Research Article

Impact of relative humidity on SARS-CoV-2 RNA extraction using Nextractor automated extraction system

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Abstract

This study investigated the influence of relative humidity (RH) on the efficiency of SARS-CoV-2 RNA extraction using the Nextractor automated system. Experiments employing clinical samples demonstrated satisfactory sensitivity and reproducibility for RNA extraction at low humidity (below 50% RH). Conversely, extractions at high humidity (above 70% RH) resulted in complete failure of reverse transcription-polymerase chain reaction assays, with neither SARS-CoV-2 RNA nor the human RNase P gene (internal control) detected. Analysis suggested that residual ethanol, incompletely evaporating due to high humidity, acted as a potent polymerase chain reaction inhibitor in these samples. These findings highlighted the importance of maintaining optimal laboratory humidity (<50% RH) for reliable SARS-CoV-2 RNA extraction using the Nextractor system. Furthermore, laboratories should implement strategies such as regular humidity monitoring, staff training on humidity's impact, and system validation under specific humidity conditions to ensure accurate molecular diagnostic workflows for COVID-19 testing.

Keywords: Humidity, COVID-19, RNA Extraction, SARS-CoV-2

1. INTRODUCTION

The COVID-19 pandemic has posed unprecedented demands on the operation of clinical and research laboratories [1]. Real-time reverse transcription-polymerase chain reaction (RT-PCR) diagnostics have emerged as the gold standard for detecting SARS-CoV-2, the virus culpable for COVID-19. To address the surge in testing needs, automation has become a cornerstone of efficient and high-throughput molecular diagnostic workflows [2,3].

The Nextractor NX-48 (Genolution Inc., Seoul, Korea) is an automated nucleic acid extraction system commonly used in conjunction with the NX-48S viral NA kit for the rapid isolation of SARS-CoV-2 RNA from clinical specimens such as nasopharyngeal (NP) swabs [4]. This walk-away system is capable of simultaneously extracting nucleic acid from up to 48 samples. This significantly improves laboratory workflow, especially in high-volume testing scenarios.

However, RNA extraction, a critical upstream step in the RT-PCR process, is significantly subject to various external factors, potentially leading to inaccurate results. No existing studies examined the impact of relative humidity (RH) on the RNA extraction efficiency of the Nextractor NX-48 system. This study specifically investigated the effect of RH on SARS-CoV-2 RNA extraction efficiency on the Nextractor NX-48 automated nucleic acid extraction system.

2. MATERIALS AND METHODS

To optimize SARS-CoV-2 RNA extraction workflows using the Nextractor automated system, this study looked at the influence of environmental RH on the extraction process. The Nextractor system's performance was evaluated under two controlled humidity conditions: a low-humidity environment maintained below 50% RH and a high humidity

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kept at over 70% RH. A digital hygrometer (Aceteo, 288-ATH, India), with a humidity range from 10% to 99% RH and an accuracy of ±3% RH, was used to monitor humidity within the controlled environment.

A total of 30 nasopharyngeal/throat swab specimens were tested, including 20 known SARS-CoV-2-positive samples, with their Ct values ranging from 16 to 35 and 10 known negative controls. The experiment strictly followed the manufacturer's instructions for the Nextractor NX-48 system (Genolution, Seoul, Korea) and NX-48S viral NA kit to ensure standardized processing across all samples.

Before extraction, each 200 µL sample mixture underwent thorough vortexing to promote homogenous mixing of the solution. Subsequently, the samples were transferred to their designated wells within an NX-48S viral NA kit plate. The Nextractor system then performed the automated RNA extraction according to the VN protocol, a default program of the Nextractor system for viral nucleic acid extraction from nasopharyngeal/throat swab specimens. The protocol possibly integrated such steps as buffering, enzymatic lysis of viral envelopes, and purification, among others. Importantly, the protocol also contained heating steps, as indicated by the activation of heating blocks within the Nextractor system. This heating is crucial for achieving optimal viral RNA extraction efficiency by enhancing enzymatic activity and promoting efficient target molecule capture.

Following extraction, the NanoDrop One Microvolume Ultraviolet–visible Spectrophotometer (Thermo Scientific, CA, USA) was used to quantify the total RNA concentration and measure the A260:A280 ratio of each sample, indicative of RNA purity. The integrity of extracted RNA, a critical factor for successful RT-PCR amplification, was subsequently evaluated. Real-time RT-PCR relies on the ability of the extracted RNA to be converted into complementary DNA (cDNA) and then amplified for detection. Here, the VIRALDTECT-II multiplex real-time polymerase chain reaction (PCR) kit (Genes2Me, India) was employed. This kit includes primers and probes specific for amplifying three key regions of the SARS-CoV-2 virus genome (E, N, and RdRp genes). The QuantStudio™ 7 Pro Real-Time PCR System (Applied Biosystems, CA, USA) served as the platform for running the RT-PCR reactions. Furthermore, the test incorporated an internal control, i.e., the human RNase P (RNP) gene. This internal control worked as a crucial check to ensure the functionality of the RT-PCR itself, independent of the presence of viral RNA.

