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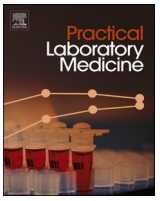

The potential relationship between EasyNAT system Tt values and Cobas z480 Ct values in the detection of SARS-Cov-2

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ABSTRACT

Objectives: This study aimed to evaluate the potential relationship between the time threshold (Tt) values of a commercial EasyNAT system, which is based on cross priming amplification (CPA) technology, and the cycle threshold (Ct) values of the Cobas z480 analyzer, which is based on a real-time fluorescence polymerase chain reaction (PCR) method, in the detection of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) from oropharyngeal swabs. Design and Methods: Data were retrospectively collected from a clinical laboratory between December 4, 2022 and July 1, 2024.

Results: A total of 277 EasyNAT-positive samples (Tt values from 3.83 to 29.5) were simultaneously investigated using the Cobas z480 analyzer (Ct values from 10.74 to 38.78). The concordance rate between the two systems was 100 %. Among the positive samples, the mean and maximum PCR Ct values of O and N genes increased in line with increasing Tt values of the left and right amplification areas of the EasyNAT system. The maximum Ct values of the O or N gene determined by the Cobas z480 analyzer were no more than 29.52 when the Tt values of the left or right amplification areas of the UC0116 analyzer were no more than 6.

Conclusions: The safe, simple, fast, accurate, and automatic EasyNAT system used in conjunction with a PCR system might be a better choice for the detection of SARS-CoV-2 in hospitals, especially in settings without sophisticated PCR facilities. The Tt value ($\leq$6) of the EasyNAT system can be a reference index for estimating the maximum Ct value (29.52) in SARS-CoV-2-positive samples.

1. Introduction

The accurate and rapid detection of SARS-CoV-2 is important for optimal treatment. Several methods have been developed for the detection of SARS-CoV-2, including methods based on nucleic acid technologies and non-nucleic acid technologies [1]. PCR methods are the molecular diagnostic gold standard; however, they require sophisticated equipment, skilled personnel, and involve complicated protocols that can be time-consuming [2]. The alternative technology of CPA is non-PCR based and can produce rapid and high-quality results to expedite the time taken to determine the appropriate treatment. It has been used for the detection of Gram-positive *Bacillus cereus*, giant salamander iridovirus, norovirus, rotavirus A, enteric adenovirus, and astrovirus [3–5]. The high sensitivity and specificity of target sequences enabled CPA technology to rapidly diagnose suspected SARS-CoV-2-infected patients

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during the COVID-19 pandemic. The EasyNAT SARS-CoV-2 Assay uses CPA to detect ORF1ab and N genes of the novel coronavirus (2019-nCoV) in infected patients [6]. The accurate and rapid SARS-CoV-2 molecular test used in the on-site EasyNAT system produced by Ustar Biotechnologies (Hangzhou) Ltd is used in more than 70 countries worldwide. However, few studies have examined the relationship between CPA Tt values and PCR Ct values in the detection of SARS-CoV-2.

2. Materials and methods

The UC0116 analyzer of the EasyNAT system detects fluorescence signals and produces curves from reactions involving specific amplification primers and fluorescent probes, extremely active reverse transcriptase, and DNA polymerase with strand displacement activity. Sample preparation and loading time is less than 1 min. Cell lysis, nucleic acid binding to magnetic beads, magnetic bead-nucleic acid cleaning, nucleic acid elution, and the amplification reaction are completed in a closed test cartridge within 1 h. In the cartridge, the left amplification area is designed to detect the ORF1ab gene and the right area is the N gene of SARS-CoV-2. The internal controls (ICs) are used to detect human *GAPDH* mRNA to monitor the whole CPA process. Regardless of IC results, the EasyNAT system reports a positive result if ORF1ab or N genes are positive. The mixed COVID-19-RNA Extraction Solution and 500 μl of oropharyngeal swab sample were added into the COVID-19 cartridge in a safety cabinet and the cartridge was then detected by the instrument. The COVID-19-negative and -positive controls in the kit were used to monitor possible reagent failure and malfunctions of the EasyNAT system. The time (min) that the fluorescent signal undergoes to reach the threshold is called the "time threshold (Tt)". These Tt values were obtained from the analyzer.

