Real-Time PCR Diagnosis of COVID-19 in Al Anbar Province, Iraq

Mohammed A. Hamad [1*], Firas R. Jameel [1], Noor Najm Abdullah [1], Muhaidi MJ [2]

Abstract

Background: Genomic sequencing has advanced COVID-19 understanding, facilitating therapeutic assessment and guiding treatment strategies. Precise diagnosis is vital for grasping transmission dynamics and detecting novel variants. The study aimed to investigate the molecular attributes, mutations, and diagnostic methodologies of the coronavirus (SARS-CoV-2) pandemic. Methods: Real-time PCR was utilized for COVID-19 diagnosis. Samples underwent RNA extraction and RT-PCR amplification. Between February and April 2020, 245 samples were collected from Anbar province, initially yielding negative results via rtPCR. Data on infection rates, gender disparities, and clinical outcomes were analyzed. Results: In 2020, the infection rate was 14%, reducing to 11% in 2021. Men exhibited higher infection rates (70%) compared to women (30%). Clinical data indicated varying severity and fatality rates between sexes. Conclusion: While RT-PCR remains widely accepted, alternative diagnostic methods show promise. Further research is essential for comprehensive COVID-19 understanding and improving diagnostic accuracy. The study underscores ongoing research's significance in combating viral epidemics, emphasizing the need for comprehensive understanding and adaptive strategies to mitigate mortality risks.

Keywords: COVID-19, Real-time PCR, diagnosis, Transmission dynamics, Viral variants

Significance | Accurate diagnosis with real-time PCR is essential for pandemic control. Understanding transmission dynamics aids in formulating effective public health measures.

*Correspondence. Mohammed A.Hamad, Department of Biotechnology, College of Applied Science University of Fallujah, Al-Anbar 31002, Iraq
E-mail: moh75mnr@uofallujah.edu.iq
https://orcid.org/0000-0003-1540-5441

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Introduction

COVID-19 is a highly infectious viral infection caused by the zoonotic novel coronavirus named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Like other coronaviruses such as SARS-CoV-1 and MERS-CoV, SARS-CoV-2 likely originated from bats, established reservoirs for various pathogenic coronaviruses. The disease initially emerged in Wuhan, China, in December 2019, rapidly spreading worldwide and causing catastrophic economic, health, and physiological effects (Khan et al., 2020).

Advancements in genomic sequencing methods have significantly improved our understanding of COVID-19 and its transmission dynamics, aiding in assessing therapeutic outcomes and shaping future treatment strategies. Detecting even subtle behavioral changes across diverse populations is crucial for understanding the transmission dynamics and potential emergence of novel variants of the COVID-19 virus (Najaflou et al., 2021). The appearance of COVID-19 caused a global epidemic, shocking economies and claiming numerous lives (Hamad et al., 2021). The disease rapid spread led to WHO stating it a pandemic in March 2020 (Singh et al., 2020). Clinical signs including respiratory symptoms are often with flu-like illnesses. The response to disease outbreak has directed to extensive terminations of schools, businesses, and community

Author Affiliation.
[1] Department of Biotechnology, College of Applied Science University of Fallujah, Al-Anbar 31002, Iraq
2 Department of Pathological Analysis, College of Applied Science University of Fallujah, Al-Anbar 31002, Iraq

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venues, impacting public communications and leading to the termination of numerous events, including gatherings and conferences (Singh et al., 2020).

According to a recent UNESCO report, governments in 61 countries have ordered school closures as a precautionary measure against the disease's spread, affecting over 420 million children and young people in more than 39 countries (Singh et al., 2020). This study aimed to investigate real-time PCR diagnosis of COVID-19 in Al Anbar province and explore the outbreak dynamics in the region.

Materıals And Methods

Clinical sample collection

A total of 245 Nasal swap sample were collected and brought to the central lab of Al-Anbar Health between February and April 2020, from the patients with respiratory symptoms, including (fever or chills, cough, shortness of breath or difficulty breathing, fatigue, muscle or body aches, headache and new loss of taste or smell)in different locations in the province of Anbar (AL-Fallujah city, AL-Ramadi city, AL-Saqlawya city). The Department of Microbiology provided archival laboratory culture isolates from public health laboratories in Ramadi city, which were used for in vitro-specific examination. All experimental methodologies utilizing live SARS-CoV-2, SARS-CoV, and MERS-CoV adhered to the biosafety level's accepted standard operating procedures.

