6-1-2024

Effectiveness of a standardized quality control management procedure for COVID-19 RT-PCR testing: A large-scale diagnostic accuracy study in China

Mengyu Liu

Cuihong Tian
Edith Cowan University

Yequn Chen

Jinxiu Zhu

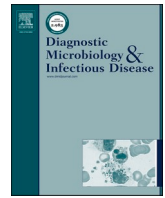Yan Zheng

See next page for additional authors

Authors

Mengyu Liu, Cuihong Tian, Yequn Chen, Jinxiu Zhu, Yan Zheng, Jianhua Chen, Zhen Li, Feng Xu, Liang Wu, Xingyu Wang, Longxu Xie, Xuerui Tan, and Yingmu Cai

Check for updates

Effectiveness of a standardized quality control management procedure for COVID-19 RT-PCR testing: a large-scale diagnostic accuracy study in China

Mengyu Liu [a,b,1], Cuihong Tian [a,c,d,1], Yequn Chen [c], Jinxiu Zhu [e,f], Yan Zheng [g], Jianhua Chen [h], Zhen Li [i], Feng Xu [i], Liang Wu [a,j,k], Xingyu Wang [a,l], Longxu Xie [b,h,***], Xuerui Tan [a,c,m,**], Yingmu Cai [b,i,*]

[a] Clinical Research Center, First Affiliated Hospital of Shantou University Medical College, Shantou, Guangdong 515041, PR China
[b] Joint Laboratory of Shantou University Medical College and Guangdong Hybribio Biotech Ltd., Shantou University Medical College, Shantou, Guangdong 515041, PR China
[c] Department of Cardiology, First Affiliated Hospital of Shantou University Medical College, Shantou, Guangdong 515041, PR China
[d] Center for Precision Health, Edith Cowan University, Perth, WA 6027, Australia
[e] Institute of Clinical Electrocardiography, First Affiliated Hospital of Shantou University Medical College, Shantou, Guangdong 515041, PR China
[f] Longgang Maternity and Child Institute of Shantou University Medical College, Shenzhen, Guangdong 518172, PR China
[g] Department of Research and Development, Guangdong Research Institute of Genetic Diagnostic and Engineering Technologies for Thalassemia, Chaozhou, Guangdong 521011, PR China
[h] Human Papillomavirus Molecular Diagnostic Engineering Technology Research Center, Chaozhou, Guangdong 521000, PR China
[i] Hybribio Medical Laboratory Group Ltd., Chaozhou, Guangdong 521000, PR China
[j] Shenzhen Key Laboratory of Single-Cell Omics, Shenzhen, Guangdong 518083, PR China
[k] BGI-Shenzhen, Shenzhen, Guangdong 518083, PR China
[l] Beijing Hypertension League Institute, Beijing 100043, PR China
[m] Phenomics Research Center, Shantou University Medical College, Shantou, Guangdong 515041, PR China

ARTICLE INFO

ABSTRACT

Background: The study aimed to construct a standardized quality control management procedure (QCMP) and access its accuracy in the quality control of COVID-19 reverse transcriptase-polymerase chain reaction (RT-PCR).
Methods: Considering the initial RT-PCR results without applying QCMP as the gold standard, a large-scale diagnostic accuracy study including 4,385,925 participants at three COVID-19 RT-PCR testing sites in China, Foshan (as a pilot test), Guangzhou and Shenyang (as validation sites), was conducted from May 21, 2021, to December 15, 2022.
Results: In the pilot test, the RT-PCR with QCMP had a high accuracy of 99.18% with 100% specificity, 100% positive predictive value (PPV), and 99.17% negative predictive value (NPV). The rate of retesting was reduced from 1.98% to 1.16%. Its accuracy was then consistently validated in Guangzhou and Shenyang.
Conclusions: The RT-PCR with QCMP showed excellent accuracy in identifying true negative COVID-19 and relieved the labor and time spent on retesting.

