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Reducing Cost and Enhancing Testing Capacity Of RT-PCR For SARS-Cov-2 Detection Using Half Reaction Volume Assay

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Abstract

The gold standard method for detecting SARS-CoV-2 is reverse transcriptase-polymerase chain reaction (RT-qPCR). Despite its effectiveness, the COVID-19 pandemic presents challenges due to the high cost and limited availability of testing reagents. Therefore, there is an increasing need for alternative methodologies that can reduce costs, preserve testing reagents, and improve the testing capacity of RT-qPCR. This study focuses on evaluating the efficacy of RT-qPCR in detecting SARS-CoV-2 using only half of the total reaction volume. This study was conducted in the Rodolphe Merieux Laboratory (RML) at the Bangladesh Institute of Tropical and Infectious Diseases (BITID) from December 2020 to December 2021. Nasopharyngeal swab samples were collected from 80 suspected and close contact COVID-19 patients. RNA extraction was performed using the Sansure Biotech kit, and both standard and half reaction RT-qPCR assays were conducted using the 2019-nCoV nucleic acid diagnostic kit (PCR-Fluorescence Probing from Sansure Biotech, China). Among the 80 samples, 39 (48.75%) tested positive in both the standard and half-reaction assays, while none of the 41(51.25%) samples that tested negative in the standard reaction showed positive results in the half-reaction. We found 100% concordant results between the two assays. Ct values were similar between the two assays, with a slight decrease observed in the half-reaction. The average drop in Ct values for the N and ORF-1ab targets was 1.4 and 1.5, respectively. The use of the RT-qPCR half-reaction would be economically advantageous for molecular diagnostic laboratories to handle the COVID-19 pandemic more effectively especially in resource-poor settings without compromising the accuracy of the results.

Keywords: Coronavirus; RT-Qpcr, COVID-19; SARS-Cov-2; RT-Qpcr Half-reaction

Abbreviations: RML: Rodolphe Merieux Laboratory; BITID: Bangladesh Institute of Tropical and Infectious Diseases; WHO: World Health Organization; IEDCR: Institute Of Epidemiology, Disease Control, And Research; NPS: Nasopharyngeal Swabs; IRB: Institutional Review Board

Introduction

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) that causes coronavirus disease 2019 (COVID-19) was first identified in Wuhan, China, in December 2019 [1]. COVID-19 was officially recognized as a worldwide public health emergency by the World Health Organization (WHO) on January 30, 2020, and later declared a pandemic on March 11, 2020 [2]. Currently, there are more than 766.44 million confirmed cases of COVID-19, including 6.93 million deaths globally [3]. The first case of COVID-19 in Bangladesh was confirmed on 8th March 2020 by the Institute of Epidemiology, Disease Control, and Research (IEDCR). Onwards, the total number of confirmed COVID-19 cases exceeds 2.04 million, with a staggering death toll of 29,457 [4]. The gold standard for diagnosis of COVID-19 from respiratory tract specimens is reverse transcriptase-polymerase chain reaction (RT-qPCR) assay. It is an accurate and sensitive molecular method that quantitively detects viral RNA from suspected clinical samples [5]. RT-qPCR is a time-consuming and costly procedure, requiring skilled manpower, advanced equipment, and special laboratory environments [6]. Given the high prevalence of SARS-CoV-2 and the need for widespread testing, it is crucial to increase the rate of RT-qPCR testing to enable rapid and accurate identification of infected individuals, thereby curbing the spread of the virus [7]. However, the cost and limited availability of testing reagents pose challenges. Therefore, there is a growing demand for alternative methodologies that can lower costs, conserve testing reagents, and enhance the testing capacity of RT-qPCR. This study aimed to

evaluate the RT-qPCR using half of the total reaction volume to detect SARS-CoV-2 in respiratory samples by using the Sansure Biotech RT-qPCR Kit.

Methods

Study Design

It was a cross-sectional study conducted in the Rodolphe Merieux Laboratory (RML) at the Bangladesh Institute of Tropical and Infectious Diseases (BITID). A total of 80 nasopharyngeal swabs (NPS) samples were collected from suspected and close contact COVID-19 patients from December 2020 to December 2021. Ethical permission was approved by the Institutional Review Board (IRB) of the BITID.

