Potential False-Negative Nucleic Acid Testing Results for Severe Acute Respiratory Syndrome Coronavirus 2 from Thermal Inactivation of Samples with Low Viral Loads

Yang Pan,[a,b,c,†] Luyao Long,[d,e,f,†] Daitao Zhang,[a,b,c] Tingting Yuan,[g] Shujuan Cui,[a,b,c] Peng Yang,[a,b,c] Quanyi Wang,[a,b,c,*] and Simei Ren[d,e,f,*]

BACKGROUND: Coronavirus disease-2019 (COVID-19) has spread widely throughout the world since the end of 2019. Nucleic acid testing (NAT) has played an important role in patient diagnosis and management of COVID-19. In some circumstances, thermal inactivation at 56°C has been recommended to inactivate severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) before NAT. However, this procedure could theoretically disrupt nucleic acid integrity of this single-stranded RNA virus and cause false negatives in real-time polymerase chain reaction (RT-PCR) tests.

METHODS: We investigated whether thermal inactivation could affect the results of viral NAT. We examined the effects of thermal inactivation on the quantitative RT-PCR results of SARS-CoV-2, particularly with regard to the rates of false-negative results for specimens carrying low viral loads. We additionally investigated the effects of different specimen types, sample preservation times, and a chemical inactivation approach on NAT.

RESULTS: Our study showed increased Ct values in specimens from diagnosed COVID-19 patients in RT-PCR tests after thermal incubation. Moreover, about half of the weak-positive samples (7 of 15 samples, 46.7%) were RT-PCR negative after heat inactivation in at least one parallel testing. The use of guanidinium-based lysis for preservation of these specimens had a smaller impact on RT-PCR results with fewer false negatives (2 of 15 samples, 13.3%) and significantly less increase in Ct values than heat inactivation.

CONCLUSION: Thermal inactivation adversely affected the efficiency of RT-PCR for SARS-CoV-2 detection. Given the limited applicability associated with chemical inactivators, other approaches to ensure the overall protection of laboratory personnel need consideration.

Introduction

The spread of new pathogen severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has caused an expanding pandemic of coronavirus disease-2019 (COVID-19) *(1–4)*. As of March 10, 2020, the global number of confirmed cases of COVID-19 had surpassed 118 000 with more than 4292 deaths due to acute respiratory failure or other related complications *(5)*. Most cases (68.42%) occurred in China; outside China, a total of 37 371 cases of COVID-19 has been confirmed in 114 countries/territories/areas with 1130 deaths *(5)*. To allow prompt patient identification and clinical treatment, the Chinese government has released seven successive editions of Guidelines for Diagnosis and Treatment of COVID-19 since January 15, 2020. Laboratory viral nucleic acid testing (NAT) has been recommended as a gold standard for COVID-19 diagnosis, together with serological examination *(6)*. NAT

[a] Institute for Infectious Disease and Endemic Disease Control, Beijing Center for Disease Prevention and Control, Beijing, P. R. China; [b] Research Centre for Preventive Medicine of Beijing, Beijing, China; [c] School of Public Health, Capital Medical University, Beijing, China; [d] National Center for Clinical Laboratories, Beijing Hospital, National Center of Gerontology, Institute of Geriatric Medicine, Chinese Academy of Medical Sciences, Beijing, China; [e] Graduate School, Chinese Academy of Medical Sciences, Peking Union Medical College, Beijing, China; [f] Beijing Engineering Research Center of Laboratory Medicine, Beijing Hospital, Beijing, China; [g] Department of Clinical Laboratory, Beijing Hospital, National Center of Gerontology, Beijing, China

* Address correspondence to: S.R. at Institute for Infectious Disease and Endemic Disease Control, Beijing Center for Disease Prevention and Control, National Center for Clinical Laboratories, Beijing Hospital, No.1 Dahua Road, Dongdan, Beijing 100730, China. Fax +86-010-65132968; e-mail rensimei4162@bjhmoh.cn. Q.W. at No.16 Heping Li Middle St, Beijing 100013, China. Fax +86-010-64407122; e-mail bjcdcxm@126.com.

[†] Yang Pan and Luyao Long contributed equally to the work.

Received March 6, 2020; accepted March 23, 2020.
DOI: 10.1093/clinchem/hvaa091

shows better performance than antibody assays as it can identify the viral RNA in the early stage of infection, even during the incubation period. Since 1988, polymerase chain reaction (PCR) technology has been used extensively in clinical examination with the prominent advantages of high sensitivity, convenience, and economy *(7)*.

