OPEN ACCESS Freely available online

Journal of Clinical Chemistry and Laboratory Medicine

Research Article

Comparison of the Performance of Six SARS-CoV-2 Nucleic Acid Detection Kit in Positive Samples Using RT-PCR

Jiankang Zhao[1*], Yulin Zhang[2], Bin Cao[3]

[1]Department of Respiratory Medicine, China-Japan Friendship Hospital, Beijing, China;[2]Department of Anesthesiology, Chinese Academy of Medical Sciences, Peking Union Medical College, Beijing, China;[3]Department of Respiratory Medicine, Capital Medical University, Beijing, China

ABSTRACT

Background: To date, several Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) nucleic acid detection kits are available in China. The virus might have a low load in specimens, resulting in false negative and under diagnosis. Therefore, it is urgently required to evaluate the sensitivities these commonly-used kits.

Methods: Six Real-time Transcription-PCR (RT-PCR) kits for SARS-CoV-2 manufactured in China were evaluated, namely, BGI, Sansure, DaAn, BioGerm, GeneoDx and Liferiver. We used 4 serial dilutions (10-fold) of 7 inactivated samples for the assay, collected from Coronavirus Disease 2019 (COVID-19) patients. Furthermore, the number of positive tests, Limit of Detection (LoD) and Cycle Threshold (CT) values were used to analyze their sensitivities.

Results: For all the 7 samples at original concentration, Samples 1-5 with 10-1 dilution, and Sample 1 with 10-2 dilutions, all 6 kits were positive. The sensitivities of the kits varied with the decreases of nucleic acid concentration. Among the 28 samples, BGI kit obtained 26 positive tests, followed by Sansure, DaAn, BioGerm, GeneoDx, and Liferiver, respectively. Furthermore, LoD of BGI kit was the lowest. Pairwise comparison of average Ct values of the above 6 kits revealed that BGI had the most significantly lower CT values for ORF 1ab gene, whereas Sansure had better performance for the detection of N gene.

Conclusions: All 6 kits can provide accurate detection results in the clinical samples with high viral loads. BGI kit was the most sensitive kits. Each kit had its own advantages and disadvantages, and further optimization is needed.

Keywords: RT-PCR; SARS-CoV-2; LoD; Sensitivity; CT value

INTRODUCTION

Coronavirus Disease 2019 (COVID-19), caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), had outbroken in Wuhan, China in December, 2019, and spread worldwide. The virus belonged to family with approximately 79% genome identity to SARS-CoV-2. The number of COVID-19 patients worldwide presently has skyrocketed. To control the further spread of the pandemic, the accurate and timely diagnosis based on the detection of SARS-CoV-2 in clinical samples, mainly nasopharyngeal swabs that often contain low viral load, is critical [1].

SARS-CoV-2 was identified from lower respiratory tract samples using Next-Generation Sequencing (NGS), which was often used in clinical diagnosis of infectious diseases. Nevertheless, high cost, long time and the complexity of sample preparation and data analysis limit its wide application [2]. Currently, RT-PCR is a widely-used tool in the identification of SARS-CoV-2. On January 21, 2020, Chinese Center for Disease Control and Prevention (China CDC) has issued the specific primers and probe sequences to detect the Open Reading Frame (ORF1ab) and nucleoprotein (N) gene regions of SARS-CoV-2 using RT-PCR. Since then, dozens of reagent manufacturers have developed nucleic acid detection kits [3]. As of January 31, 6 of these kits have been applied for the diagnosis of COVID-19 in

Corresponding Author: Jiankang Zhao, Department of Respiratory Medicine, China-Japan Friendship Hospital, Beijing, China, Tel no. 86-010-84206257; E-mail: zjk4265296@163.com

Received: April 20, 2021; **Accepted**: May 04, 2021; **Published**: May 11, 2021

Citation: Zhao J, Zhang Y, Cao B (2021) Comparison of the Performance of Six SARS-CoV-2 Nucleic Acid Detection Kit in Positive Samples Using RT-PCR. J Cliin Chem Lab Med. 4: 170.