Ethics

The study adhered to the ethical standards. Pseudonymization techniques were employed to register and process medical records to safeguard against the identification of data subjects. The Research Ethics Committee approved the study protocol. This examination followed the guiding principles outlined in the 1964 Helsinki Declaration.

Method of extraction and amplification protocol

The Protocol for nucleic acid extraction, described by (BIORON Diagnostics GmbH/Germany RNA extraction kits), involves an initial step where the specimen undergoes treatment with a pre-heated multicomponent Lysis Reagent. This treatment is designed to induce the destruction of cellular structures and nucleoprotein complexes. Subsequently, nucleic acids are absorbed onto silica gel-covered magnetic particles through binding. Following this, the specimen undergoes multiple washings to remove contaminants, ensuring the purification of the nucleic acids. The concentration of the extracted nucleic acid is quantified using a spectrophotometer, providing a quantitative measure of the nucleic acid yield.

Real-time PCR detection

The initial step of the laboratory. The work will be initiated in the sampling room. The collected samples are transported to a designated location and subjected to refrigeration for 72 hours, maintaining a constant temperature of -20 degrees Celsius.

According to (BIORON Diagnostics GmbH/Germany RNA extraction kits), prepare and label tubes for specimens, Positive Control (PC), and Negative Control (NC). Add 30 µl of Internal Control (IC) solution to each tube. For NC, add 100 µl of Negative Control sample; for PC, add 70 µl of Negative Control sample and 30 µl of Positive Control sample. Add patient specimens (100 µl, or 200 µl for increased sensitivity, 50 µl for sample) to labeled tubes. Apply 300 µl of Lysis Reagent with Sorbent, vortex, and incubate at 65°C and 1300 rpm for 10 minutes. Precipitate with 400 µl of Solution for NA precipitation, vortex, and centrifuge. Wash with Wash Solution № 1 (500 µl), centrifuge, and repeat with Wash Solution № 2 and № 3. Dry the pellet for 2-3 minutes at (18 - 25) °C, then resuspend in Specimen Diluent (200 µl for 1-3 assays, 600 µl for more). Vortex incubate at 56°C and 1300 rpm for 10 minutes, then centrifuge for 1 minute at 13000 rpm.

If required, the resultant RNA can be kept frozen for one thaw before storage at -20 °C for no more than a week of reverse transcription, but be aware that the direct use of freshly prepared RNA will bring a more reliable result, especially with low RNA contents.

Reverse transcription-PCR (RT-PCR) amplification

According to (BIORON Diagnostics GmbH/Germany SARS-CoV-2 Detection Kit), label tubes for samples, Positive Control (PC), and Negative Control (NC), ensuring correct allocation. Thoroughly mix RT-PCR buffer and Enzyme Taq/RT, then prepare the mixture according to sample quantity. Carefully add the mixture to tubes, ensuring paraffin layer integrity. Place a drop of mineral oil in each tube, seal tightly, and ensure no leakage. Vortex tubes briefly, spin to settle contents, and transfer to the Spectrop.0 for further processing Table 1.

Results

In the context of the reverse transcription-polymerase chain reaction (RT-PCR) procedure, it is imperative to utilize the extracted RNA promptly, as it is not amenable to long-term storage. If necessary, the resulting RNA can be cryopreserved for one thaw cycle before being stored at -20 °C for a maximum of one week before starting reverse transcription. However, it should be noted that using freshly generated RNA will yield more accurate results, particularly when dealing with low RNA concentrations. As shown in below Figure 1, Figure 2).

The data on COVID-19 infection rates in 2020 reveals that 576 persons tested positive for the virus, while 2821 individuals tested negative. The comparative analysis revealed that the infection rate observed in the year 2020 surpassed that observed in the subsequent year of 2021, with the latter being quantified at 11%. The infection rate observed in the year 2020 amounted to 14%. The observed phenomenon can be attributed to a combination of individuals'

Table 1. PCR resultsaccording to BIORON Diagnostics GmbH protoccol

Detection Channel				
FAM/Green	**HEX/Yellow**	**ROX/Orange**	**Cy5/Red**	**Interpretation**
SARS-CoV	IC	SARS-CoV-2 E-gene	SARS-CoV-2 N-gene	
Analyzed samples				
+	Not considered	+	+	RNA of SARS-CoV-2 is detected✯
+	Not considered	-	-	RNA of SARS-like Coronaviruses is detected, RNA of SARS-CoV-2 is not detected
-	+	-	-	RNA of SARS-like Coronaviruses and RNA of SARS-CoV-2 is not detected
Positive Control sample				
+	Not considered	+	+	Positive result
Negative Control sample				
-	+	-	-	Negative result

