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Assessment of Chip-Based Real-Time RT-PCR (TrueNat) Versus Conventional Real-Time RT-PCR for Swift Diagnosis of SARS-CoV-2: A Comparative Study

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Abstract- The emergence of SARS-CoV-2, a novel coronavirus, has resulted in a global pandemic with rapid spread and significant public health implications. Diagnostic testing plays a pivotal role in identifying and managing COVID-19 cases. This study describes a chip-based real-time reverse transcriptase polymerase chain reaction (RT-PCR) method, TrueNat, for diagnosing SARS-CoV-2. The protocol involves RNA extraction followed by amplification and detection of viral genes using micro PCR chips. A comparison with conventional real-time RT-PCR was conducted, evaluating sensitivity, specificity, positive predictive value, negative predictive value, and turnaround time. Out of 500 samples screened, TrueNat demonstrated a sensitivity of 100% and specificity of 99.12%. The positive predictive value was 91.84%, and the negative predictive value was 100%. TrueNat exhibited a shorter turnaround time compared to conventional RT-PCR, providing results within 75 minutes for negative samples and approximately two hours for positive samples. High concordance was observed between TrueNat and conventional RT-PCR, with a 97% correlation in cycle threshold values. The study concludes that TrueNat offers a reliable and cost-effective solution for rapid diagnosis of COVID-19, particularly in settings with limited testing capacity and urgent clinical needs.

Keywords- SARS-CoV-2, TrueNat, Molecular diagnostics, RT-PCR, COVID-19 testing

1. INTRODUCTION

The emergence of SARS-CoV-2, a novel coronavirus, in December 2019 marked the onset of a global health crisis. Originating in Wuhan, China, this virus swiftly escalated into a pandemic, prompting the World Health Organization (WHO) to declare it a Public Health Emergency of International Concern by

January 2020, followed by a pandemic declaration in March of the same year. Characterised by its rapid transmission and significant morbidity and mortality rates, COVID-19, the disease caused by SARS-CoV-2, posed unprecedented challenges to healthcare systems worldwide. Distinguishing COVID-19 patients from those with other respiratory illnesses became paramount for effective management and containment strategies. Studies revealed that within 5-6 days of symptom onset, COVID-19 patients exhibited substantial viral loads in their upper and lower respiratory tracts, distinguishing them from individuals with other respiratory diseases. This study focuses on developing and evaluating a comprehensive diagnostic approach utilising molecular diagnostics, specifically chip-based real-time RT-PCR (TrueNat), for detecting SARS-CoV-2. The methodology involves utilising viral lysis media, RNA extraction cartridges, and micro PCR chips to extract and amplify viral RNA from patient samples efficiently. Statistical analyses were conducted to assess the performance of TrueNat compared to conventional real-time RT-PCR, demonstrating high sensitivity, specificity, and predictive values. The results highlight the potential of TrueNat as a reliable and rapid diagnostic tool, particularly in settings where timely interventions are crucial, and testing capacities are limited.

2. MATERIALS

The materials utilised in this study encompass essential components for the efficient extraction and analysis of viral RNA, which are crucial for detecting SARS-CoV-2. These materials include:

1. Viral Lysis Media and Sample Buffer: These reagents facilitate the breakdown of viral particles and preserve RNA integrity during sample processing.
2. RNA Extraction Cartridge: This cartridge is designed to extract RNA from patient samples, efficiently isolating viral genetic material.
3. Micro PCR Chip: The micro PCR chip serves as a platform for amplifying and detecting viral RNA through polymerase chain reaction (PCR) technology, enabling high-throughput analysis of multiple samples simultaneously.

These materials are integral to the methodology employed in this study, which involves a systematic process of sample preparation, RNA extraction, and amplification. The study aims to develop a reliable and efficient diagnostic approach for detecting SARS-CoV-2 by utilising these materials in conjunction with established protocols. By utilising these materials, the study seeks to address the urgent need for accurate and rapid diagnostic methods amidst the ongoing COVID-19 pandemic, thereby contributing to the enhancement of testing capacities and the facilitation of timely interventions in affected populations.