1. Introduction

As of July 12, 2023, the coronavirus disease 2019 (COVID-19) has spread to over 200 countries with approximately 768 million cumulative confirmed cases and 6.95 million cumulative deaths [1]. Although the

World Health Organization (WHO) declared the COVID-19 pandemic emergency over on May 5, 2023, it is still considered a global health threat [2–9]. There are still new scattered and small clustered cases in many places due to the existence of novel Omicron variants, *e.g.*, XBB and its subvariants, which accounted for 99.2% of cases in China on May

28, 2023 [10]. Therefore, reverse transcriptase-polymerase chain reaction (RT-PCR) is still significant in diagnosing COVID-19 cases, especially in countries with huge population bases like China.

The RT-PCR is the gold standard for the identification of genetic material of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in upper and lower respiratory specimens [11,12]. It has been widely applied in community screening and clinical diagnosis, effectively containing the transmission of the epidemic. As the gold standard for diagnosing COVID-19, RT-PCR can achieve 95% sensitivity and specificity [13]. Nevertheless, there are still 20%-67% false negative cases in practice [14–16]. A study involving 365 laboratories in 36 countries showed a false positive rate of 0.7% for COVID-19 RT-PCR testing [17]. Even this 0.7% false positive rate can heavily destroy the prevention and management of COVID-19 in countries with a huge population density, *e.g.*, China. Both false positive and false negative cases pose a huge challenge for the diagnosis and deployment of the epidemic [18,19]. Especially, false positive results are not only detrimental to the mental and physical health of misdiagnosed people, but also place a heavy burden on the economy and health care system. Therefore, ensuring the accuracy of COVID-19 RT-PCR testing, identifying the possible factors which result in false positive and false negative, and providing targeted intervention are beneficial to the prevention and control of the epidemic.

A complete quality control management system and a standardized handling procedure can guarantee the high quality of COVID-19 RT-PCR testing. To improve the quality of RT-PCR testing and reduce false positive and false negative rates, every procedure in the process of RT-PCR testing must be strictly controlled. Based on the long-term COVID-19 RT-PCR testing practices of Hybribio Medical Laboratory Group Ltd., we formulated a standardized quality control management procedure (QCMP) to eliminate the factors leading to false positive and false negative and assessed its performance in the quality control of COVID-19 RT-PCR testing in Foshan, China, as a pilot test. Afterwards, its accuracy was validated in two real-world scenarios, Guangzhou and Shenyang, China, aiming to improve the diagnostic accuracy of COVID-19 RT-PCR testing and prevent further spread of the epidemic.

2. Materials and Methods

2.1. Design, setting and participants

537,920 oropharyngeal samples from Foshan, 1,037,172 from Guangzhou, and 2,810,833 from Shenyang were collected in this diagnostic accuracy study from May 21, 2021 to December 15, 2022 in China to assess and validate the performance of RT-PCR with the application of QCMP in diagnosing COVID-19. Specifically, Foshan was a pilot site, while Guangzhou, the representative city in Southern China, and Shenyang, the representative city in Northern China, were validation sites. The Human Medical Ethics Committee of the First Affiliated Hospital of Shantou University Medical College approved this study and exempted the need for informed consents of participants because all samples were de-identified in the study (No. B-2021-263).

2.2. Results interpretation of RT-PCR

The open reading frame 1ab (ORF1ab) and gene coding for nucleo-capsid protein (N) are two targeted genomic sequences of SARS-CoV-2 in RT-PCR [20]. All detections were conducted in SLAN-96P/S RT-PCR system (Hongshi Medical Techonology Co., Ltd., Shanghai, China) with COVID-19 RT-PCR kits (Hybribio Biotech Co., Ltd., Guangdong, China) in initial RT-PCR testing. The samples were categorized into positive, indefinite, and negative cases according to the following interpretation principles of RT-PCR in the process of initial RT-PCR testing:

a. Positive: 10-35 cycle threshold (Ct) values and sigmoidal amplification curves of both ORF1ab and N genes.

b. Indefinite: <10 or >35 Ct values and abnormal curves of ORF1ab and (or) N gene(s).
c. Negative: undetermined Ct values and no sigmoidal amplification curves of both ORF1ab and N genes.

