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Comparative Evaluation of Conventional RT-PCR with Chip Based Real Time RT- PCR (TrueNat) for Detection of COVID-19

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ABSTRACT

The outbreak of SARS-CoV-2 has required the creation of high-speed, sensitive, and convenient molecular diagnostic solutions. This paper compares the diagnostic capability of the chip-based real-time RT-PCR (TrueNat) with the traditional real-time RT-PCR in detection of COVID-19. Nasopharyngeal qualitative samples of 400 samples were tested at an ICMR-certified laboratory. TrueNat reportedly identified 51 and 349 positive and negative samples respectively, compared to the conventional RT-PCR that reported 47 and 353 positives and negative samples, respectively. In comparison with RT-PCR that was used as the reference standard, the TrueNat assay had a sensitivity of 100, specificity of 98.8 and a combined diagnostic accuracy of 99. Ct values of the E and RdRp genes showed good correlation of the two methods ($r = 0.97$ and $r = 0.96$, respectively; $p < 0.001$). TrueNat also identified a small subset of low viral-load samples that were negative using the conventional RT-PCR, which suggests better analytical sensitivity. TrueNat is a viable molecular platform in decentralized testing due to its reduced turnaround time, closed-cartridge workflow and low requirements on biosafety. The results confirm that chip-based RT-PCR can be a useful and valid diagnostic platform to laboratory-based systems, especially in resources-constrained and peripheral healthcare systems, which can play a role in the quicker disease identification and enhanced capacity to respond to hazards to the public health.

Keywords: SARS-CoV-2, TrueNat, RT-PCR, Diagnostic Accuracy, Viral Load.

INTRODUCTION

SARS-CoV-2 has become one of the most dangerous world-recognized public-health crises of the twenty-first century. Its unparalleled spread of the infectious disease, along with the lack of therapeutic or preventive measures to address it at the moment, caused a global crisis that shook the social and healthcare fabric on an unprecedented scale. The transmissibility and adaptability of this virus is exceptionally high and can be seen in the efficient movement of the virus across continents and in remote societies. The initial COVID-19 diagnosis in India was announced on 30 January 2020, and the country began a nationwide reaction. The Government of India realized that there was a possibility of an uncontrolled spread in the communities; therefore, a total lockdown was imposed on 24 March 2020 to curb the infection and position themselves in anticipation of the

rise in cases. By 10 August 2022, some 4.42 crore confirmed infections and 5.27 lakh deaths had been recorded in India, which is one of the nations with the highest disease burden in the world [1]. The coronavirus has posed urgent diagnostic and epidemiological threats. Fast, sensitive and extensive diagnostic testing is essential, as it is essential to good patient management, as well as to break the chain of transmission, early isolation, and epidemiological monitoring [2]. At the beginning of the pandemic, it was obvious that the quality of laboratory-based measures would largely define the national and international opportunities to overcome outbreaks. Clinical suspicion was not reliable in making the diagnosis of the disease since it is clinically heterogeneous, ranging between asymptomatic carriers and severe pneumonia and multi-organ failure, which underscores the inseparable role of molecular diagnostics in confirming the presence of the disease [3,4].

Quantitative real-time reverse-transcription polymerase chain reaction (qRT-PCR) was one of the technologies, and it quickly became the standard of SARS-CoV-2 detection. It is a technique based on reverse transcription of viral RNA and amplification of selected genomic targets, giving it high analytical sensitivity and virtually absolute specificity, which can be 100 percent [5]. The amplification cycle threshold (Ct) values produced in the qRT-PCR have been useful in diagnosis as well as a semi-quantitative measure of viral load [6]. Nonetheless, this process is labor intensive in nature with several steps of sample processing, RNA extraction, reagent preparation, amplification and data analysis being done under biosafety level-2 (BSL-2) conditions by highly trained personnel. Moreover, logistical issues, including shortages of reagents, cold chain reliance, and 24-48 hours turnaround times, have constrained its scale during the surge of epidemic, especially in resource-limited or rural environments [7]. To address these limitations of operation, molecular diagnostics developed quickly in the COVID-19 context leading to point-of-care molecular platforms, which focus on portability, fast turnaround, and biosafety. It has been shown that miniaturized thermocyclers can be used to achieve detection within an hour and that they can be as analytically reliable as their benchtop counterparts [8] because of technological advances, such as microfluidic and chip-based PCR systems. This speedy innovation process has been stated to be a technological ripple point in the history of PCR development.