Nucleic Acid Isolation System EXM6000 (Zybio Inc. Chongqing) is based on a magnetic bead method of nucleic acid isolation and purification. Novel Coronavirus (2019-nCoV) Nucleic Acid Detection Kit was provided by Shanghai BioGerm Medical Technology Co., Ltd, and the Ct values of the O and N genes of 2019-nCoV were obtained by real-time fluorescence PCR using the Cobas z480 PCR analyzer (Roche) according to the manufacturer's instructions.

GraphPad Prism 9 software was used to perform statistical analysis.

3. Results

The EasyNAT SARS-CoV-2 Assay using the UC0116 analyzer detected 277 SARS-CoV-2-positive samples between December 4, 2022 and July 1, 2024. All of these samples were also positive by the Cobas z480 system. The positive samples were from 131 males and 146 females. Among them, the minimum age was 16 year, the maximum age was 99 year, and the mean age was 65.45 year.

As shown in Table 1 and Fig. 1, the maximum and mean Ct values of O and N genes determined by the Cobas z480 analyzer increased in line with both valid Tt values of the left and right amplification areas from the UC0116 analyzer, respectively. The mean PCR Ct values of the O and N genes of positive samples increased from 21.96 ± 3.77 to 30.8 ± 4.33 and from 19.71 ± 3.8 to 29.78 ± 4.43, respectively, in line with the increasing valid Tt values of the UC0116 analyzer. Student's t-test for unpaired measures was applied using GraphPad Prism 9 to compare the Ct values for O and N genes among the four groups ($Tt \leq 6$, $6<Tt<8$, $8\leq Tt \leq 10$, and $Tt > 10$). Any two group of them have statistically ($p < 0.05$), respectively. The maximum PCR Ct values of the O and N genes from positive samples increased from 29.52 to 38.78 and from 25.32 to 38, respectively, in line with the increasing valid Tt values of the UC0116 analyzer.

The UC0116 analyzer detected 15 samples with left amplification area invalid/right amplification area valid, and 18 samples with right amplification area invalid/left amplification area valid. Among the latter samples, two in which the O gene was detected by PCR with Ct values of 36.71 and 37.34, but the N gene was not detected.

4. Discussion

Reliable, accurate diagnostic testing is important for the detection of SARS-CoV-2 and patient management. In 2020, the World Health Organization called for the development of simple, highly specific, and easy-to-use detection platforms to interrupt SARS-CoV-2

Table 1
The relationship between Tt values of the UC0116 analyzer and the Ct values of the Cobas z480 among patients during the study period.

Tt values (Left)	Total (277)	Ct values for O gene			Tt values (Right)	Total (277)	Ct values for N gene		
		Min	Max	Mean ± SD			Min	Max	Mean ± SD
Tt≤6	45 (16.25 %)	11.82	29.52	21.96 ± 3.77	**Tt≤6**	17 (6.14 %)	10.74	25.32	19.71 ± 3.80
6<Tt<8	83 (29.96 %)	14.78	33.59	26.16 ± 3.91	**6<Tt<8**	54 (19.49 %)	17.47	32.82	24.33 ± 3.93
8≤Tt≤10	77 (27.80 %)	17.31	33.86	27.73 ± 4.19	**8≤Tt≤10**	93 (33.57 %)	14.77	35.02	27.13 ± 4.14
Tt>10	57 (20.58 %)	20.71	38.78	30.80 ± 4.33	**Tt>10**	95 (34.30 %)	18.27	38.00	29.78 ± 4.43
Left Invalid but Right Valid	15 (5.42 %)	21.38	37.00	33.46 ± 3.93	**Right Invalid but Left Valid**	18 (6.50 %)	18.38	37.03	30.39 ± 5.41

Left: left amplification area results of the UC0116 analyzer; Right: right amplification area results of the UC0116 analyzer.