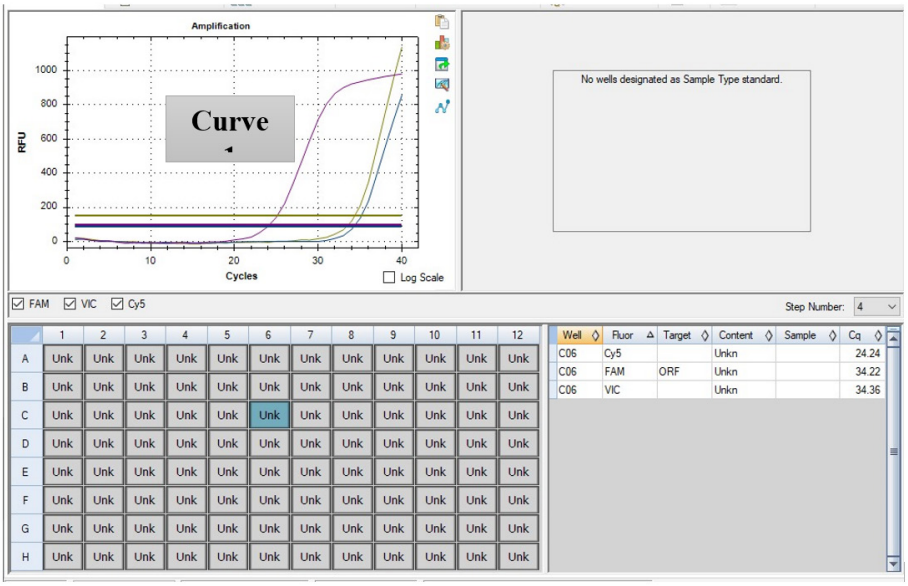

Figure 1. Curves analysis in positive status, the ORF and N genes if appears indicating infection to COVID-19 and The range (20 - 40) it also indicates infection.

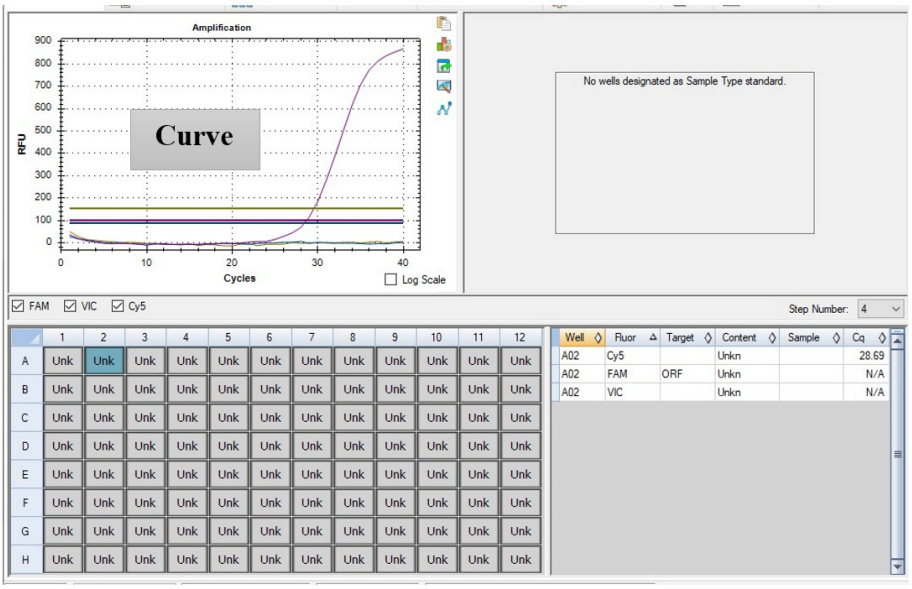

Figure 2. Curve analysis in negative status, appear negative due absence of genes (ORF and N gene) that indicate infection.

Table 2. The males are more vulnerable to the virus than females.