China. The false negative rate of RT-PCR challenged the accurate diagnosis of COVID-19, which might be related to the quality of kits. The comparison and evaluation of these reagents should be performed. In this study, we compared the sensitivities of 6 nucleic acid detection kits for SARS-CoV-2 available in China [4].

MATERIALS AND METHODS

Biosafety procedures

Our study were performed in a biosafety level 2 laboratory maintained under negative pressure to surroundings. Virus inactivation of all samples had been conducted with water bath for 30 minutes before nucleic acid extraction. Nucleic acids extraction and preparation of PCR reagents were conducted in a class II, Type A2 biological safety cabinet. Furthermore, additional biosafety level 3 precautions, including protective laboratory clothing, gowns, goggles, N95 masks, double gloves, and shoe covers, were taken [5].

Study design and samples preparation

We collected 6 nasopharyngeal swabs and one Broncho Alveolar Lavage Fluid (BALF) from laboratory-confirmed COVID-19 patients, inactivated before, and nucleic acids were re-extracted and store at -80 °C for further study [6]. Cycle Threshold (CT) values, defined as the number of cycles required for the generated fluorescent signal to cross the threshold and positive rate of RT-PCR were used for comparing the effectiveness of different kits to detect these viral RNAs with serial dilutions.

Viral nucleic acid extraction

Viral Total Nucleic Acid Extraction kit (HEAS BioTech, Guangzhou, China) and the SMART32 system (Liferiver, Shanghai, China) were used for nucleic acid extraction in line with their instruction manuals by using an automated magnetic bead extraction method. Viral RNA extracted from 200 µl samples were transferred into a 1.5 ml Eppendorf tubes and stored at -80 °C until use [7].

RT-PCR analysis

In the current study, 6 nucleic acid detection kits for SARS-CoV-2 were evaluated, manufactured by DaAn, Liferiver, BioGerm, GeneoDx, BGI and Sansure respectively. Serial dilutions (from 100 to 10-3) of extracted viral RNA of 7 clinical samples by sterile nuclease-free water were used as templates. RT-PCR amplification was performed on LightCycler 480 II real-time PCR system [8]. Positive and negative control were provided by each kit and set for each PCR reaction. Furthermore, all reactions were performed in triplicate. Amplification conditions and interpretation of the results referred to the instructions of each kits, as shown in **Table 1**.

Kits	Batch number	Time (h: min)	Template (µl)	LoDb (copies/ml)	Cycling and programming information					
					In the manual	Actually detected	Reverse transcription	Transcriptase inactivation	Denaturing/ Extending	RT-PCR cycles
BGI	602020021 7	0.052778	10	None	500	50 oC/ 20min	95 oC/ 10min	95 oC/ 15s and 60 oC/ 30s	40	
Sansure	2020003	0.057639	5	200	1000	50 oC/ 30min	95 oC/ 1min	95 oC/ 15s and 60 oC/ 30s	45	
DaAn	2020018	0.065972	5	500	2000	50 oC/ 15min	95 oC/ 15min	94 oC/ 15s and 55 oC/ 45s	45	
BioGerm	20200210A	0.046528	5	1000	1000	50 oC/ 10min	95 oC/ 5min	95 oC/ 10s and 55 oC/ 40s	40	
GeneoDx	COV2020013	0.053472	5	500	8000	50 oC/ 5min	95 oC/ 20s	95 oC/ 10s and 57 oC/ 60s	45	
Liferiver	P2020020 3	0.041667	5	1000	8000	45 oC/ 10min	95 oC/ 3min	95 oC/ 15s and 58 oC/ 30s	40	

Table 1: Comparison of amplification time, the amount of template needed, detection limit, target genes and the cycling

and programming information between 6 nucleic acid detection kits for SARS-CoV-2.