The Indian Council of Medical Research (ICMR) acknowledged the necessity of the existence of accessible but quality molecular testing on 19 May 2020, when it approved the TrueNat system as a complete chip-based, real-time RT-PCR platform to screen and confirm COVID-19 cases [9]. TrueNat is a native technology that was initially created to diagnose tuberculosis and subsequently modified to SARS-CoV-2. The device is a battery-powered system that combines automated RNA extraction and amplification in microchips and pre-loaded reagents that provide a very high reduction in the risk of contamination and operator error [10]. TrueNat has a turnaround time of 3060 minutes and has low biosafety needs, which enables testing in peripheral and field laboratories that do not have standard infrastructure to do molecular testing [11]. Although RT-PCR will always be an indispensable part of a centralized lab, TrueNat can fill an important void between the quality and the availability. Every TrueNat module is capable of 1-4 samples per run allowing 24-48 tests per day but at the trade-off of throughput of increased speed, portability, and safety. Comparative determinations have revealed that TrueNat can give out sensitivity of 5080 percent and specificity of 9095 percent which is highly reliant on the amount of viral load and the quality of samples. Its closed-cartridge design reduces aerosol exposure as well as predisposes it to be used in low-resource settings.

The studies recently provided have also

highlighted the need to learn about the interpretation of Ct values across molecular assays in order to direct inter-platform consistency. The Ct-based estimation of viral load is becoming a significant parameter used in assessing the transmissibility, severity and monitoring of treatment [12]. Nonetheless, assay chemistry and target genes may vary between assays of the same sample, and this difference may cause Ct value to vary indicating the importance of standardization and assays comparison [13]. Besides, it has been demonstrated using computational models that a combination of various diagnostic approaches including RT-PCR, RT-LAMP, antigen detection, and microchip-based systems can be used to improve surveillance efficiencies and optimize tests coverage during pandemics.

TrueNat is, therefore, a significant innovation in this dynamic diagnostic environment. It has been a useful instrument in the containment of pandemics, due to its ease of field application, the combination of nucleic-acid extraction, and amplification, and compatibility with the Indian public-health infrastructure. With the trend of a global diagnostic sphere shifting to portable, integrated molecular diagnostics, TrueNat can be considered an example of a decentralized, scaling diagnostics that can provide credible results even in the non-tertiary care environment. Both conventional RT-PCR and TrueNat SARS-CoV-2 testing have been performed regularly in the Department of Microbiology, Government Medical College (GMC), Rajouri, since July 2020, which gives a chance to conduct a systematic comparison of the diagnostic performance of both methods. Therefore, the current research was conducted in order to conduct a comparative assessment of the analytical performance of chip-based real-time RT-PCR (TrueNat) against real-time RT-PCR, to detect the presence of SARS-CoV-2 infection. The aim of the study is to undertake an evaluation of the diagnostic accuracy, concordance and practical practicality of TrueNat platform as an alternative of molecular diagnostic tool in the region and resource- constrained settings.

MATERIAL AND METHODS

It was a cross-sectional observational study that was carried out in an ICMR-approved RT- PCR/TrueNat COVID-19 testing laboratory in the Department of Microbiology, GMC Rajouri. The study has used 400 randomly selected nasopharyngeal swabs collected between July 2020 and July 2022. The purpose of conducting the study was to compare the diagnostic quality of real-time RT-PCR with the use of conventional methods and chip-based real-time RT-PCR (TrueNat) to detect SARS-CoV-2 infection.