Gender	Positive	Negative
Man	10	140
Women	3	92

limited awareness of health precautions and their diminished inclination to utilize masks and engage in sterilization practices during the initial stages of the epidemic. The statistical data about COVID-19 infections in 2021 reveals a total of 1033 confirmed positive cases and 8229 confirmed negative cases. The observed disparity in occupational exposure to the Coronavirus between men and women has led to identifying a corresponding discrepancy in infection rates, with a higher prevalence observed among men. Indeed, the prevalence of infection among males throughout 2020 and 2021 was almost 70%, in contrast to approximately 30% among females as shown in Table 2.

Discussion

The effectiveness of RT-PCR as the gold standard for diagnosing COVID-19 has been questioned, leading to the exploration of alternative methods for evaluating diagnostic efficacy (Hag-Ali et al., 2021). Studies have demonstrated that dogs can be trained to detect human diseases, with explosives-detecting canines successfully identifying individuals negative for SARS-CoV-2 odor in perspiration samples (Hag-Ali et al., 2021). The bayesian analysis further revealed the K9 test's superior sensitivity compared to RT-PCR, indicating its potential as a non-invasive, cost-effective, and efficient screening tool for asymptomatic individuals (Hag-Ali et al., 2021). To speech sensitivity and specificity subjects, novel molecular diagnostic approaches like multiplex PCR have been developed (Ali et al., 2020). widespread PCR application in numerous fields, including molecular biology and medical diagnostics (Dong *et al*., 2021, Bala et al. 2024a, Bala et al. 2024b, Nithya et al. 2024, Jayanthi et al. 2024, Jeha et al. 2024, Hana et al. 2024).

In 2021, researchers extensively documented various aspects of the COVID-19 pandemic, including its medical concerns, causes, diagnosis, preventative measures, and treatment possibilities (Hamad et al., 2021, Esra et al. 2024, Moniruddin et al. 2023, Sasi et al. 2021, Zahra et al. 2022). The results indicated a higher infection rate among men due to increased exposure to the virus in the office, with nearly 70% of males contracting the virus compared to 30% of females in 2020 and 2021. Clinical outcomes from COVID-19 infections may vary between sexes due to differences in behavior and susceptibility to co-morbidities, age is an important factor in COVID-19 severity and prognosis, with increased mortality risk associated with chronic diseases such as obesity, diabetes, and cardiovascular disease (Mukherjee *et al.,* 2021). While infections in children are rare and typically mild, the severity of illness among COVID-19 hospitalizations is linked to advanced age and the prevalence of chronic medical conditions.

Conclusion

The researchers hope to learn more about the Coronavirus, which has been responsible for several deaths worldwide. The study's overarching goal is to learn more about the virus, its molecular features, and the various diagnostic techniques used to identify it. The spread of COVID-19 has had a devastating effect on people's standard of living. Directly or indirectly, people all throughout the world are feeling the effects of this illness. The RT-PCR test has emerged as the gold standard in molecular diagnostics because of its high degree of accuracy, sensitivity, and dependability. The severity of COVID-19 varies widely; some people may have very modest symptoms, while others may suffer fatal consequences. Because of the gender gap in occupational exposure to the Coronavirus, men have a higher risk of catching the virus than women do. Males accounted for over 70% of the infected population in 2020 and 2021, while females comprised roughly 30% of the affected population. Children have a much lower risk of contracting an infection; when they do, the illness is usually mild and short-lived. Because of their robust immune systems, these people can avoid getting sick from viruses. According to the studies above, we still do not know much about COVID-19 in epidemiology, virology, and clinical practice. A better understanding of viruses is necessary because viral epidemics occasionally cause human mortality. The global COVID-19 epidemic has highlighted the urgency of responding to and recovering from a public health emergency of historic proportions. It is generally agreed that nucleic acid amplification techniques are the gold standard for identifying COVID-19 infections. Regulatory bodies on both the national and international levels have approved several RT-PCR-based diagnostic tests. Diagnostic procedures for COVID-19 have progressed greatly since the first methods of viral culture analysis and de novo sequencing of the SARS-CoV2 genome, greatly hindering the ability to diagnose infections quickly and reliably. Despite RT-PCR's excellent sensitivity, the diagnostic capacities for COVID-19 have improved substantially thanks to these improved testing and clinical and radiographic investigations.

Author contributions

M.A.H. developed the concept and the design of the study. F.R.J. conducted data analysis, N.N.A, M.M.J. conducted the study design, analyzed the data, and wrote the draft of the manuscript.

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Competing financial interests

The authors have no conflict of interest.

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