The quality management of negative samples shown in the initial RT-PCR testing were conducted by both Chinese Center for Disease Control (CDC) and Hybribio Medical Laboratories via random sampling inspection. For example, a negative sample was randomly picked out from each 96-well plates and re-inspected to evaluate the specificity and accuracy of RT-PCR, while the remined negative samples from each 96-well plates were reported as negative instantaneously, which is consistent with the sampling inspection standard of Chinese CDC. The specificity and accuracy of sampling inspection were 100% both in CDC and Hybribio Medical Laboratories. The positive and indefinite samples shown in the initial RT-PCR testing were retested by other kits produced by different manufacturers and only confirmed by the identical results being obtained by two different kits. Only the positive RT-PCR results shown in the retesting process were diagnosed as COVID-19. The RT-PCR kits used for retesting were listed in Supplementary Table S1.

2.3. Instructions for QCMP

To improve the quality and biological safety in COVID-19 RT-PCR testing, a standardized QCMP was formulated, which consisted of both pre-lab (specimen collection, transportation and storage) and in-lab workflows (RT-PCR operations and results interpretation). In the present study, owing to the intricate environmental characteristics and different infectious status among these three cities, the heavy requirements of large-scale nucleic acid detection on human, material and financial resources, as well as the difficulties in collecting two samples in one participant, only one sample was collected from each participant, and all samples were collected by using unified standardized swabs, preservation solution, and tubes to exclude the bias of pre-lab operations on RT-PCR results. Therefore, the difference between RT-PCR with QCMP and RT-PCR without QCMP lies in the in-lab protocol. The in-lab quality control measures and strict retesting criteria for positive and indefinite samples were particularly vital effect factors for the accuracy of QCMP. The detailed instructions of QCMP were listed in the Supplementary Materials.

2.4. RT-PCR without QCMP as the gold standard

In the first round RT-PCR, the samples were categorized into positive, indefinite and negative cases according to the interpretation principles, which reflected the real-world scenario without the influence of QCMP. In the second round RT-PCR, the QCMP was used to retest these samples. In this process, the negative samples shown in the first round RT-PCR were re-inspected via random sampling, while the positive and indefinite results indicated in the first round RT-PCR were retested by other brands of RT-PCR kits. Therefore, the first round RT-PCR testing without applying QCMP was served as a benchmark and considered the gold standard in the diagnostic accuracy study. The comparison of the RT-PCR results obtained with and without applying QCMP allowed for a direct evaluation of the actual effectiveness and improvement achieved by QCMP application.

2.5. Statistical analysis

To evaluate the performance of QCMP, the COVID-19 RT-PCR results were divided into two categories, no retest required (*i.e.*, the negative samples) and retest required (*i.e.*, the positive and indefinite or suspicious positive samples) [21]. Considering the RT-PCR results in the first round screening without QCMP as the gold standard, the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), accuracy, 95% confidence interval (95%CI), and Cohen's kappa

of RT-PCR with QCMP were calculated by applying an online statistical tool [22].

3. Results

To evaluate the performance of the QCMP, the RT-PCR results of 537,920 samples in the pilot site, Foshan, were analysed before and after the application of QCMP. The results of first round RT-PCR testing without QCMP were divided into positive, indefinite, and negative. The negative results were inspected via random sampling, while the positive and indefinite results were retested by other RT-PCR kits provided by different manufacturers. Of the 537,920 oropharyngeal samples in Foshan, 9,855 (1.83%) were positive, 772 (0.14%) were indefinite, and 527,293 (98.02%) were negative in initial RT-PCR testing (Table S2). Without the support of QCMP, 10,627 (1.98%) participants in Foshan were required to be retested. When the QCMP was applied, the rate of retesting was decreased to 1.16% in Foshan, showing the effective role of the QCMP in decreasing manpower and time input (Table 1). Additionally, the application of QCMP demonstrated 58.58% (95%CI, 57.63%-59.52%) sensitivity, 100.00% specificity, 100.00% PPV, 99.17% (95%CI, 99.15%-99.19%) NPV, and 99.18% (95%CI, 99.16%-99.21%) accuracy, with a Cohen's kappa of 0.735 in Foshan (Table 2).