Inclusion Criteria

Samples that had been stored at the appropriate temperature and previously tested by real-time RT-PCR were included in the study. Only those samples with valid test results and adequate volume for repeat testing were selected to ensure accuracy and comparability between both testing platforms.

Exclusion Criteria

Samples that were insufficient in quantity or not stored at the appropriate temperature were excluded from the study. Samples showing evidence of contamination or degradation, or those that had undergone repeated freeze–thaw cycles, were also excluded to maintain analytical precision.

Sample Processing

The samples were handled under the standard precautions and infection-control practice in a biosafety level-2 (BSL-2) lab. All the samples were processed by laboratory personnel under proper protective conditions to avoid contamination and to be safe. The Cold chain integrity was observed throughout the period of receiving the samples until they were processed. All the samples were given a distinct identification number and aliquots were made to test using both methods. Samples were kept at -80 C till the analysis to maintain the integrity of RNA.

Conventional Real Time RT-PCR Test

An amplification/detection experiment of the amplification and detection of the E-gene of Sarbecovirus and RdRp gene of SARS-CoV-2 was done using an ICMR-approved kit through quantitative RT-PCR. The test was conducted in accordance with the instruction of the manufacturer. A real time PCR system was established to amplify the reaction using extracted RNA and amplification was done according to the thermal profile of the kit protocol. It took around 2.5 to 3 hours to accomplish the whole process. Samples that tested positive or E-gene and RdRp gene was identified between threshold cycle (Ct) range were considered positive. Samples where neither of the targets was amplified and an internal control was positive were considered negative.

True-Nat Testing

TrueNat is a chip-based RT-PCR that is utilized to detect SARS-CoV-2. This assay was conducted in two sequential tests; a screening test which was able to identify the E gene, and a confirmatory test which was able to identify the RdRp gene. RNA was isolated with the help of the TrueNat extraction module and inserted into the particular microchips to amplify the target genes. The amplification was performed automatically using 40 cycles and output of the amplification was presented on-screen in the shape of amplification curves. The positive sample was a sample on which the E gene and the RdRp gene were identified. TrueNat test had an overall turnaround time of 45-60 minutes.

Truenat Nucleic Acid Amplifier

The Truenat nucleic acid amplifier was used for the amplification of viral RNA. The extracted RNA was loaded onto chips pre-coated with primers and probes for the E gene and RdRp gene. The amplification process was fully automated and performed in a closed system. Each run underwent 40 thermal cycles, and amplification was monitored through real-time fluorescence detection. The software automatically generated the Ct value for each target gene. A sigmoidal amplification curve indicated a positive result, while the absence of fluorescence amplification indicated a negative sample.

Viral Load Evaluation Using Ct Value

The viral load in each sample was evaluated using the Ct values obtained from both TrueNat and conventional RT-PCR assays. The Ct value, defined as the number of cycles required for the fluorescent signal to cross the detection threshold, was inversely proportional to the viral RNA concentration. For analytical comparison, viral loads were categorized as follows: high viral load (Ct < 25), moderate viral load (Ct 25–30), low viral load (Ct 30–35), and very low viral load (Ct > 35). The concordance between TrueNat and conventional RT-PCR was determined by comparing the amplification results and analyzing the correlation between the Ct values of both methods.

Statistical Analysis

All the data were tabulated and processed with the help of statistical packages. The parameters calculated by the diagnostic parameters were: sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and the overall diagnostic accuracy of the TrueNat assay when compared to conventional RT-PCR as the reference parameter. The degree of agreement between the two tests was obtained due to the level of Cohen Kappa coefficient (K). The correlation coefficient (r) between the values of Ct recorded by both assays was determined by Pearson correlation test to assess the correlation of the two assays. P-value of less than 0.05 was taken to be statistically significant.

RESULTS

The diagnostic performance of the chip-based real-time RT-PCR (TrueNat) assay is evaluated against conventional real-time RT-PCR, which serves as the reference standard. A total of 400 nasopharyngeal samples are analyzed in this study.