Currently, more than 100 enterprises in China have developed real-time PCR (RT-PCR) detection kits for COVID-19, and 9 of these kits have been approved by National Medical Products Administration (NMPA) of China *(8)*. In clinical trials subjected to the NMPA approval procedure, all kits exhibited a high sensitivity of over 90% with samples from confirmed cases. However, in real clinical settings, the positive diagnosis rates of suspected patients were not as high as previously evaluated *(9)*. Many suspected patients exhibited typical clinical symptoms or imaging studies consistent with pneumonia, but were not found positive in RT-PCR testing. This raised the question of what caused these missing positive results in clinical practice?

Given the high quality of NMPA approved kits, false-negative test results were less likely to have arisen from the procedures of nucleic acid extraction and detection. The types of samples collected clinically appeared to be one of the main causes of the lower performance of RT-PCR tests than expected *(10)*, but other factors need to be considered, particularly appropriate sample pretreatments before testing.

Considering that SARS-CoV-2 is a single-stranded RNA, isolation of its genome requires cautious handling of samples and good laboratory practices. Owing to the contagiousness of SARS-CoV-2, many Chinese laboratories have inactivated the virus at 56–60°C for 30–60 min before RNA extraction. Thermal inactivation at 56°C has also been recommended to ensure the security of medical inspectors in the Chinese expert's consensus *(11)*. The consensus also raises the uncertainty of whether heat inactivation may decrease the sensitivity of NAT *(11)*. Although it is suspected that such pretreatment may affect the detection of SARS-CoV-2 in samples with low viral loads, quantitative comparisons on its impact are still unclear. Herein, we investigated the effects of thermal inactivation on the quantitative RT-PCR results of SARS-CoV-2 and evaluated the false-negative rates due to thermal inactivation. We further investigated the effects of different specimen types, sample preservation times, and a chemical inactivation approach on NAT.

Materials and Methods

SOURCES OF SPECIMEN

Twenty-three confirmed patients with SARS-CoV-2 infection in Beijing were included in this study. The criteria for confirmed cases with SARS-CoV-2 infection included: *(1)* A positive result of the nucleic acid sequence of SARS-CoV-2 by real-time RT-PCR or *(2)* the virus gene sequence being highly homologous to the known SARS-CoV-2 *(12)*. A total of 19 throat swabs, 2 sputum samples, and 2 stool samples were collected from enrolled patients when diagnosed. Four throat swabs and sputum samples were stored in 3 mL standard virus transport media (VTM) (modified Hank's buffer, Yocon, China). The other 15 weak positive throat swabs were stored in 3 mL VTM or 3 mL chemically inactivated buffer (lysis buffer, Qiagen) and the stool samples were kept in the sampling tube. All samples were kept at 4°C and transferred to the laboratory within 4 h.

SPECIMEN DILUTION AND INACTIVATION

The throat swabs were serially diluted using VTM or chemically inactivated buffer. The sputum samples were treated with an equal volume of Sputasol (ThermoFisher) before serial dilution. For stool samples, 3 mL VTM were used to resuspend the sample and the supernatant was collected for the subsequent dilution. All samples were inactivated by incubation in a water bath at 56°C for 30 min.

RNA EXTRACTION AND QUANTITATIVE RT-PCR ASSAY

Samples were pretreated with heat incubation, followed by RNA extraction. Viral RNA purification kit (QIAamp Viral RNA Mini Kit, Qiagen) was used as instructed by the manufacturer. For all RNA extractions, RNA was extracted from 140 μL of sample and eluted in 60 μL elution buffer containing poly(A) carrier RNA. The quantitative RT-PCR assay was performed by TaqMan Fast Virus 1-Step Master Mix (ThermoFisher) in an ABI 7500 fast system. 0.5 μmol/L of forward primer, 0.5 μmol/L of reverse primer, 0.25 μmol/L of probe, and 5 μl of RNA sample were mixed in a 25 μL monoplex quantitative RT-PCR reaction. The primer and probe were generated following the national guidelines *(12)*, and the reaction conditions were set according to the manufacturer's protocol. All reactions were run in duplicate. Ct (threshold cycle) value was determined by default threshold settings. A Ct value >37 was determined as an undetectable value. $\Delta Ct = Ct\ i - Ct\ n$, where Ct i was defined as the Ct value of the inactivated group, and Ct n was defined as the Ct value of the non-inactivated group.

STATISTICAL ANALYSIS

Data are presented as mean ± standard deviation (SD) or median, range. Paired two-tailed Student's *t*-tests were used to analyze the differences between paired samples in inactivated and non-inactivated groups. Mann-Whitney *U* tests were used to compare the differences of detectable data between low and high viral load

groups. SPSS 20.0 (IBM) and Prism 7 (GraphPad) were used for all statistical determinations. *P*-values less than 0.05 were considered significant.