The accuracy of QCMP in the quality control of COVID-19 RT-PCR was then validated in two mega cities, Guangzhou and Shenyang. Of the 1,037,172 oropharyngeal samples in Guangzhou, 25,333 (2.44%) were positive, 9,821 (0.95%) were indefinite, and 1,002,018 (96.61%) were negative in initial RT-PCR testing; of the 2,810,833 oropharyngeal samples in Shenyang, 17,754 (0.63%) were positive, 1,954 (0.07%) were indefinite, and 2,791,125 (99.30%) were negative in initial RT-PCR testing (Table S2). Without the support of QCMP, 35,154 (3.39%) participants in Guangzhou and 19,708 (0.70%) participants in Shenyang were required to be retested. When the QCMP was applied, the rate of retesting was decreased to 1.77% in Guangzhou and 0.32% in Shenyang (Table 1). In addition, the application of QCMP demonstrated 52.09% (95%CI, 51.56%-52.61%) sensitivity, 100.00% specificity, 100.00% PPV, 98.35% (95%CI, 98.33%-98.36%) NPV, and 98.38% (95%CI, 98.35%-98.40%) accuracy, with a 0.677 Cohen's kappa in Guangzhou; 45.62% (95%CI, 44.92%-46.31%) sensitivity, 100.00% specificity, 100.00% PPV, 99.62% (95%CI, 99.61%-99.62%) NPV, and 99.62% (95%CI, 99.61%-99.63%) accuracy, with a Cohen's kappa of 0.625 in Shenyang and a *P*-value under 0.001 (Table 2), suggesting a perfect accuracy of RT-PCR with the application of QCMP in real-world environments.

Table 1
COVID-19 RT-PCR results before and after the application of QCMP, classified as retest required and no retest required.

After application of QCMP		Initial RT-PCR results without QCMP		Total
		Retest required[a]	No retest required[b]	
Foshan	Retest required[a]	6,225	0	6,225
	No retest required[b]	4,402	527,293	531,695
	Total	10,627	527,293	537,920
Guangzhou	Retest required[a]	18,310	0	18,310
	No retest required[b]	16,844	1,002,018	1,018,862
	Total	35,154	1,002,018	1,037,172
Shenyang	Retest required[a]	8,990	0	8,990
	No retest required[b]	10,718	2,791,125	2,801,843
	Total	19,708	2,791,125	2,810,833

[a] Retest required: positive and indefinite
[b] No retest required: negative

Table 2
Evaluation for the diagnostic accuracy of QCMP.

	Foshan	Guangzhou	Shenyang
Sensitivity	58.58% (95%CI, 57.63%-59.52%)	52.09% (95%CI, 51.56%-52.61%)	45.62% (95%CI, 44.92%-46.31%)
Specificity	100%	100%	100%
Youden index	0.5858	0.5209	0.4562
PPV	100%	100%	100%
NPV	99.17% (95%CI, 99.15%-99.19%)	98.35% (95%CI, 98.33%-98.36%)	99.62% (95%CI, 99.61%-99.62%)
Accuracy	99.18% (95%CI, 99.16%-99.21%)	98.38% (95%CI, 98.35%-98.40%)	99.62% (95%CI, 99.61%-99.63%)
Cohen's kappa	0.735	0.677	0.625
P-value	<0.001	<0.001	<0.001
Prevalence	1.98% (95%CI, 1.94%-2.01%)	3.39% (95%CI, 3.35%-3.42%)	0.70% (95%CI, 0.69%-0.71%)

Abbreviations: PPV, Positive predictive value; NPV, Negative predictive value; 95%CI, 95% confidence interval.

