elute card strategy (Figure 5b).

To determine the sensitivity of FTA Elute cards, wastewater samples were spiked with serial dilutions of RNA (10^3 – 10^9 copies μL^{-1}). The strategy achieved a detection limit of 10^3 – 10^4 copies μL^{-1} for SARS-CoV-2, influenza A, and influenza B (Figure 5c), which aligned with prior studies reporting enteroviruses on FTA cards.^[39] The reduced sensitivity for low-concentration samples can be attributed to the binding kinetics of nucleic acids to the FTA Elute card matrix.^[40–42] The proprietary chemical reagents (e.g., guanidine salts and chelating agents) can efficiently lyse virions and immobilize nucleic acids at moderate to high concentrations.^[43] However, at low concentrations, the probability of RNA molecules encountering active binding sites on the matrix may be reduced. This limitation emphasized the need for preconcentration when applying FTA Elute cards to actual wastewater samples.

3. Conclusion

In this work, a portable and user-friendly strategy was developed for room-temperature storage of RNA in wastewater using FTA Elute cards. Although refrigeration (4 °C) and freezing (–20 °C) storage conditions optimized RNA preservation, FTA Elute cards still maintained RNA stability for over one month at room temperature, eliminating the need for cold-chain transport. This preservation capability was consistent across multiple viruses, including SARS-CoV-2, influenza A virus, and influenza B virus, highlighting the versatility of FTA Elute cards for RNA stabilization. Such features positioned FTA Elute cards as a valuable tool for the identification of viral pathogens in wastewater. However, it's noted that virus detection in actual wastewater samples remained challenging due to the low viral loads. Effective recovery highlighted the necessity of preconcentration when analyzing environmental samples with low viral loads. Further field testing will be performed to validate FTA Elute card's feasibility across diverse climates and logistical scenarios. With low cost, simple operation, and preservation performance, FTA Elute cards will facilitate the storage and transport of samples in remote areas, thus improving the feasibility of pathogen monitoring.

4. Experimental Section

Materials and Reagents: FTA Elute card, RNase-free water, and phosphate-buffered saline (PBS) were purchased from Sigma-Aldrich

(USA). RNA extraction was performed using the QIAamp Viral RNA Mini Kit (Qiagen, Germany), while RT-qPCR assay was performed with the AgPath-ID One-Step RT-PCR Kit (Thermo Fisher Scientific, UK). Synthetic RNA templates were generated using the HiScribe T7 High-Yield RNA Synthesis Kit and purified with the Monarch RNA Cleanup Kit (New England Biolabs, UK). Custom oligonucleotides, including primers, probes, and gene fragments, were synthesized by Eurofins Scientific and Sigma-Aldrich. Chemicals were of analytical grade unless Periodicalwise specified.

Preparation of Target RNA Templates: The analytical performance of FTA Elute cards was evaluated using N, HA, and NS1 RNA targets. At first, a linear DNA template containing the target gene sequence flanked by a T7 promoter was amplified by PCR, digested with restriction enzymes, and purified with cleanup reagents. A 20 µL in vitro transcription reaction mixture was incubated at 37 °C for 2 h, consisting of DNA template, T7 RNA polymerase, nucleoside triphosphates (NTPs), and reaction buffer. To remove residual DNA, 1 µL RNase-free DNase I was added, followed by incubation at 37 °C for 15 min. The transcribed RNA was subsequently purified and eluted in RNase-free water. Finally, the RNA was stored at −20 °C until further analysis.

Wastewater Sample Collection and Processing: Wastewater samples were collected from Anglian Water (collected in 2020, representing urban areas with mixed residential, commercial, and industrial discharges) and Cranfield WWTP (collected in 2022, primarily serving residential areas). Samples were collected using an autosampler over a 24-h period and transported to the laboratory on ice to preserve sample integrity. Upon arrival, samples were centrifuged at 3000 × g for 10 min at 4 °C to remove large suspended solids. The resulting supernatant was stored at −20 °C until further analysis. For downstream applications, wastewater samples were applied to FTA Elute cards and processed later, followed by RNA quantification via RT-qPCR assay. Wastewater samples used for stability study are provided in Table S1 (Supporting Information).