Diagnostic Findings

Among the 400 samples tested, 51 (12.8%) are positive by the TrueNat assay and 349 (87.2%) are negative. When analyzed by the conventional RT-PCR method, 47 (11.8%) samples are positive and 353 (88.2%) are negative. All TrueNat-negative samples are also negative by conventional RT-PCR, demonstrating complete concordance for negative results. However, four samples that are positive by TrueNat yielded negative results on conventional RT-PCR (Table 1, Figure 1).

Table 1: Compares the cross-tabulated results of both diagnostic assays.

TrueNat Result	RT-PCR Positive	RT-PCR Negative	Total
TrueNat Positive	47	4	51
TrueNat Negative	0	349	349
Total	47	353	400

Figure 1 presents the graphical comparison of TrueNat and RT-PCR positivity rates across all tested samples, showing close agreement in overall detection pattern. Taking conventional RT- PCR as the gold standard, TrueNat achieves a sensitivity of 100%, specificity of 98.8%, and an overall diagnostic accuracy of 99%, confirming its reliability for SARS-CoV-2 detection.

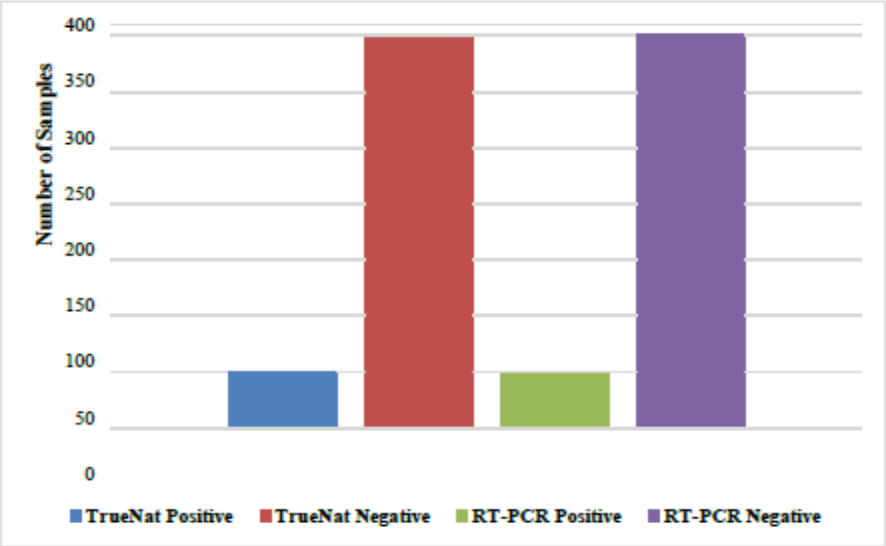

Figure 1. Comparison of overall positive and negative detection rates between TrueNat and conventional RT-PCR assays.

Statistical Evaluation

Predictive values and confidence intervals of the detailed diagnostic indices are presented in Table 2. Diagnostic indices indicate that TrueNat and conventional RT-PCR have an excellent agreement. The coefficient of Cohen Kappa ($\kappa = 0.97$) shows almost perfect concordance whereas the small 95% intervals of confidence prove statistical precision. The findings confirm the strength in the reliability of TrueNat results in a clinical test setting, highlighting its ability to be considered a reliable point-of-care molecular platform.

Table 2: Statistical evaluation of TrueNat assay compared with conventional RT-PCR.