Specimen Collection

Nasopharyngeal swab samples were collected in a 2mL sample storage buffer (Sansure Biotech, Changsha, China) for SARS-CoV-2 RT-qPCR. Collected specimens to be tested were immediately processed or stored at 40 C for testing within 24 hours of sample collection. All suspected specimens were treated in a biosafety cabinet with full personal protective equipment.

SARS-Cov-2 Viral RNA Extraction

A sample-release reagent kit (Sansure Biotech, Changsha, China) was used to extract total RNA from 20 µl of nasopharyngeal swab samples. The extraction of RNA was carried out as per the manufacturer's instructions.

SARS-CoV-2 Viral RNA Detection via Real-Time RT-PCR

We conducted the RT-qPCR assays for the standard and half-reactions concurrently using the novel Coronavirus 2019-nCoV nucleic acid diagnostic kit (PCR-Fluorescence Probing from Sansure Biotech, China). Briefly, to perform the standard RT-qPCR reaction, 20 µl extracted RNA was added to 30 µl of 2019-nCoV-PCR master mix (2019-nCoV-PCR Mix + 2019-nCoV-PCR-Enzyme Mix). For the half-reaction of RT-qPCR, half of the volumes of the reagents were utilized. Therefore, our proposed reaction comprised 15 µl of reagents and 10 µl of RNA, adding up to 25 µl in each well. We conducted both assays (standard and half-reactions) in 96-well plates using the CFX96Touch™ Real-time PCR Detection System (Bio-Rad Laboratories, Inc., Hercules, CA, USA). The manufacturer's instructions were followed for conducting the reaction, amplification conditions, and interpretation of the results. RT-qPCR result was reported as positive when the cycle threshold (Ct) value for the N and ORF-1ab targets was less or equal to 40. If the Ct was greater than 40 or undetectable, the result was considered negative [8].

Statistical analysis

SPSS (Statistical Package for Social Science) for Windows version 23 software was used for the analyses. Continuous variables were given as means ± SD, whereas categorical variables were presented as percentages.

Results

A total of 80 NPS samples were analyzed with RT-qPCR using both standard and half reaction assays, where 53 (66.25%) were male and 27 (33.75%) were female. The mean age of study individuals was 36.13 (±13.24). Regarding symptom status, 69 individuals (86.25%) reported experiencing symptoms, while 11 individuals (13.75%) were asymptomatic close contacts (Table 1). Among 80 samples, 39 (48.75%) tested positive for both standard and half reaction assays. In addition, none of the 41(51.25%) samples that yielded negative results in the standard reaction showed positive results in the half-reaction (Table 2). The Ct values of the SARS-CoV-2 N gene and the ORF-1ab gene obtained in the half-reaction and standard-reaction tests were compared (Figure 1 & 2). For the N gene target in the standard reaction assay, out of the 39 positive samples, 3 samples (7.7%) had Ct values ≤ 20, 4 samples (10.3%) had Ct values >20-25, 10 samples (25.6%) had Ct values >25-30, and 22 samples (56.4%) had Ct values > 30. Similarly, when the tests were conducted at half-reaction, the distribution of Ct values was similar, with 3 samples (7.7%) having Ct values ≤ 20, 4 samples (10.3%) having Ct values >20-25, 9 samples (23.1%) having Ct values >25-30, and 23 samples (58.9%) having Ct values > 30 (Table 3).

Table 1: Demographic characteristics of study participants.

Gender	n (%)
Male	53 (66.25)
Female	27 (33.75)
Total	80 (100)
Age, Years (Range)	14 to 70
Mean (±SD)	36.13 (±13.24)
Symptoms Status	
Symptomatic	69 (86.25)
Asymptomatic	11 (13.75)

Table 2: Distribution of RT-qPCR results (n=80) for both standard and half-volume reactions.

Reaction Volume of RT-qPCR	Positive (n, %)	Negative (n, %)
Standard reaction	39 (48.75%)	41 (51.25%)
Half reaction	39 (48.75%)	41(51.25%)

For the ORF-1ab gene, none of the samples had a Ct value ≤ 20 in either the standard or half-volume reactions. 3 samples (7.7%) had Ct values >20-25, 4 samples (10.3%) had Ct values >25 -30, and the majority of samples, 32 (82.1%), had Ct values > 30 (Table 3). Out of 39 positive samples, half reaction assay showed

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a decrease in Ct values of 36 samples for the N gene target and all 39 for the ORF-1ab gene target. In comparison to the standard reaction testing, the average drop in Ct values was 1.4 and 1.5 for both targets respectively (Table 4).