Determination of detection limit

The Limit of Detection (LoD) of each kit was determined using positive control with known copy number. Briefly, SARS-CoV-2 nucleic acid from clinical sample was quantified using QX200 Droplet Digital PCR system and then diluted into different copy number with sterile nuclease-free water, including 200, 500, 1,000, 2,000, 4,000 and 8,000 copies/ml. A total of 20 parallel RT-PCR reactions were performed for each concentration of the positive control [9]. The LoD was defined as the lowest concentration at which 80% of the reactions or more obtained a positive test.

Statistics analysis

The CT values of RT-PCR were compared using one-way ANOVA analysis in order to assess the kit-to-kit variability of these 6 kits. When the differences were significant, pairwise comparisons of CT values were performed. Tukey's multiple comparison test was used for homogeneous variance, and independent samples t-test was used for heterogeneous variance [10]. All probabilities were 2-tailed, with statistical significance defined as P value lower than 0.05. All analyses were performed using SPSS Statistics version 21 (IBM).

RESULTS

Amplification conditions for each kits

We compared the amplification conditions of 6 nucleic acid detection kits for SARS-CoV-2. The cycle number of denaturing and annealing/extending was 45 for DaAn, GeneoDx and Sansure kits, and 40 for BioGerm, BGI and Liferiver kits, respectively. The total time of PCR amplification was calculated based on the reaction conditions of each kits and the heating and cooling rate of LightCycler 480 II real- time PCR system [11]. DaAn kit took the longest time for 1 hour and 35 minutes, followed by Sansure (1 hour 23 minutes), GeneoDx (1 hour 17 minutes), BGI (1 hour 16 minutes), GeneoDx (1 hour 07 minutes) and Liferiver (1 hour), respectively.

Comparison of the number of positive tests

In this study, a total of 84 tests were performed for each kit, including 7 original samples, 4 serial dilutions (from 100 to 10-3) of these samples, and 3 replicates for each dilution setting (**Table 2**). Finally, BGI kit obtained 26 positive tests, more than other kits (ranged from 13 to 20), as shown in Figure 1. For each SARS-CoV-2 nucleic acid sample at original concentration, all kits got positive tests. As the concentration decreases, fewer kits tested positive [12].

Kits	Number of positive tests				
	100	10-1	10-2	10-3	Total
BGI	7	7	7	5	26
Sansure	7	6	6	1	20
DaAn	7	7	5	1	20
BioGerm	7	6	2	0	15
GeneoDx	7	6	1	0	14
Liferiver	7	5	1	0	13

Table 2: Number of positive tests by RT-PCR in 4 serial dilutions (10-fold) of the 7 samples.

At the lowest concentration (10-3 dilution) of each sample, BGI, Sansure and DaAn kits obtained positive test for Sample 1, and only BGI kit got positive results for Sample 3, Sample 4, Sample 5 and Sample 7. At the 10-2 dilution, BGI got positive tests for all the 7 samples, Sansure, DaAn, BioGerm, GeneoDx and Liferiver got 6, 5, 2, 1 and 1 positive tests, respectively, and only BGI got positive results for Sample 6 and Sample 7. For the 10-fold diluted Sample 7, only BGI and DaAn kits got positive tests. As a result, BGI kit got the most positive tests at low concentrations, revealing its higher sensitivity [13].

LoD of each kit

The actual LoD for all kits are equal to or higher than those indicated in the instructions (**Table 1**). BGI kit had the lowest LoD, which was 500 copies/ml, followed by Sansure (1,000 copies/ml), BioGerm (1,000 copies/ml), DaAn (2,000 copies/ml), GeneoDx (8,000 copies/ml) and LifeRiver (8,000 copies/ml), respectively.