Preparation of FTA Elute Card: The FTA Elute card protocol was optimized for wastewater analysis by modifying the manufacturer's instructions. To maximize RNA binding efficiency, each sampling spot on the card was pretreated with 200 µL PBS and incubated at room temperature for 5 min. The PBS was then aspirated using a pipette to prevent physical disruption of the card matrix. The pretreated card was air-dried in the fume hood for 30 min to eliminate residual moisture and preserve the functional integrity of the card's embedded chemistries. Finally, the dried card was sealed in the foil bags and stored at varying temperatures until further analysis.

RNA Elution from FTA Elute Card: For spiked wastewater samples, RNA was added to a final concentration of 10⁹ copies µL^{−1}. A 20 µL aliquot was applied to each sampling spot and incubated at room temperature for 2 h to facilitate RNA adsorption. Excess liquid was removed, and the card was air-dried for 10 min. Afterward, sampling spots were excised using sterile scissors (pre-autoclaved at 121 °C for 20 min) and transferred into 1.5 mL RNase-free microcentrifuge tubes. Next, each sampling spot was washed twice with 200 µL RNase-free water to remove unbound contaminants. After elution buffer was added to the tube, the mixture was vortexed for 15 s, incubated at 95 °C for 30 min, and vortexed for 15 s. Finally, the mixture was centrifuged at 15000 × g for 10 min to pellet debris. The RNA-containing supernatant was stored at −20 °C until further analysis.

RT-qPCR Quantification of Eluted RNA: RNA eluted from FTA Elute Card was quantified using RT-qPCR. Primer and probe sequences for N, HA, or NS1 RNA targets are provided in Table S2 (Supporting Information). Reactions were performed in 25 µL, containing 12.5 µL 2 × RT-qPCR buffer (with ROX reference dye for fluorescence normalization), 1 µL enzyme mix (ArrayScript Reverse Transcriptase and AmpliTaq Gold DNA Polymerase), 0.5 µL each of forward and reverse primers (10 µM), 0.25 µL probe (10 µM), 5 µL template RNA, and 5.25 µL RNase-free water. RT-qPCR amplification was performed on a CFX96 Touch Real-Time PCR System, involving reverse transcription (45 °C, 10 min), initial denaturation (95 °C, 15 min), 40 cycles of denaturation (95 °C, 15 s) and annealing/extension (60 °C, 60 s). Quantification was achieved using a standard curve generated

from serial dilutions of in vitro transcribed RNA. Recovery was calculated as:

$$\text{Recovery (\%)} = (\text{Measured RNA copies} / \text{Incorporated RNA copies}) 100\% \quad (1)$$

Statistical Analysis: All experiments were performed in triplicate, with data expressed as mean ± standard deviation (SD). Each RT-qPCR run included positive and negative controls to ensure assay validity. Baseline and threshold values were maintained for consistency. Viral RNA copy numbers were determined by extrapolation from a standard curve based on the mean C_t values. Comparative statistical analysis of RNA recovery between different wastewater samples was performed using an unpaired, two-tailed Student's *t*-test (GraphPad Prism).

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Q.H. and Y.P. contributed equally to this work. The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript. Q.H. and Z.Y. conceived the study and designed experiments. Q.H. performed experiments with the supervision of Z.Y., Q.H., Y.P., and Z.Y. wrote the manuscript with input from all co-authors. Z.Y. supervised and acquired funding for the study. D.C. provided support for technical development and edited the manuscript.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Keywords

FTA Elute card, influenza virus, RNA storage, SARS-CoV-2, wastewater-based epidemiology

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