4. Discussion

RT-PCR is the gold standard for the detection of SARS-CoV-2 genetic material, and it is the most commonly used method in mass COVID-19 screening [23]. However, the false positive and false negative results have been often reported in the mass community screening, causing huge panic in citizens and affecting the prevention and control strategies in combating COVID-19. Therefore, a standardized operation procedure of RT-PCR testing is necessary to reduce the false positive and false negative cases.

Both pre-lab and in-lab factors can contribute to suspicious results. The factors giving rise to the false negative of RT-PCR testing mainly attribute to the method and timing of sampling. Study showed that the positive rate in nasopharyngeal swabs is higher than that of in oropharyngeal swabs (65% vs. 22.5%) and that positive rate dropped further for oropharyngeal swabs in the later stages of COVID-19 infection (65% vs. 2.5%) [24]. The false negative rate also varies from infection exposure, onset of clinical symptoms to recovery period. A systematic retrospective study showed a U-shaped change of COVID-19 false negative rate, *i.e.,* 100% on the first day of infection, 67% on the fourth day, 38% on the fifth day, 20% on the eighth day, and 66% on the twenty-first day [16]. In addition, virus mutations can also contribute to false negative results. For example, a case with repeated negative RT-PCR tests due to virus mutations was reported in Guangzhou, China in February 2020 [25]. Other factors such as a long-time storage and long-distance transportation for specimens, mismatch between extraction reagents and amplification reagents, and non-standard operation also increase the false negative rate. In contrast, contamination in the process of specimen collection and detection, such as pollution of reagents, amplification products, aerosol, and clonal plasmid, is the main reason contributing to false positive of COVID-19 [26].

To minimize the false negative and false positive results, we listed the key points of pre-lab and in-lab operations that merit close attention and formulated a detailed QCMP for laboratory workflow including the instructions of samples collection, storage and transportation, and the corresponding retesting criteria and strategies. Subsequently, the effectiveness of QCMP was tested in Foshan and validated in Guangzhou and Shenyang in routine daily tests and several large-scale community screenings. The RT-PCR results of COVID-19 screened in Chinese communities revealed that the application of QCMP reduced the retesting rate from 1.98% to 1.16% in Foshan, from 3.39% to 1.77% in Guangzhou, and from 0.70% to 0.32% in Shenyang. These data suggested that the application of QCMP is both labor-saving and time efficient.

The specificity and accuracy of QCMP were perfect, which indicated that QCMP was excellent in correctly identifying negative cases, *i.e.,* true negatives. While the sensitivity of QCMP was unsatisfactory, revealing

that it was not good at recognizing positive cases, *i.e.,* true positives. However, this cannot be problematic, because the goal of QCMP was to identify all negative cases accurately, which can be reported directly, while these samples being identified as true positive and false negative cases, need to be retested for further assessment.

This study was conducted as a pilot in Foshan and further validated in representative cities in both Southern China (Guangzhou) and Northern China (Shenyang), aiming to assess the generalizability and applicability of QCMP in settings with variable testing conditions, laboratory practices, and geographical environmental characteristics. The results of the study revealed that QCMP exhibited effective and reliable performance in ensuring the accuracy and precision of COVID-19 RT-PCR testing, highlighting its potential for widespread implementation and adoption in diverse healthcare settings across China.

However, a limitation in this study should be noted. The QCMP was formulated based on the situation and practice of large-scale COVID-19 screening in China, and only tested and validated in three Chinese cities, whether it keeps the perfect accuracy worldwide remains to be further investigated.

5. Conclusions

In conclusion, we formulated a standard QCMP covering every step of COVID-19 RT-PCR testing by summarizing potential factors that caused the false positive and false negative cases in COVID-19 RT-PCR testing and tested its performance in the quality control of COVID-19 RT-PCR in Foshan as a pilot test. Subsequently, it was validated in two cities, Guangzhou and Shenyang. This study revealed that QCMP not only relieved labor and time spent on retesting, but also showed a high accuracy in COVID-19 RT-PCR testing.