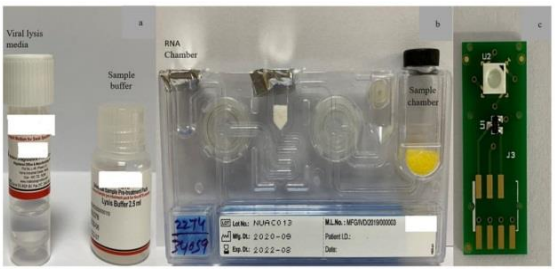

Figure 1: Schematic Representation of the RNA Extraction Process

3. METHODS

The method for efficient RNA extraction and detection of SARS-CoV-2 entailed a systematic approach to ensure accuracy and reliability. Initially, the RNA extraction cartridge

underwent meticulous labelling with pertinent patient identification details and the test run's date and time. Simultaneously, the elute collection tube received appropriate labelling. In order to initiate the process, viral lysis media was introduced into the sample buffer to facilitate the breakdown of viral particles and RNA preservation. A precise volume of the viral transport medium (VTM) was then added to the lysis buffer using a provided dropper, followed by incorporating a mixed lysis buffer into the sample chamber of the cartridge. All used items were disposed of in 0.5% hypochlorite solution to maintain safety protocols. The cartridge chamber was then securely closed with a provided black cap and inserted into the amplification machine. Upon initiation of the process, the run button was activated, and after 20 minutes, a beep signalled the completion of RNA extraction. Subsequently, the cartridge was transferred to a designated stand, and the elute chamber was opened. With utmost care, the elute was transferred to a pre-labelled elute collection tube, while the cartridge was discarded in 0.5% hypochlorite solution. Hand hygiene was rigorously maintained throughout the procedure, with sanitisation occurring after each step. A portion of the elute, six microliters precisely, was then transferred into a cuvette containing RT enzyme, followed by a brief 10-30 second incubation period. The elutes were further transferred to the elute chamber in the Beta CoV chip, which was subsequently loaded with elute. After discarding the micropipette tip in hypochlorite, the Beta CoV chip was transferred to the amplification machine, with patient details entered before test initiation. Forty minutes later, a beep indicated the completion of the test. Initial screening of all samples was conducted using the E-gene for Sarbecovirus assay, with negative results considered true negatives. Positive samples underwent confirmation through the RdRp gene assay for SARS-CoV-2, with positive results deemed true positives. The results were promptly available within the same 40-minute timeframe. Additionally, conventional real-time RT-PCR testing was performed using the ICMR-approved PathoDetectTM Coronavirus (COVID-19) PCR kit from Mylab Discovery Solutions. This quantitative RT-PCR test targeted the amplification and detection of both the E-gene of Sarbecovirus and the RdRp gene of SARS-CoV-2, following the manufacturer's instructions. The PCR was conducted in the CFX96 Touch BioRad real-time PCR system, with a thermal profile completion time of 2.5-3 hours. Samples detecting both genes were considered positive.

4. STATISTICAL ANALYSIS

Statistical analysis was conducted to compare the performance of chip-based real-time RT-PCR (TrueNat) with conventional real-time RT-PCR to detect SARS-CoV-2 genes. All results were entered into a Microsoft Excel sheet, and correlation tables were generated. TrueNat's sensitivity, specificity, and positive and negative predictive values were calculated. Out of 500 samples screened, TrueNat exhibited a sensitivity of 100% and specificity of 99.12%. The positive predictive value was 91.84%, and the negative predictive value was 100%. TrueNat also demonstrated a shorter turnaround time, providing results within 75 minutes for negative samples and approximately two hours for positive samples, compared to 4-6 hours for conventional RT-PCR. A high correlation of 97% was observed between TrueNat and conventional RT-PCR in

cycle threshold values. These findings suggest that TrueNat offers a reliable and cost-effective solution for rapid diagnosis of COVID-19, particularly in settings with limited testing capacity and urgent clinical needs.

5. RESULTS ANALYSIS

The performance of chip-based real-time RT-PCR (TrueNat) was assessed using conventional real-time RT-PCR as a benchmark, with 500 samples screened. TrueNat results indicated 49 positive and 451 negative samples out of the 500 tested. Compared to conventional real-time RT-PCR, 45 samples tested positive, and 455 tested negative. All samples identified as negative by TrueNat also tested negative by real-time RT-PCR.