Results

THERMAL INACTIVATION REDUCED THE DETECTABLE AMOUNT OF SARS-COV-2 IN RT-PCR DETECTION

To determine the impact of thermal inactivation, parallel NAT was performed using clinical specimens from 4 confirmed COVID-19 patients. Each specimen was serially diluted by a factor of 10^{-5} and then determined by RT-PCR. The line chart presented contrasts the Ct values of each specimen with or without incubation at 56°C for 30 min (Fig. 1). We classified the original and diluted concentration of 10^{-1} and 10^{-2} as the high viral load (HVL) group and all remaining dilutions (10^{-3} to 10^{-5}) as the low viral load (LVL) group. In general, most of the inactivated samples exhibited higher Ct values (mean 33.07 ± SD 5.00) than those with non-inactivated treatment (mean 32.69 ± SD 4.92) with a mean increase of 0.38 ($P = 0.017$, Supplemental Table 1). We further calculated the ΔCt of each sample with or without inactivation. The ΔCts of detectable samples in the LVL group (median 1.37, range 0.81 to 2.17) were much higher than those (median 0.14, range 0.38 to 1.57) in the HVL group ($P = 0.02$, Supplemental Table 1). These data suggested that in detectable samples of the LVL group, the tendency for increased Ct by thermal inactivation was more substantial than that in the HVL group. Notably, a few diluted samples showed negative results after inactivation. These results demonstrated thermal inactivation could cause an increased Ct value in the RT-PCR tests of SARS-CoV-2 and might affect the qualitative results of samples carrying low viral loads.

CLINICAL WEAK POSITIVE SPECIMENS WERE MORE SUSCEPTIBLE TO THERMAL INACTIVATION

To further verify the effects of thermal inactivation on specimens with low viral loads, 15 specimens with viral loads near the limit of detection were collected from confirmed cases and tested in parallel. The Ct values of non-inactivated specimens ranged from 33.37 to 36.89. Again, the inactivated group (mean 36.48 ± SD 1.48) showed higher mean Ct values than the non-inactivated group (mean 35.26 ± SD 1.24) with a mean increase of 1.22 ($P < 0.001$, Fig. 2). Moreover, in duplicate RT-PCR tests, positive results in 7 of 15 specimens (46.7%) were converted into undetectable values (false negatives) in at least one parallel testing after thermal inactivation (Table 1). The mean Ct value of the 7 specimens with non-inactivation was 36.25, providing a possible threshold for thermal susceptibility in viral NAT.

EFFECTS OF THERMAL INACTIVATION ON DIFFERENT TYPES OF SAMPLES

Clinical samples for COVID-19 tests included throat swab, sputum, bronchoalveolar lavage fluid, stool, and blood. To explore the effect of thermal inactivation in