S. No.	Statistic	Value	95 % CI
1	Sensitivity	100 %	92.4 – 100
2	Specificity	98.8 %	97.1 – 99.6
3	Positive Predictive Value	92.1 %	81.7 – 97.0
4	Negative Predictive Value	100 %	98.9 – 100
5	Diagnostic Accuracy	99%	97.9 – 99.7

Ct Value Correlation Between TrueNat and Conventional RT-PCR

A strong positive correlation is observed between the Ct values of both assays for the detection of the E gene and the RdRp gene (Figures 2 and 3). Among the 51 E-gene-positive samples detected by TrueNat, 46 are confirmed positive by RT-PCR. The correlation coefficient between their Ct values is $r = 0.97$ ($p < 0.001$), indicating a highly consistent amplification dynamic. The mean ± SD Ct value for the E gene is 21.62 ± 6.64 with TrueNat and 26.02 ± 7.19 with RT-PCR. Similarly, among the 51 RdRp-gene-positive samples detected by TrueNat, 47 are positive by RT-PCR. The correlation between Ct values for the RdRp gene is $r = 0.96$ ($p < 0.001$). The mean ± SD Ct for the RdRp gene is 23.40 ± 6.68 for TrueNat and 25.96 ± 7.20 for RT-PCR. These findings indicate that both platforms demonstrate comparable amplification efficiency, with TrueNat showing slightly lower mean Ct values, suggesting marginally higher analytical sensitivity. Figure 2 illustrates the linear regression analysis of E-gene Ct values between TrueNat and RT-PCR ($r = 0.97$, $p < 0.001$), and Figure 3 depicts the correlation plot for RdRp gene Ct values between the two assays ($r = 0.96$, $p < 0.001$).

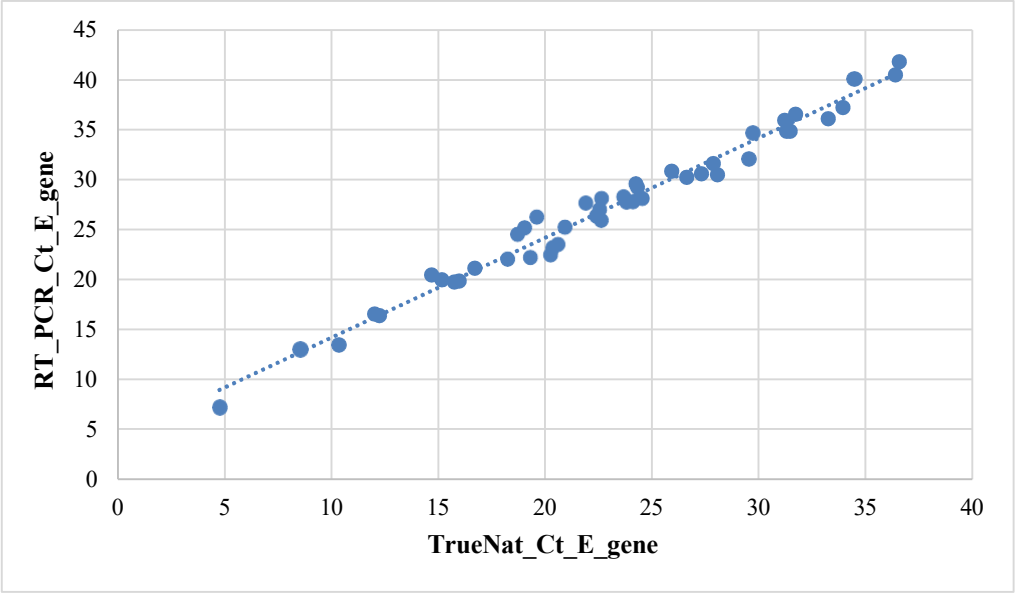

Figure 2. Linear regression analysis of Ct values for E gene between TrueNat and RT-PCR ($r = 0.97$, $p < 0.001$).