DISCUSSION

COVID-19 is spreading around the world at an extremely fast speed, and a rapid and accurate detection of the virus is crucial. RT-PCR was the most widely applicable method due to its convenience and high sensitivity. Dozens of SARS-CoV-2 kits based on RT-PCR have been manufactured in mainland China. This study is an attempt to compare the sensitivities of 6 most commonly-used kits for COVID-19 diagnosis. In the present study, we evaluated the amplification reaction time of each kits. DaAn kit required the longest reaction time, followed by Sansure and GeneoDx kits, and Liferiver kit took the shortest time. Therefore, in terms of reaction time, the Liferiver kit was more advantageous. To date, the diagnosis of COVID-19 is made mainly on the basis of nucleic acid detection from nasopharyngeal swabs. False negative test increases the workload and challenges clinical diagnosis. As reported in a previous study, among the 20 laboratory-confirmed COVID-19 patients, only 17 (70%) and 3 (15%) were positive for the first and second RT-PCR tests with nasopharyngeal swabs, respectively.

In our study, three replicates were performed for each sample at each serial dilution in order to get more credible results. It was important to note that for some samples, only one or two out of three replicates were positive even at original concentration of nucleic acids, as was the case with almost all kits, which showed a varied repeatability of different kits. Considering that we have

performed the same operation on the three repeated tests, this problem has to attract our great attention. According the New Coronavirus Pneumonia Prevention and Control Program of China, a negative nucleic acid test cannot rule out SARS-CoV-2 infection. Clinically, for patients who are highly suspected of SARS-CoV-2 infection, multiple examinations and sampling from different parts should be perform to confirm or exclude diagnosis. Sensitivity is one of the most important indicators for evaluating the performance of a kit. In this study, for all the 7 original samples, Sample 1-5 at 10-fold dilution, and high-concentration Sample 1 with 10-2 dilutions, positive results were obtained for all kits. However, with the concentration of nucleic acid decreased, the sensitivities of different kits showed differences. For the samples with lower concentrations, BGI kits had the best performance, followed by Sansure and DaAn.

The LoD of each kit were detected using positive control, whose viral load was quantified by droplet digital PCR. BGI kit had the lowest LoD, consistent with its higher sensitivity. The LoD of other 5 kits ranged from 1,000 to 8,000 copies/ml. GeneoDx and Liferiver kit had the highest LoD. Moreover, in line with our study, all kits had higher LoD than written in the manuals except for BioGerm kit, which may be partially explained by the clinical samples used in our study. The CT value is correlated to the amount of target RNA. A lower Ct value is interpreted as higher viral load. For testing on the same sample, the lower the CT value, the better the PCR sensitivity. In our study. BGI and Sansure had the most significantly lower Ct values for ORF1ab and N genes, respectively. Therefore, the two kits had higher sensitivities than other 4 kits. The differences in sensitivity between 6 kits may be related to the following factors. A previous study demonstrated that CT value can be affected by amplification efficiency, higher amplification efficiency can lead to lower CT value and higher sensitivity. The 6 kits included in this study were set at different times and temperatures for reverse transcription, transcriptase inactivation of reverse, denaturing and annealing/extending, which may correlate to the amplification efficiency. PCR instrument. Each kit has its own recommended instrument. Light Cycler 480 II real-time PCR system, which was used uniformly in this study, may slightly affect the amplification results of some kits.

For BGI kit, only ORF1ab gene is tested, the PCR conditions might be more specifically designed and therefore, it has the best sensitivity. However, this might lower its specificity, which is not evaluated in the present study. Sansure had higher sensitivity for N gene but relatively poor performance for ORF1ab gene. DaAn got more positive tests for ORF1ab gene but took the longest time. The sensitivity of GeneoDx and BioGerm kits should be improved. Liferiver got the lowest sensitivity but took the shortest time.