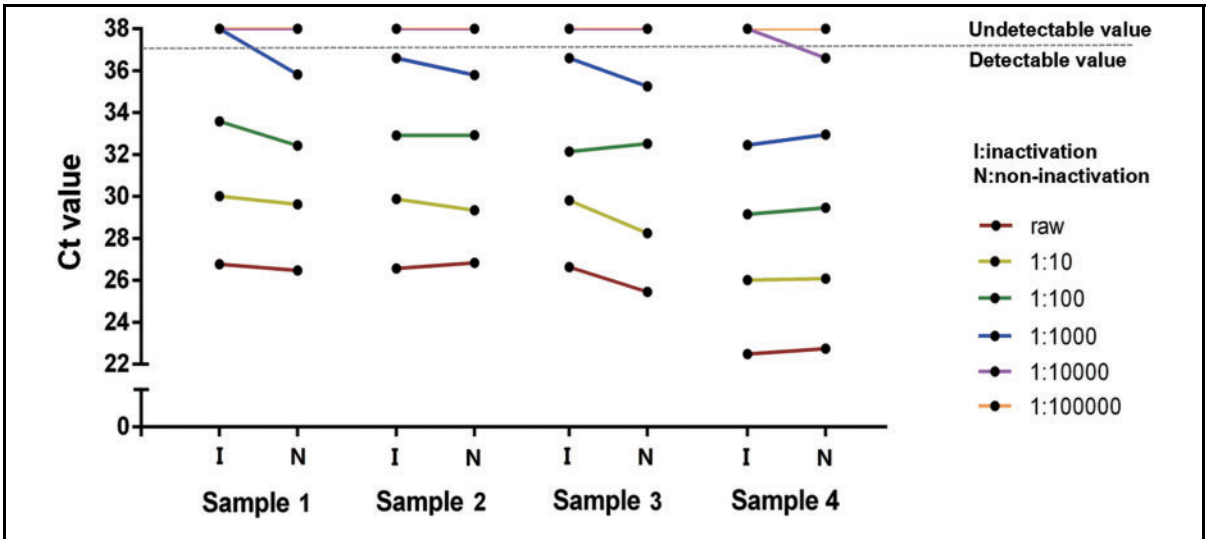

Fig. 1. Effects of thermal inactivation on the RT-PCR tests of SARS-CoV-2 in throat swabs samples. Each sample (n = 4) was detected in raw solution or after a series of dilutions by virus transport media from 10^{-1} to 10^{-5}, followed by treatment with or without incubation at 56°C for 30 min. The black dot represents the mean Ct value of the duplicate quantitative RT-PCR experiments. The dotted line represents Ct value 37 and Ct values of undetectable results were determined as 38.

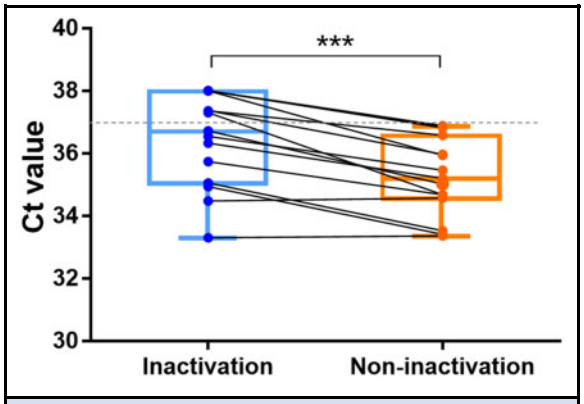

Fig. 2. Effects of thermal inactivation on RT-PCR tests of SARS-CoV-2 in clinical throat swabs carrying low viral loads. Each sample (n = 15) was detected with or without incubation at 56°C for 30 min. Each dot represents the mean Ct value of duplicate quantitative RT-PCR experiments. The dotted line represents Ct value 37 and Ct values of undetectable results were determined as 38. The comparison between the inactivation and non-inactivation groups was tested by paired two-tailed Student's *t*-test. *** represents $P < 0.001$.

Table 1. Ct value of RT-PCR for detecting SARS-CoV-2 in throat swab specimens with low viral loads.

	Inactivation		Non-inactivation	
	Ct 1	Ct 2	Ct 1	Ct 2
Sample A	35.64	35.84	34.65	34.71
Sample B	33.12	33.49	33.34	33.39
Sample C	34.47	34.50	34.55	34.58
Sample D	36.72	NA	36.55	36.62
Sample E	NA	NA	36.75	36.94
Sample F	36.70	36.39	35.51	35.42
Sample G	36.61	36.06	35.41	35.00
Sample H	NA	36.74	36.02	35.92
Sample I	NA	36.61	34.69	34.67
Sample J	34.89	34.98	33.33	33.50
Sample K	35.14	34.97	33.64	33.41
Sample L	36.50	36.93	34.95	35.09
Sample M	NA	NA	36.88	36.90
Sample N	NA	NA	36.80	36.84
Sample O	NA	NA	35.84	36.04

NA, not available, represents undetectable value of Ct > 37.

different specimen types, we deployed 4 throat swabs, 2 sputum samples, and 2 stool samples, and serially diluted each specimen by a factor of 10^{-5}. Compared to the non-inactivated group (mean 33.64 ± SD 4.61), thermal inactivation raised the Ct values of most samples (mean 34.08 ± SD 4.68) by a mean increase of 0.44 ($P < 0.001$, Fig. 3, Supplemental Table 2). We then performed a further analysis within different sample types. While the susceptibility to thermal inactivation in throat swabs has been described above (Fig. 1, Supplemental Table 3), the mean Ct values of inactivated stool samples (mean 36.29 ± SD 2.87) were also higher than those of the non-inactivated group (mean 35.55 ± SD 3.24) by a mean increase of 0.74 ($P = 0.014$, Supplemental Table 3). However, the difference in mean Ct values in sputum between inactivated (mean 33.90 ± SD 5.06) and non-inactivated groups (mean 33.62 ± SD 4.85) was not found to be significant ($P = 0.308$, Supplemental Table 3).

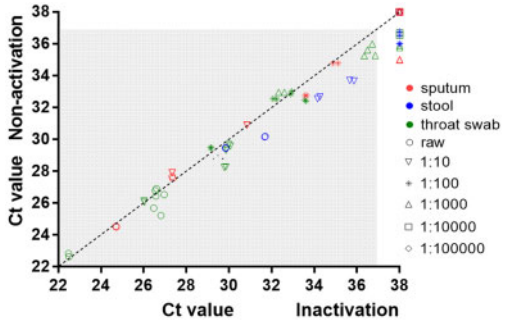

Fig. 3. Effects of thermal inactivation on the quantitative RT-PCR tests of SARS-CoV-2 in different sample types. Specimens of sputum (n = 2), stool (n = 2), and throat swabs (n = 4) were incorporated. Each sample was detected twice in raw solution or dilution by virus transport media from 10^{-1} to 10^{-5}. Each symbol represents Ct value from one test. The dotted line represents equal Ct value between the inactivated and non-inactivated group. Shaded areas represent detectable values in both two groups. Ct values of undetectable results were determined as 38.