Data availability

The dataset generated and analyzed during the study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The Human Medical Ethics Committee of the First Affiliated Hospital of Shantou University Medical College approved this study and exempted the need for informed consents of participants because all samples were de-identified in the study (No. B-2021-263). All methods were carried out in accordance with relevant regulations and guidelines.

CRediT authorship contribution statement

Mengyu Liu: Writing – original draft, Conceptualization, Data curation, Visualization. **Cuihong Tian:** Formal analysis, Writing – original draft, Conceptualization, Visualization. **Yequn Chen:** Writing – review & editing. **Jinxiu Zhu:** Writing – review & editing. **Yan Zheng:** Data curation. **Jianhua Chen:** Data curation. **Zhen Li:** Data curation. **Feng Xu:** Data curation. **Liang Wu:** Data curation. **Xingyu Wang:** Writing – review & editing. **Longxu Xie:** Supervision. **Xuerui Tan:** Funding acquisition, Project administration, Supervision, Writing – review & editing. **Yingmu Cai:** Conceptualization, Data curation, Methodology, Project administration, Resources, Supervision, Writing – review & editing.

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.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.diagmicrobio.2024.116287.

References

[1] WHO. Coronavirus (COVID-19) dashboard - Global Situation WHO. WHO 2023. Available from https://covid19.who.int/.

[2] Lenharo M. WHO declares end to COVID-19's emergency phase. Nature 2023. https://doi.org/10.1038/d41586-023-01559-z.

[3] Tian C, Balmer L, Tan X. COVID-19 lessons to protect populations against future pandemics by implementing PPPM principles in healthcare. EPMA J 2023;14(3):329–40. https://doi.org/10.1007/s13167-023-00331-7.

[4] Bakar Siddiq M, Rasker J. COVID-19, long COVID, and psychosomatic manifestations: a possible burden on existing rheumatology facilities. Heart Mind 2022;6(3):195–202. https://doi.org/10.4103/hm.hm_63_21.

[5] Li J, Sun L, Wang F, Liu B, Li H, Tang G, et al. Relation between cardiac injury and elevated levels of inflammatory biomarkers in patients with severe COVID-19. CVIA 2021;5(3):165–72. https://doi.org/10.15212/cvia.2019.1264.

[6] Lv T, Meng F, Yu M, Huang H, Lin X, Zhao B. Defense of COVID-19 by Human Organoids. Phenomics 2021;1(3):113–28. https://doi.org/10.1007/s43657-021-00015-0.

[7] Nicholson JK. Molecular phenomic approaches to deconvolving the systemic effects of SARS-CoV-2 infection and post-acute COVID-19 syndrome. Phenomics 2021;1(4):143–50. https://doi.org/10.1007/s43657-021-00020-3.

[8] Huang S, Wang Y, Zhou Z, Yu Q, Yu Y, Yang Y, et al. Distribution atlas of COVID-19 pneumonia on computed tomography: a deep learning based description. Phenomics 2021;1(2):62–72. https://doi.org/10.1007/s43657-021-00011-4.

[9] Molina-Mora JA, González A, Jiménez-Morgan S, Cordero-Laurent E, Brenes H, Soto-Garita C, et al. Clinical profiles at the time of diagnosis of SARS-CoV-2 infection in costa rica during the pre-vaccination period using a machine learning approach. Phenomics 2022;2(5):312–22. https://doi.org/10.1007/s43657-022-00058-x.

[10] CDC C. Prevalence of COVID-19 in China. Chinese CDC 2023. Available from https://www.chinacdc.cn/jkzt/crb/zl/szkb_11803/jszl_13141/202306/t20230611_266656.html.

[11] Esbin MN, Whitney ON, Chong S, Maurer A, Darzacq X, Tjian R. Overcoming the bottleneck to widespread testing: a rapid review of nucleic acid testing approaches for COVID-19 detection. RNA 2020;26(7):771–83. https://doi.org/10.1261/rna.076232.120.

[12] Goudouris ES. Laboratory diagnosis of COVID-19. J Pediatr (Rio J) 2021;97(1):7–12. https://doi.org/10.1016/j.jped.2020.08.001.