CONCLUSION

Our study is limited by several factors. First, the sample size is small. The conclusions should be interpreted with caution. Second, the specificity of the detection kits was not compared due to the rarity of clinical positive samples during the study period. Third, the RT-PCR system may affect experimental results to some extent, and almost each kit has its own recommendation. However, it is not practical to buy an instrument in order to use a nucleic acid detection kit, and the main purpose of this study is to assess the sensitivity of different kits, therefore, results produced by the same RT-PCR system are more likely to reflect the performance of different kits. In the current study, Roche Light Cycler 480 II was used for PCR detection, which is one of the most commonly-used SARS-CoV-2 detection PCR platforms in China and worldwide. Moreover, the parameters used on this system in the study were provided by the 6 manufactures. Finally, the contents and PCR conditions of the above kits are continuously optimized. More clinical samples and more kits with different serial Number should be included for further study. In conclusion, we evaluated the sensitivity of 6 SARS-CoV-2 nucleic acid detection kits. All kits are operator-friendly and provided accurate results in the samples with relatively high viral load. BGI kit demonstrated the highest sensitivity based on comparison of Ct values and number of positive tests. Sansure had better performance for detection of N gene. Each kit has its own advantages and disadvantages, further optimization is needed for all the 6 kits.

REFERENCES

1. Lu R, Zhao X, Li J, Niu P, Yang B, Wu H, et al. Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. The Lancet. 2020;395(10224):565-574.
2. Xiaojing W, Ying C, Xu H, Xin Y, Li Z, Fan W, et al. Co-infection with SARS-CoV-2 and Influenza A Virus in Patient with Pneumonia, China. Emerg Infect Dis. 2020;26(6):1324-1326.
3. Chu DKW, Pan Y, Cheng SMS, Hui KPY, Krishnan P, Liu Y, et al. Molecular Diagnosis of a Novel Coronavirus (2019-nCoV) Causing an Outbreak of Pneumonia. Clin Chem. 2020;66(4):549-555.
4. Tay JY, Lim PL, Marimuthu K, Sadarangani SP, Ling LM, Ang BSP, et al. De-isolating COVID-19 Suspect Cases: A Continuing Challenge. Clin Infect Dis. 2020;71(15):883-884.
5. Winichakoon P, Chaiwarith R, Liwsrisakun C, Salee P, Goonna A, Limsukon A, et al. Negative Nasopharyngeal and Oropharyngeal Swab Does Not Rule Out COVID-19. J Clin Microbiol. 2020.
6. Bai Y, Yao L, Wei T, Tian F, Jin DY, Chen L, et al. Presumed Asymptomatic Carrier Transmission of COVID-19. JAMA. 2020;323(14):1406-1407.
7. Ai JW, Zhang HC, Xu T, Wu J, Zhu MQ, Yu YQ, et al. Optimizing diagnostic strategy for novel coronavirus pneumonia, a multi-center study in Eastern China. medRxiv. 2020.
8. Zou X, Chang K, Wang Y, Li M, Zhang W, Wang C, et al. Comparison of the Cepheid Xpert Xpress Flu/RSV assay and commercial real-time PCR for the detection of influenza A and influenza B in a prospective cohort from China. Int J Infect Dis. 2019;80:92-97.
9. Chiu CH, Guu YK, Liu CH, Pan TM, Cheng W. Immune responses and gene expression in white shrimp, Litopenaeus vannamei, induced by Lactobacillus plantarum. Fish Shellfish Immunol. 2007;23(2): 364-377.
10. Jevsnik M, Ursic T, Zigon N, Lusa L, Krivec U, Petrovec M, et al. Coronavirus infections in hospitalized pediatric patients with acute respiratory tract disease. BMC Infect Dis. 2012;12:365.
11. Rose HL, Dewey CA, Ely MS, Willoughby SL, Parsons TM, Cox V, et al. Comparison of eight methods for the extraction of Bacillus atrophaeus spore DNA from eleven common interferents and a common swab. PLoS One. 2011;6:e22668.

12. Tang F, Hajkova P, Barton SC, Lao K, Surani MA. MicroRNA expression profiling of single whole embryonic stem cells. Nucleic Acids Res. 2006;34(2):e9.

13. Hilscher C, Vahrson W, Dittmer DP. Faster quantitative real-time PCR protocols may lose sensitivity and show increased variability. Nucleic Acids Res. 2005;33(21):e182.