HIGHER TEMPERATURE AND LONGER TIME OF SPECIMEN PRESERVATION PARTIALLY CONTRIBUTED TO FALSE-NEGATIVE RESULTS IN SPECIMENS CARRYING LOW VIRAL LOADS

To explore the effects of time and temperature on laboratory detection, we diluted 4 throat swab specimens by factors of 10^{-2} and 10^{-4}. All original and diluted samples were kept at 4°C or room temperature (RT) and harvested at different time points of 12, 24, 36, and 48 h. Time and temperature of preservation only exhibited sight impact on the increase of detected Ct value. However, higher Ct values were more prominent with longer time of preservation, especially in diluted samples

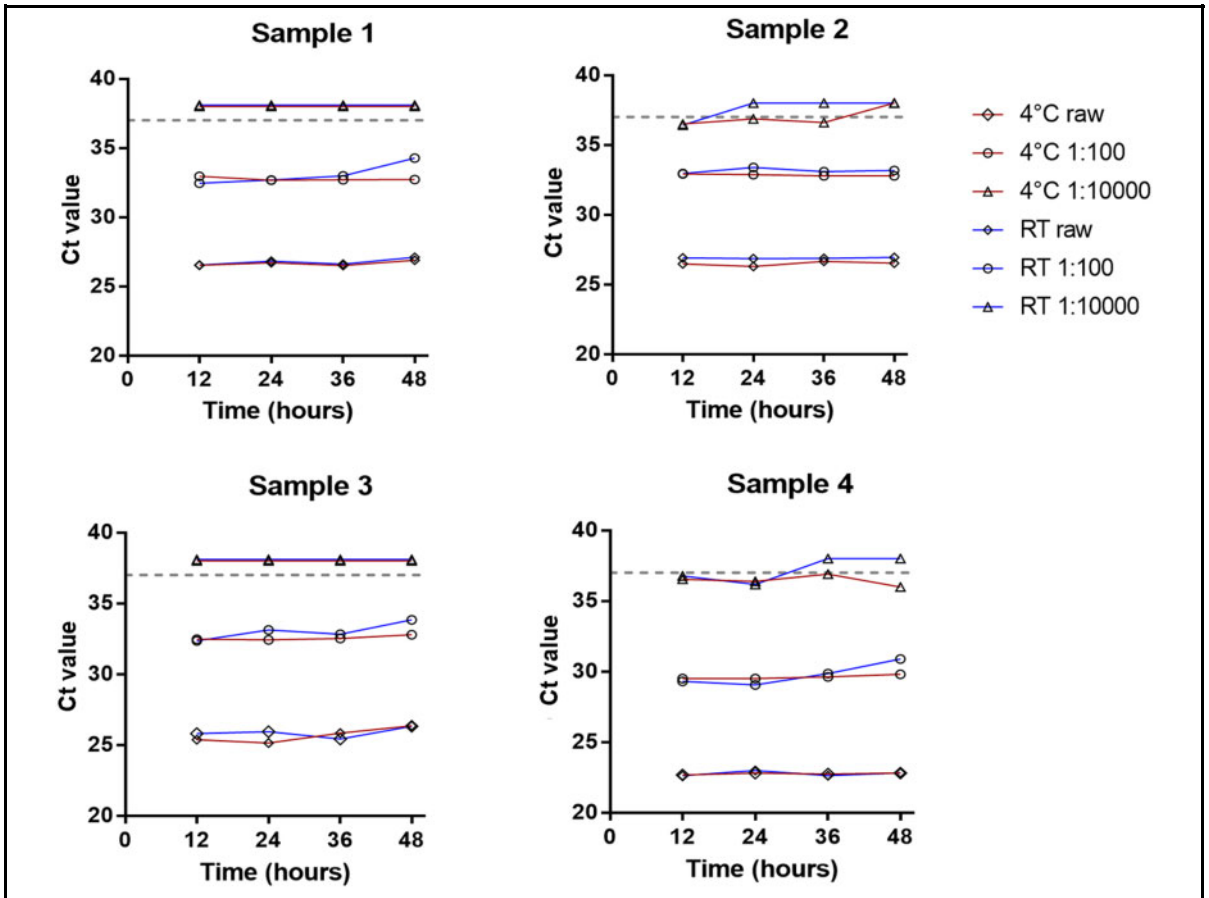

Fig. 4. Effects of temperature and time of specimen preservation on the RT-PCR tests of SARS-CoV-2 in throat swabs samples. Each sample (n = 4) was detected in raw solution or diluted by virus transport media by factors of 10^{-2} and 10^{-4} and then preserved at 4°C or room temperature (RT). The samples were detected every 12 h in the next 2 days. Each symbol represents mean Ct of duplicate quantitative RT-PCR experiments. The dotted line represents Ct value 37 and Ct values of undetectable results were determined as 38.