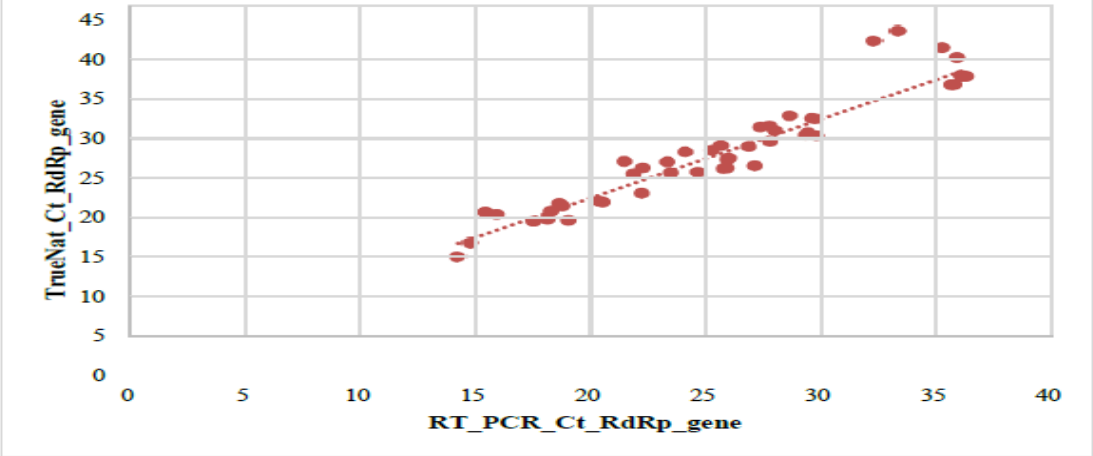

Figure 3. Linear regression analysis of Ct values for RdRp gene between TrueNat and RT- PCR ($r = 0.96$, $p < 0.001$).

Viral Load Distribution

To evaluate performance across viral loads, the Ct values are categorized into four groups: high (< 25), medium (25–30), low (30–35), and very low (> 35) viral loads. The positivity rates for E gene and RdRp gene detection by both assays are summarized in Table 3 and visualized in Figure 4.

Table 3: Positivity rate of E-gene and confirmatory RdRp-gene assays according to viral- load categories.

Viral Load Category	TrueNat (E gene)	TrueNat (RdRp gene)	Conventional RT- PCR (E gene)	Conventional RT- PCR (RdRp gene)
Very low viral load	4	1	6	2
Low viral load	16	12	–	–
Medium viral load	13	13	–	–
High viral load	25	25	–	–
Total	58	51	53	47

TrueNat detects a slightly higher number of positives in low and very-low viral-load groups compared with RT-PCR, while both methods show identical results in medium and high viral- load categories. The overall distribution pattern (Figure 4) emphasizes TrueNat's ability to identify additional low-copy samples that conventional RT-PCR occasionally misses.

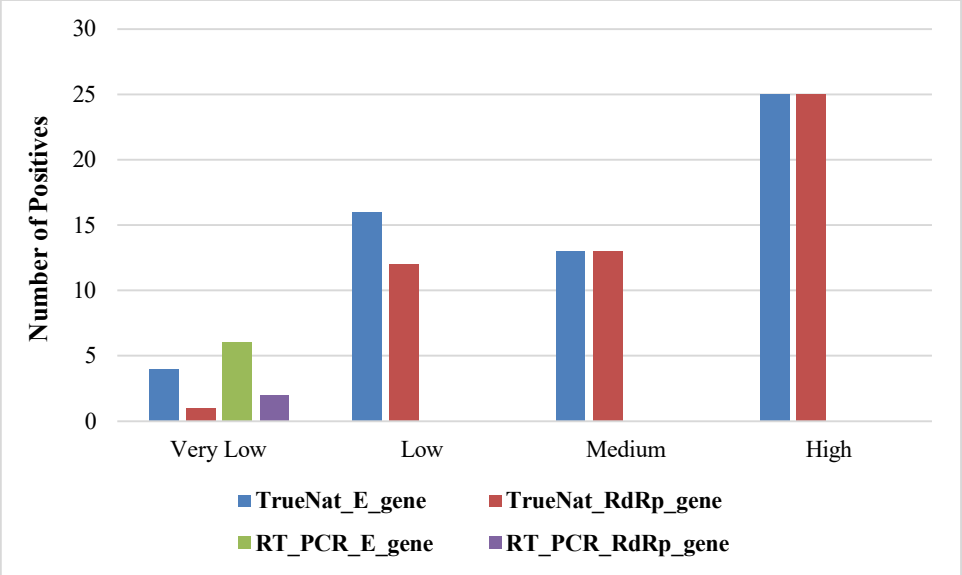

Figure 4. Distribution of E-gene and RdRp-gene positivity rates across viral-load categories for both assay