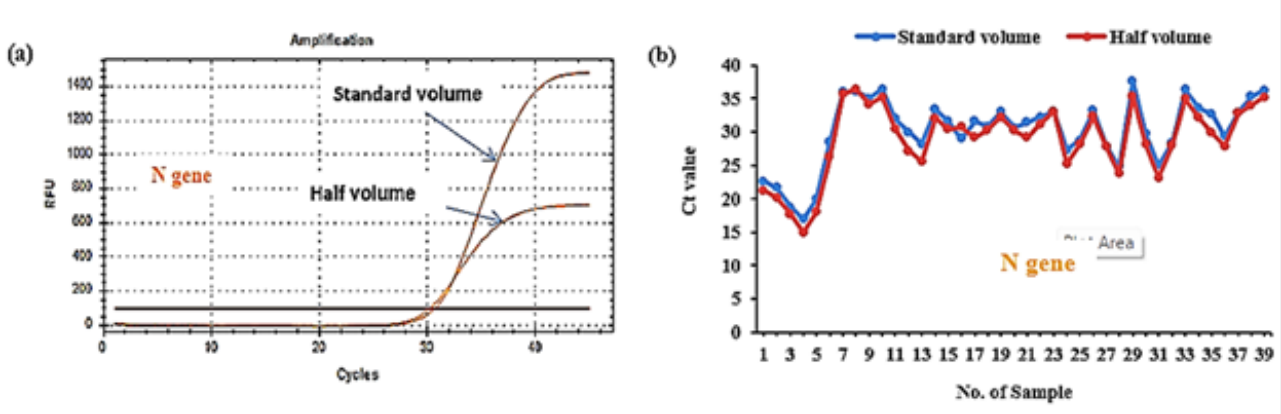

Figure 1: Corresponding single RT-qPCR amplification curves of the N gene assay with standard volume and half volume reagents (a), and cycle threshold (Ct) of the N gene assay with standard volume and half volume reagents (b).

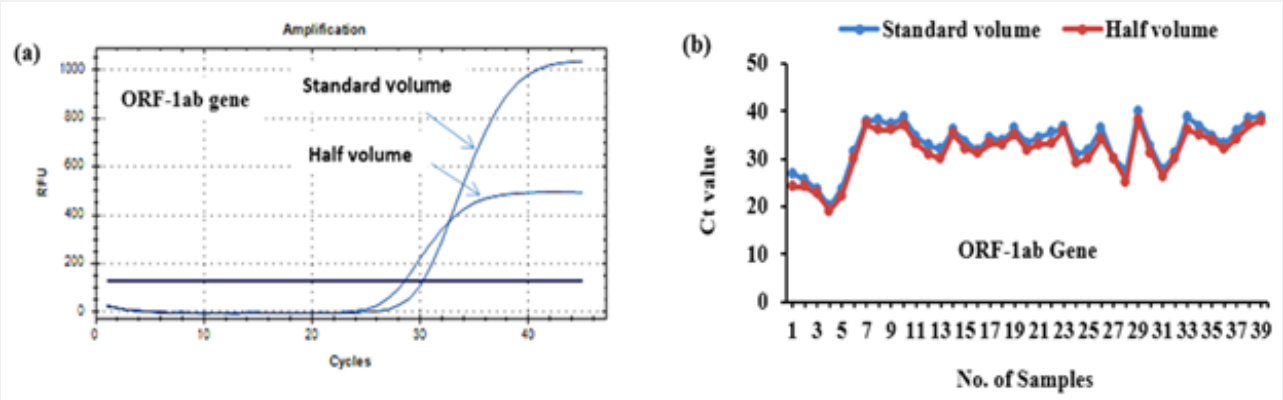

Figure 2: Corresponding single RT-qPCR amplification curves of the ORF-1ab gene assay with standard volume and half volume reagents (a), and Cycle threshold (Ct) of the ORF-1ab gene assay with standard volume and half volume reagents (b).

Table 3: Ct value ranges of positive RT-qPCR tests for two genes at standard volume and half volume reactions.