with lower viral loads. Notably, two positive samples (sample 2 and 4) diluted by a factor of 10^{-4} were converted into negative ones with longer storage or preservation at RT (Fig. 4). Altogether despite the fact that the influence of storage time and temperature on NAT was slight, an extended time and high temperature of preservation were able to cause false-negative results in low viral load specimens.

VIRAL INACTIVATION BY GUANIDINIUM-BASED LYSIS EXHIBITED LESS EFFECTS ON THE DETECTABLE AMOUNT OF SARS-COV-2 THAN THERMAL INACTIVATION

As genomic RNA of SARS-CoV-2 is unstable and easily degraded by environmental nuclease, the solutions used to preserve specimens are of great importance to protect the viral genomic integrity. Guanidinium-based buffer, a common solution for specimen preservation, has been shown to have the dual function of viral inactivation through chemical destruction of the viral protein *(13)*. Therefore, we collected 15 throat swab specimens to investigate the effect of chemical inactivation by guanidinium-based solutions on the RT-PCR tests for SARS-CoV-2. Each sample was divided into subgroups based on chemical inactivation by guanidinium-based lysis (GL group), and by thermal inactivation at 56°C for 30 min (VTM group). VTM was used for specimen preservation before thermal inactivation. The Ct values of most samples in the VTM group (mean 36.48 ± SD 1.48) were higher than those in GL group (mean 35.40 ± SD 1.33) by a mean increase of 1.08 ($P < 0.001$, Fig. 5A, Supplemental Table 4), demonstrating that GL provided better protection of viral

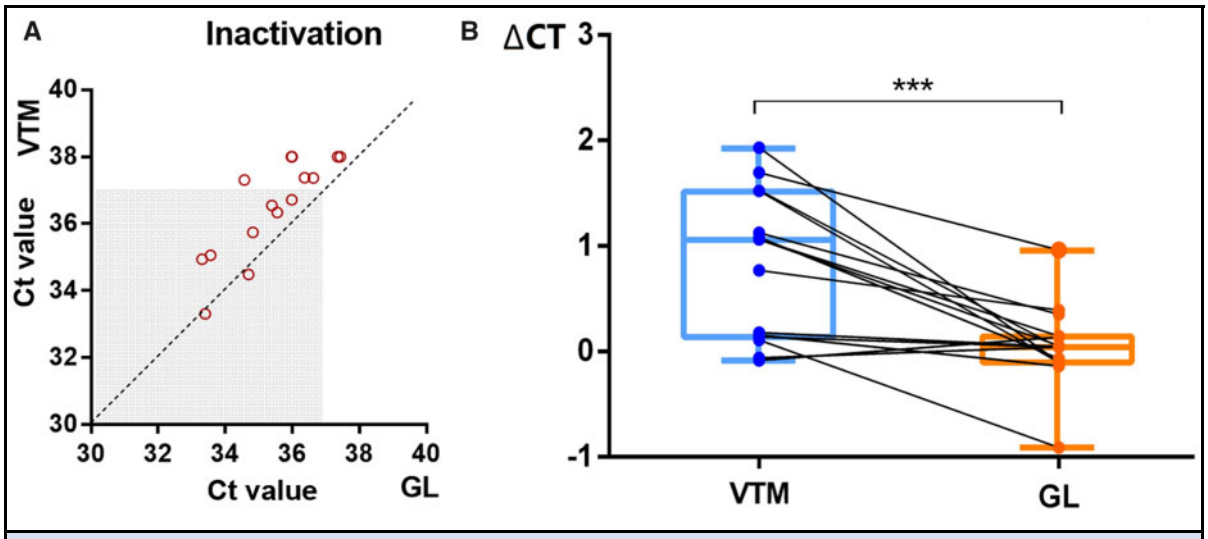

Fig. 5. Effects of guanidinium-based lysis on the quantitative RT-PCR tests of SARS-CoV-2. All throat swabs samples (n = 15) were placed into guanidinium-based lysis (GL) or virus transport media (VTM). Each sample in the VTM group was divided into subgroups with or without incubation at 56°C for 30 min. A) Comparative analysis of Ct values in specimens between chemical inactivation (preserved in GL) or thermal inactivation (preserved in VTM). Shaded areas represent detectable values in both inactivation methods. B) Comparative analysis of ΔCt values in specimens preserved in GL or VTM. ΔCt value was calculated by the difference of Ct values between inactivation and non-inactivation specimens. Each symbol or dot represents the mean Ct of duplicate quantitative RT-PCR experiments. Ct values of undetectable results were determined as 38. The comparison of ΔCt values between GL and VTM group was tested by paired two-tailed Student's *t*-test. *** represents $P < 0.001$.