[13] Watson J, Whiting PF, Brush JE. Interpreting a covid-19 test result. BMJ (Clinical research ed) 2020;369:m1808. https://doi.org/10.1136/bmj.m1808.

[14] Wang W, Xu Y, Gao R, Lu R, Han K, Wu G, et al. Detection of SARS-CoV-2 in different types of clinical specimens. JAMA 2020;323(18):1843–4. https://doi.org/10.1001/jama.2020.3786.

[15] Sethuraman N, Jeremiah SS, Ryo A. Interpreting diagnostic tests for SARS-CoV-2. JAMA 2020;323(22):2249–51. https://doi.org/10.1001/jama.2020.8259.

[16] Kucirka LM, Lauer SA, Laeyendecker O, Boon D, Lessler J. Variation in false-negative rate of reverse transcriptase polymerase chain reaction-based SARS-CoV-2 tests by time since exposure. Ann Intern Med 2020;173(4):262–7. https://doi.org/10.7326/m20-1495.

[17] Matheeussen V, Corman VM, Donoso Mantke O, McCulloch E, Lammens C, Goossens H, et al. International external quality assessment for SARS-CoV-2 molecular detection and survey on clinical laboratory preparedness during the COVID-19 pandemic, April/May 2020. Euro Surveill 2020;25(27):2001223. https://doi.org/10.2807/1560-7917.Es.2020.25.27.2001223.

[18] Dramé M, Tabue Teguo M, Proye E, Hequet F, Hentzien M, Kanagaratnam L, et al. Should RT-PCR be considered a gold standard in the diagnosis of COVID-19? J Med Virol 2020;92(11):2312–3. https://doi.org/10.1002/jmv.25996.

[19] Teymouri M, Mollazadeh S, Mortazavi H, Naderi Ghale-Noie Z, Keyvani V, Aghababaei F, et al. Recent advances and challenges of RT-PCR tests for the diagnosis of COVID-19. Pathol Res Pract 2021;221:153443. https://doi.org/10.1016/j.prp.2021.153443.

[20] NHCOTPsRO China. Protocol for prevention and control of COVID-19 (Edition 6). China CDC Wkly 2020;2(19):321–6. https://doi.org/10.46234/ccdcw2020.082.

[21] Cai Y, Liu M, Wu Z, Tian C, Qiu S, Li Z, et al. Diagnostic accuracy of autoverification and guidance system for COVID-19 RT-PCR results. EPMA J 2022;14(1):119–29. https://doi.org/10.1007/s13167-022-00310-4.

[22] Ltd MS. Diagnostic test evaluation calculator (Version 20.2). MedCalc Software Ltd 2024. Available from https://www.medcalc.org/calc/diagnostic_test.php.

[23] Benda A, Zerajic L, Ankita A, Cleary E, Park Y, Pandey S. COVID-19 testing and diagnostics: a review of commercialized technologies for cost, convenience and quality of tests. Sensors (Basel) 2021;21(19):6581. https://doi.org/10.3390/s21196581.

[24] Liu M, Li Q, Zhou J, Ai W, Zheng X, Zeng J, et al. Value of swab types and collection time on SARS-COV-2 detection using RT-PCR assay. J Virol Methods 2020;286:113974. https://doi.org/10.1016/j.jviromet.2020.113974.

[25] Li Z, Li Y, Chen L, Li S, Yu L, Zhu A, et al. A Confirmed Case of SARS-CoV-2 pneumonia with negative routine reverse transcriptase-polymerase chain reaction and virus variation in Guangzhou, China. Clin Infect Dis 2021;73(2):e426–ee33. https://doi.org/10.1093/cid/ciaa941.

[26] Martinez RJ, Pankratz N, Schomaker M, Daniel J, Beckman K, Karger AB, et al. Prediction of false-positive severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) molecular results in a high-throughput open-platform system. J Mol Diagn 2021;23(9):1085–96. https://doi.org/10.1016/j.jmoldx.2021.05.015.