DISCUSSION

In this research, the chip-based real-time RT-PCR (TrueNat) assay was able to have a diagnostic performance equivalent to the conventional real-time RT-PCR, showing a sensitivity of 100, specificity of 98.8 and overall diagnostic accuracy of 99. The near consistency of Ct values of both assays (high concordance, 0.97) and the high correlation of the two confirm the analytical reliability and reproducibility of TrueNat as a diagnostic platform that can be used reliably to detect SARS-CoV-2. These results support the accumulating body of evidence that chip-based portable amplification systems can match the diagnostic performance of centralized molecular laboratories, in addition to providing a quicker turnaround and operational ability. The diagnostic concordance observed supported the validation studies that were performed in numerous independent cohorts. As Ghoshal et al. [14] have determined, the TrueNat assay has the same diagnostic sensitivity as traditional RT-PCR, and it is thus suitable in routine clinical laboratory

settings to screen SARS-CoV-2 infections. This validation was later extended by Chandwani et al. who showed that TrueNat retained similar detection efficiency with stored nasopharyngeal samples highlighting its strength under varying preanalytical conditions [15]. Likewise, Chakraborty et al. have also proved that TrueNat had equal throughput performance with high-throughput RT-PCR systems such as COBAS-6800 and much faster turnaround time, an aspect that has made the technology attractive as a rapid molecular architecture to be used in large-scale epidemiological surveillance [16].

The current findings are an extension of these results as they show that TrueNat not only matches conventional RT-PCR regarding its analytical sensitivity but also detects a small group of other positives not identified by conventional RT-PCR. These four TrueNat-positive and RT-PCR- negative samples probably represented those with borderline concentrations of the viral RNA within the detection limits of the standard RT-PCR assays. This further increased sensitivity on low-copy samples has been reported in previous multicentric analyses of the TrueNat system, which credited this to the optimization of microvolume thermocycling, as well as, minimized loss of RNA during closed-cartridge amplification. This current data is therefore an addition to the previous findings that TrueNat is of a high sensitivity in low viral-load specimen, which is vital in the early detection and isolation of infection. Correlation analyses of Ct values of e and rdrp genes showed near-linear relationships ($r = 0.97$ and $r = 0.96$, respectively), which showed that TrueNat assay was quantitatively equivalent to the reference RT-PCR method. This consistency of amplification dynamics proves that the microthermal control and fluorescence detection system of TrueNat has similar reaction kinetics as the benchtop RT-PCR systems. According to Chen et al., microfluidic chip PCR technologies maintain high quality of amplification fidelity due to improved efficiency of thermal transfer and reduced reaction variability [17]. The stability of the current research, then, is in line with these tenets in a mechanistic sense, which indicates that integrated microchip reactors can effectively measure viral load without affecting the accuracy of the analysis. Similarly, Hosokawa and Ohmori showed that chip-based digital RT-PCR assays can retain the same cycle accuracy as standard thermocyclers proving that miniaturized reaction systems can be reliable in detecting RNA virus [18].