nucleic acid than thermal inactivation. Moreover, the number of specimens with undetectable results was 2 of 15 in the GL inactivated group, but 7 of 15 in the thermal inactivated group. We calculated the ΔCt values of the thermally and chemically inactivated groups versus the non-inactivated group. The ΔCt values of thermally inactivated specimens were much higher than those in GL inactivated group ($P < 0.001$, Fig. 5B). These results suggested that GL preservation could attenuate the increased Ct value compared to thermal inactivation.

Discussion

SARS-CoV-2 is a novel member of beta-coronavirus with high homology to severe acute respiratory syndrome coronavirus (SARS-CoV) *(14, 15)* that has attracted high attention worldwide. Owing to its high sensitivity, specificity, and efficiency, RT-PCR has played a pivotal role in the public health response to pathogen identification, including coronavirus *(16, 17)*. Previous studies on the application of thermal inactivation have provided evidence that treatment at 56°C for at least 20 min or 30 min was sufficient to inactivate SARS-CoV *(18)* or the Middle East respiratory syndrome coronavirus (MERS-CoV) *(19)*, thereby attenuating their infectivity. During the current rapid emergence of COVID-19, inactivation of 56°C for 30–60 min has been recommended in a few Chinese consensus.

Whether thermal inactivation affects nucleic acid results is controversial *(9, 20)*. We found that thermal inactivation could result in a decreased detectable amount of viral nucleic acid and increased Ct values in RT-PCR detection, consistent with the results of a recent study that established a model of porcine epidemic diarrhea virus *(9)*. Thermal inactivation showed no significant influence on qualitative results for specimens carrying high viral loads. However, for the weak positive specimens (Ct value range from 33.37 to 36.89), thermal inactivation could be one of the possible causes of false-negative results in laboratory detection of SARS-CoV-2. It has been speculated that thermal inactivation might have a potential impact on detection in patients with early infection, causing NAT to lose its advantage of identifying patients with low viral loads. In our studies, nearly half of the weak positive specimens (7 of 15 samples, 46.7%) exhibited false-negative qualitative results after thermal inactivation in at least one parallel RT-PCR test. The mean Ct values of those 7 specimens (36.25), equivalent to about 700 copies/mL *(21)*, might provide a possible threshold for thermally susceptible

results. Furthermore, we found the effects of thermal inactivation varied between different sample types, demonstrating that the composition of tested samples might influence their viral thermal stability. Such variation in thermal stability has also been reported in previous studies that found thermal inactivation of SARS-CoV could be attenuated in the presence of fetal calf serum (20%) *(22)* or antithrombin III *(23)*. Considering the limited sample size in our study, a larger number of specimens might lead to a more significant result in sputum samples. We also observed that longer storage or preservation at room temperature could cause false-negative results in a portion of weak positive samples.

In addition to the above factors, clinical examination itself involves a series of complex processes that could alter the detection rates. Factors that could play a role include improper materials or nonstandard operation in sampling, imperfect preservation or prolonged turn-around time in transportation, and unqualified kits or incompatibility of reagents and apparatus used for detection. Such factors require comprehensive and meticulous management to improve the laboratory quality control system.

With the worldwide spread of COVID-19, false-negative laboratory detection could lead to a missed diagnosis of infective patients, falsely discharged patients, and some potential safety problems in blood transfusion. Therefore, improvement in the laboratory detection rate is an urgent need. Chemical inactivators, such as guanidinium-based lysis, are considered as an alternative approach to avoid biosafety risk by some manufacturers or laboratories. Our results have shown that these inactivators might contribute to better assay performance and detection rates than thermal inactivation in specimens with low viral loads. However, the narrow applicability, limited availability, and potential environmental contamination of chemical inactivators restrict their application in SARS-CoV-2 testing.