3. RESULTS

Low humidity conditions (<50% RH) yielded satisfactory performance in terms of sensitivity and reproducibility (**Table 1**). This indicated that the Nextractor system effectively extracted RNA from clinical specimens at low humidity, allowing for accurate detection of SARS-CoV-2 using RT-PCR. Furthermore, the stable amplification of the internal control RNP gene across all samples in this group further supported the validity of the extraction process. The consistent RNA concentration and A260:A280 ratios further suggested that impurities in the eluted RNA were marginal, thereby ensuring optimal quality for downstream RT-PCR applications.

In stark contrast, the high humidity environment (above 70% RH) rendered the RNA extraction difficult. Most strikingly, the RT-PCR failed to detect SARS-CoV-2 RNA in all the positive control samples. This complete detection failure suggests a substantial compromise in the quality or quantity of the extracted RNA, making it unsuitable for RT-PCR. Similarly, the failure with the internal controls indicated a more systemic issue impairing the entire RT-PCR process, not just the viral target detection. Potential explanations include the degradation of RNA due to high humidity or the co-extraction of PCR inhibitors along with the RNA.

The observed inconsistency in total RNA concentration and A260:A280 ratios among samples in the high-humidity group further strengthened the notion that high humidity leads to incomplete or impaired extraction. This inconsistency might be ascribed to variable RNA yield and potential contamination with impurities that can alter the A260:A280 ratio.

4. DISCUSSION

The complete absence of amplification in the high-humidity group strongly suggests the presence of PCR inhibitors in the eluted RNA samples. Our investigation revealed that the association between humidity and RNA extraction might be attributed to the residual ethanol in the extracted RNA. Ethanol, a common component in RNA extraction workflows, is well documented to inhibit PCR [5]. With the Nextractor system, ethanol evaporation is a crucial step preceding elution, typically lasting for around 5 min. We hypothesize that high RH (above 70%) hinders complete ethanol evaporation [6].

Table 1. A260:A280 ratios and Ct values of SARS-CoV-2-positive samples (*n*=20) under different humidity conditions

Humidity conditions	<50% RH	>70% RH
A260:A280 ratio (mean±SD)	2.10±0.01	1.60 ± 0.02
Ct value (mean±SD)		
E gene	22.20±5.7	ND
RdRp gene	24.85±6.2	ND
N gene	21.96±5.2	ND
RNase P gene	18.60±2.6	ND

ND: Not detected; SD: standard deviation.

This incomplete evaporation could lead to residual ethanol contaminating the eluted RNA. As a default protocol of the Nextractor system, ethanol evaporation is set at 5 min and cannot be set to last more than the default setting. Previous studies [7,8] supported the notion that humidity exerts an effect on ethanol evaporation rates. The presence of even trace amounts of residual ethanol can significantly impede RT-PCR amplification, which explains the observed failure to detect SARS-CoV-2 RNA and the internal control gene. Further investigations are warranted to explore the possibility and to have an in-depth understanding of how humidity affects RNA extraction efficiency through alternative paths besides ethanol evaporation.

Our findings underscore the critical importance of maintaining optimal RH within laboratories for reliable SARS-CoV-2 RNA extraction using the Nextractor system. Our study led to the following recommendations: (i) for optimal Nextractor performance, laboratory humidity should be regularly monitored, and RH should be kept below 50%. Installation of dehumidifiers or air conditioning systems is desirable to achieve this target range. Furthermore, hygrometers should be deployed throughout the laboratory to provide real-time data on humidity levels; (ii) laboratory personnel should be educated or trained regarding the impact of humidity on RNA extraction and the importance of humidity control. Training should focus on the proper handling of samples and strict adherence to the manufacturer's protocols to minimize the sources of contamination; and (iii) laboratories should validate the Nextractor system for SARS-CoV-2 RNA extraction under the specific humidity conditions prevalent within their facilities. This validation process helps ensure the reliability of the system in the actual laboratory environment.

By putting these recommendations into practice, laboratories can minimize the risk of false negatives of COVID-19 test results and ensure the reliability of molecular diagnostic workflows. Our study contributed to the optimization of SARS-CoV-2 RNA extraction using the Nextractor system and highlights the importance of environmental factors like humidity in guaranteeing the accuracy of COVID-19 testing. Future research efforts should be directed at exploring the strategies to mitigate the negative effects of humidity on RNA extraction, with an attempt to work out more robust and environmentally resilient automated extraction protocols.

5. CONCLUSION

This study identified humidity as a critical factor in achieving accurate COVID-19 testing using the Nextractor system. Tests worked well at low humidity, but high humidity led to interference due to leftover cleaning solution and eventually to complete test failure. Laboratories should monitor humidity, train staff, and validate the Nextractor system for their specific humidity conditions. These are essential for optimal performance and reliability of COVID-19 testing.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

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ETHICS APPROVAL AND CONSENT TO PARTICIPATE

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Not applicable.

AVAILABILITY OF DATA

Data not available due to commercial restrictions.

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