The marginally reduced average Ct of TrueNat-positive samples relative to conventional RT- PCR (21.62 vs. 26.02 of E gene; 23.40 vs. 25.96 of RdRp gene) is indicative of higher amplification efficiency of TrueNat, potentially through smaller reaction volume, or workflow RNA-amplification. Shrestha et al. made a comparable interpretation of the on-chip MEDIC- PCR system that produced lower Ct distributions relative to standard RT-PCR, indicating improved template-reagent interaction in the miniaturized microfluidic channels [19]. Together, these results demonstrate that microchip amplification technologies do not just preserve the diagnostic accuracy of the technology, but can be even more effective in lower-copy viral RNA detection by having better reaction kinetics than traditional technologies. TrueNat is operationally advantageous in terms of biosafety, portability and turnaround time. Its aerosol-contamination-free, cartridge-based workflow allows testing in decentralized locations without the infrastructure needs of the biosafety level-2 lab, and has eliminated the risk of aerosols contamination. The given attributes are a direct reaction to the problem of diagnostics accessibility during the COVID-19 pandemic. Swarn et al. pointed out that the portability of the TrueNat system and its low level of operator reliance contributed to its special usefulness in the field-based and low-resource healthcare environment [20]. On the same note, Basawararajappa et al. indicated that the closed amplification system of TrueNat

caused less biohazard exposure and technical error and still delivered diagnostic quality that was the same as centralized molecular testing [21].

In addition to having a pandemic implication, the TrueNat performance profile highlights the overall trend to miniaturized, automated, and field-deployable molecular diagnostics. The development of microchip and digital PCR platforms is transforming the landscape of diagnosis by allowing an accurate quantification of nucleic acid in the point-of-care setting. The high concordance and reproducibility of this study confirms that this type of technology may provide a complement to current RT-PCR infrastructure as scalable means of infection disease response to any infectious disease outbreak and genomic surveillance programs in the future. Still, there are some constraints that deserve to be mentioned. TrueNat is limited in its ability to be applied in a high-demand laboratory environment due to its relatively small sample throughput (four samples per run). Also, its increased sensitivity increases ease of detection of the low-level of the virus, but further multicentric studies on an asymptomatic or post-symptomatic population would be necessary to confirm consistency. This comparison would be enhanced by extending to variant-specific assays and whole-genome load quantification to enable it to be applied to human cells in a translational manner. The current research paper has confirmed that the RT-PCR assay in the form of TrueNat chip had the same diagnostic performance as the conventional real-time RT-PCR in addition to being faster, safer, and flexible to field use. 1 The relationship between TrueNat and ct is high, its sensitivity is considerable, and it is able to detect low viral-load samples-this makes TrueNat a decentralized diagnostic method. With the world strategy of global health moving to the rapid molecular decentralization, microchip-based PCR systems like TrueNat are the future of specific diagnostics that can maintain the testing capacity even after the centralized laboratory system loses power.

CONCLUSION

The present study establishes that the chip-based real-time RT-PCR (TrueNat) assay performs with diagnostic precision equivalent to the conventional real-time RT-PCR for the detection of SARS-CoV-2. With a sensitivity of 100%, specificity of 98.8%, and an overall diagnostic accuracy of 99%, the TrueNat platform demonstrated excellent agreement ($\kappa = 0.97$) and strong correlation of Ct values for both *E* and *RdRp* genes ($r > 0.95$) compared with the gold-standard RT-PCR. These findings confirm that TrueNat is a reliable molecular tool capable of delivering high analytical sensitivity while substantially reducing processing time and biosafety risks. Beyond equivalence, the system exhibited enhanced detection capability in low and very low viral-load specimens, suggesting improved analytical response near the detection threshold. Its closed-cartridge design, automation, and portability enable safe, rapid, and decentralized testing, making it particularly valuable for resource-limited and peripheral healthcare settings where conventional RT-PCR infrastructure is not feasible. In the broader context of infectious-disease diagnostics, TrueNat exemplifies the emerging class of microchip-based nucleic acid amplification technologies that bridge the gap between laboratory precision and point-of-care accessibility. Integration of such platforms into national and global diagnostic networks could strengthen pandemic preparedness, improve early case identification, and expand equitable access to molecular testing. The findings of this study therefore support TrueNat as a practical, high-performing, and scalable diagnostic solution for ongoing and future infectious-disease surveillance efforts.

Declaration by Authors

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