Table 1. Comparison of TrueNat and RT-PCR Results for SARS-CoV-2 Detection

	RT-PCR Positive	RT-PCR Negative	Total
TrueNat Positive	45	4	49
TrueNat Negative	0	451	451
Total	45	455	500

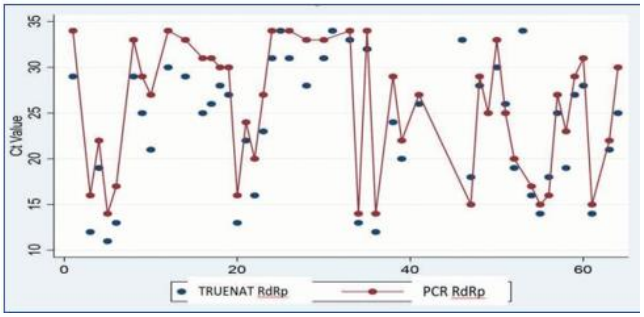

Figure 2. Representation of TrueNat RdRp with PCR RdRp

TrueNat exhibited a sensitivity of 100% and a specificity of 99.12% at a 95% confidence interval. The positive predictive value was determined to be 91.84%, while the negative predictive value was 100%. Turnaround time for TrueNat was approximately 75 minutes for samples negative for the E-gene and approximately two hours for samples positive for both the E-gene and RdRp gene, in contrast to the 4-6 hours required for processing a sample by real-time RT-PCR.

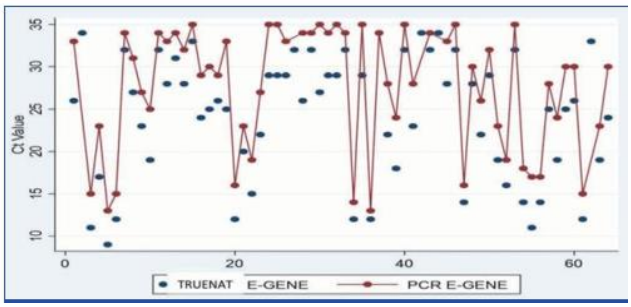

Figure 3. Representation of TrueNat E-GENE with PCR E-GENE

Figure 4 Representation of PCR Devices

Among the 64 samples that tested positive for the E-gene by TrueNat, 59 were also found to be positive by conventional real-time RT-PCR. A strong correlation of 97% was observed among the cycle threshold (Ct) values between TrueNat and real-time RT-PCR ($p<0.001$). The average

Ct value for the E-gene detected by TrueNat was 21.62 ± 6.64, whereas for real-time RT-PCR, it was 26.02 ± 7.19. In conclusion, the TrueNat RT-PCR test for SARS-CoV-2 demonstrated rapid turnaround time, high sensitivity, and specificity, making it a reliable and efficient option for urgent interventions and augmenting testing capacity, particularly in peripheral settings with lower sample loads.

6. CONCLUSION AND FUTURE SCOPE

The findings of this study underscore the effectiveness of chip-based real-time RT-PCR (TrueNat) as a rapid and reliable diagnostic tool for detecting SARS-CoV-2. With a sensitivity of 100% and a specificity of 99.12%, TrueNat demonstrates robust performance in identifying both positive and negative cases. Its positive predictive value of 91.84% and negative predictive value of 100% further affirm its diagnostic accuracy. Moreover, TrueNat offers a significantly shorter turnaround time compared to conventional RT-PCR, with results available within 75 minutes for negative samples and approximately two hours for positive samples. This swift diagnostic capability facilitates timely interventions and augments testing capacities, particularly in peripheral settings with lower sample loads.

Additionally, the high concordance observed between TrueNat and conventional RT-PCR, along with the strong correlation in cycle threshold values, validates its reliability as a diagnostic tool. Overall, TrueNat emerges as a cost-effective solution that can enhance COVID-19 testing capacity and provide rapid results, aiding in effective disease management and containment efforts. The promising performance of TrueNat in this study opens avenues for its further implementation and refinement in COVID-19 diagnostics. Future research could focus on expanding the utilisation of TrueNat in diverse healthcare settings, including remote and resource-limited areas where access to sophisticated laboratory infrastructure is limited. Additionally, ongoing efforts to optimise TrueNat protocols and enhance scalability could lead to even greater efficiency and affordability in COVID-19 testing. Furthermore, longitudinal studies tracking the performance of TrueNat over time, including its ability to detect emerging variants of SARS-CoV-2, would provide valuable insights into its continued utility in managing the evolving landscape of the pandemic. Overall, continued investment in developing and deploying TrueNat can significantly impact global efforts to combat COVID-19 by enabling widespread, rapid, and accurate diagnosis.

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