RT-PCR Ct Values	Standard Volume		Half Volume	
	N Gene	ORF-1ab Gene	N Gene	ORF-1ab Gene
≤ 20	3 (7.7%)	None	3 (7.7%)	None
>20-25	4 (10.3%)	3 (7.7%)	4 (10.3%)	3 (7.7%)
>25-30	10 (25.6%)	4 (10.3%)	9 (23.1%)	4 (10.3%)
>30	22 (56.4%)	32 (82.1%)	23 (58.9%)	32 (82.1%)
Total	39	39	39	39

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Table 4: Comparison of the Ct values for SARS-CoV-2-positive clinical samples that showed a reduction in Ct between the standard and half-reactions assay of RT-qPCR.

RT-PCR Ct values	The Average Reduction in Ct values of RT-qPCR (Ct Standard Volume - Ct Half-Reactions Volume)	
	Target (N) gene	Target (ORF-1ab) gene
≤ 20	1.6 ± 0.50 (n= 3)	None
>20-25	1.4 ± 0.30 (n= 4)	1.2± 0.40 (n= 3)
>25-30	1.5 ±1.01 (n= 9)	1.9 ± 0.70 (n= 4)
>30	1.2 ± 0.70 (n= 20)	1.4 ± 0.60 (n= 32)
Average decrease in Ct values	1.4 ± 0.60 (n= 36)	1.5 ± 0.60 (n= 39)

Discussion

RT-qPCR assays for SARS-CoV-2 RNA detection in clinical samples are widely used in COVID-19 diagnostic laboratories due to their high sensitivity and specificity [9]. To effectively address the widespread presence of SARS-CoV-2, enhancing the rate of RT-PCR testing is crucial. Due to the expensive and limited availability of reagents, there is a pressing demand for a new methodology to lower costs, preserve testing reagents, and enhance the testing capacity of RT-qPCR. In this study, we evaluated the RT-qPCR using half of the total reaction volume to detect SARS-CoV-2 in respiratory samples collected from COVID-19 suspected and close contact patients at BITID. The gender distribution of the study participants revealed a higher proportion of males (66.25%) compared to females (33.75%). This finding aligns with previous studies that have shown a higher prevalence of COVID-19 in males [10,11]. Additionally, a significant majority (86.25%) of the individuals reported experiencing symptoms, highlighting the importance of timely and accurate testing to identify and isolate infected individuals.

We analyzed the cycle threshold (Ct) values of the clinical samples for SARS-CoV-2 using the standard and half reaction assays. We found 100% concordant results between the two assays. Among the positive SARS-CoV-2 specimens, we noticed a slight decline in the values of Ct for the majority of samples, either for the N (36 cases [92%] or ORF-1ab (39 cases [100%] targets in the half-reaction assay which was similar to the previous study [7,12]. This decrease in Ct values had no impact on the RT-qPCR result. So, according to the above-mentioned data, the half-reaction volume assay is a promising strategy for the detection of SARS-CoV-2, and it can help to reduce the cost of the test in resource-poor settings without compromising the accuracy of the results. The study possessed certain limitations such as a limited sample size and a single-center study design. Hence, additional investigations with larger sample sizes and multicenter designs using the same RT-qPCR test kit are necessary to validate the study's results.

Conclusion

Due to a shortage of test reagents, each laboratory's ability to conduct tests is still limited and the number of patients tested for COVID-19 is increasing globally, it would be economically advantageous for molecular diagnostic laboratories to implement the half-reaction methodology to handle the COVID-19 pandemic more effectively. However, further studies are needed to validate these findings in different settings and with different RT-PCR kits.

Author Contributions

Md. Shakeel Ahmed was the principal investigator responsible for the study conception, design, validation, review, and editing of the manuscript. Md. Zakir Hossain was responsible for the laboratory resources, investigation, and supervision of the laboratory staff. Md. Mamunur Rashid's role was the laboratory research methodology, investigation, and validation. Istiak Ahmad's role was COVID-19 case selection. Md. Zahirul Islam's role was laboratory research methodology, study, validation, formal data analysis, original draft writing, and editing of the manuscript. Farzana Begum, Meherab Hossain, and Hasan Rabbi assisted in patient specimen collection and laboratory methodology.

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