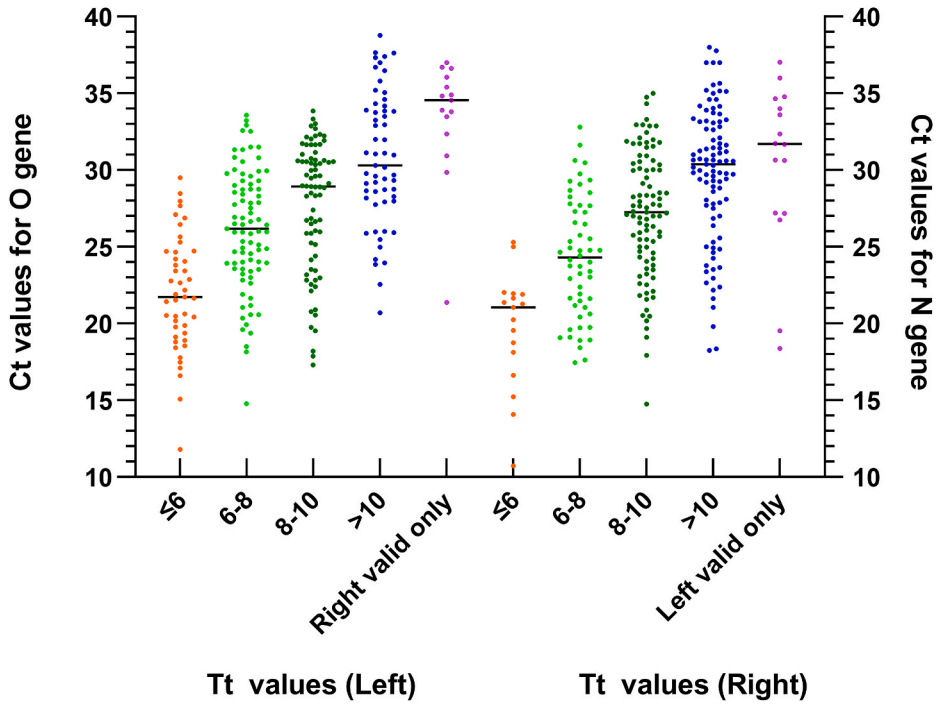

Fig. 1. Distribution of the Tt values of the UC0116 analyzer and the Ct values of the Cobas z480 of the patients in this study.

transmission and identify close contacts. As an internal policy of most hospitals in China, EasyNAT-positive specimens were routinely tested again using the Cobas z480 analyzer. Compared with Nucleic Acid Isolation System EXM6000 and the Cobas z480 analyzer, the Ustar EasyNAT Novel-Coronavirus Real-Time Molecular Diagnostic system (the UC0116 analyzer) integrates the processes of nucleic acid extraction, amplification, and detection, and it only requires a one-step operation to realize "sample in, result out". In the field of COVID-19 screening, the CPA technology advantages of simple operation and fast amplification make it an ideal candidate for point-of-care testing [7].

The EasyNAT system is a safe, simple, fast, accurate, and automatic analyzer for the detection of SARS-CoV-2. The concordance rate between test results from the UC0116 analyzer and the Cobas z480 system was 100 % in this study. Among the positive tests were 33 samples with only left or only right amplification area results using the UC0116 analyzer, and two samples with only the O gene detected using the Cobas z480 analyzer. These data indicate that the design of the UC0116 analyzer (with left and right amplification areas) successfully avoided false-negative results.

The retrospective data in this study can be applied to device-to-device comparisons and quality control of SARS-CoV-2 detection. In addition, based on a limit of 200 copies/ml in the initial phase, there was a good concordance rate between EasyNAT-negative and PCR-negative detection. Considering the advantages and disadvantages of both methods, the integration of the EasyNAT system with the Cobas z480 system may provide a new, effective tool for the detection of SARS-CoV-2.

We found a potential connection between the Ct and Tt values: the mean and maximum Ct values of the O and N genes determined by the Cobas z480 analyzer increased in line with increasing corresponding Tt values (for valid left and right amplification area results) determined using the UC0116 analyzer. The Ct values of the O and N genes above were statistically different among groups. We conclude that UC0116 analyzer Tt values ≤ 6 correlate with maximum Ct values (29.52) in positive samples. These data may help clinicians make a preliminary judgment for a patient's treatment. A study has shown that RT-PCR Ct values can predict the infectious status of COVID-19 patients, with an ideal cut-off of 30.4 for any significant secondary transmission [8].

We acknowledge the limitations of this study. First, a small number of samples were collected, and the results should be confirmed in other settings. Second, the Ct values were attained by analysis of the PCR amplification curve, which did not reach an ideal standardized status.

5. Conclusion

We believe that our study shows that integration of the EasyNAT system based on the CPA method with PCR testing has great potential in the detection of SARS-CoV-2. The UC0116 analyzer (CPA technology) Tt values ≤ 6 may provide a preliminary assessment index for significant secondary transmission of SRAS-CoV-2.

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CRediT authorship contribution statement

Lu-Qing Zheng: Writing – original draft, Investigation, Conceptualization. **Qing-Yong Wang:** Writing – review & editing, Supervision, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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