The proper use of validated biological safety cabinets (BSCs) was stressed by WHO, as a core requirement in SARS-CoV-2 laboratory testing *(24)*. Competent personnel in clinical laboratories will achieve full protection during sample handling using BSCs and personal protective equipment (PPE) in biosafety level 2 labs. In view of the non-negligible influence of thermal inactivation on the RT-PCR assay, and the potential contamination of chemical inactivators, the overall approach taken to ensure the protection of laboratory personnel, rather than viral inactivation itself, should receive more attention in all diagnostic laboratories *(24)*.

Supplemental Material

Supplemental material is available at *Clinical Chemistry* online.

Nonstandard Abbreviations: COVID-19, coronavirus disease-2019; NAT, nucleic acid test; SARS-CoV-2, severe acute respiratory syndrome voronavirus 2; RT-PCR, real-time polymerase chain reaction; NMPA, National Medical Products Administration; VTM, virus transport media; SD, standard deviation; HVL, high viral load; LVL, low viral load; RT, room temperature; GL, guanidinium-based lysis; SARS-CoV, severe acute respiratory syndrome coronavirus; MERS-CoV, Middle East respiratory syndrome coronavirus; BSCs, biological safety cabinets; PPE, personal protective equipment.

Author Contributions: *All authors confirmed they have contributed to the intellectual content of this paper and have met the following 4 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; (c) final approval of the published article; and (d) agreement to be accountable for all aspects of the article thus ensuring that questions related to the accuracy or integrity of any part of the article are appropriately investigated and resolved.*

Y. Pan performed the experiments, collected data, and edited the manuscript. L. Long analyzed the data, generated the figures, and wrote the manuscript. D. Zhang, S. Cui, and P. Yang collected specimens and data. T. Yuan analyzed data. Q. Wang supervised this study. S. Ren designed and supervised this study, analyzed data, wrote and edited the manuscript.

Authors' Disclosures or Potential Conflicts of Interest: *No authors declared any potential conflicts of interest.*

Role of Sponsor: No sponsor was declared.

References

1. Sun K, Chen J, Viboud C. Early epidemiological analysis of the coronavirus disease 2019 outbreak based on crowdsourced data: a population-level observational study. Lancet Digit Health 2020;2:e2010–8.
2. Leung GM, Leung K. Crowdsourcing data to mitigate epidemics. Lancet Digit Health 2020;2;e156–7.
3. Yang XB, Yu Y, Xu J, Shu H, Xia J, Liu H, et al. Clinical course and outcomes of critically ill patients with SARS-CoV-2 pneumonia in Wuhan, China: a single-centered, retrospective, observational study. Lancet Respir Med February 24, 2020 as doi: 10.1016/S2213-2600(20)30079-5.
4. Zhou P, Yang XL, Wang XG, Hu B, Zhang L, Zhang W, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature 2020;579:270–3.
5. World Health Organization. Coronavirus Disease 2019 (COVID-19) Situation Report–46. World Health Organization; 2020. https://www.who.int/docs/default-source/coronaviruse/situation-reports/20200306-sitrep-46-covid-19.pdf?sfvrsn=96b04adf_4 (Accessed March 2020).
6. National Health Commission (NHC) of the PRC and National Administration of Traditional Chinese Medicine of the PRC. Guidance for Corona Virus Disease 2019: Prevention, Control, Diagnosis and Management (Seventh Edition). 2020. http://www.nhc.gov.cn/yzygj/s7653p/202003/46c9294a7dfe4cef80dc7f5912eb1989.shtml (Accessed March 2020) [Chinese].
7. Saiki RK, Gelfand DH, Stoffel S, Scharf SJ, Higuchi R, Horn GT, et al. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science 1988;239:487–91.
8. National Medical Products Administration. Emergent Approval of Novel Coronavirus Testing Products by National Medical Products Administration. National Medical Products Administration; 2020. http://www.nmpa.gov.cn/WS04/CL2056/375802.html (Accessed March 2020) [Chinese].

9. Zhang Q, Zhao Q. Inactivating porcine coronavirus before nuclei acid isolation with the temperature higher than 56°C damages its genome integrity seriously. A version of this article is available as a preprint at https://www.biorxiv.org/content/10.1101/2020.02.20.958785v1 (2020).
10. Zou LR, Ruan F, Huang MX, Liang LJ, Huang HT, Hong ZS, et al. SARS-CoV-2 viral load in upper respiratory specimens of infected patients. N Engl J Med 2020;382:1177–9.
11. Chinese Society of Laboratory Medicine. The experts' consensus on nucleic acid detection of 2019-nCoV. Zhonghua Yi Xue Za Zhi 2020;100:E003. [Chinese].
12. National Health Commission (NHC) of the PRC and National Administration of Traditional Chinese Medicine of the PRC. Guidance for Corona Virus Disease 2019: Prevention, Control, Diagnosis and Management (Sixth Edition). 2020. http://www.nhc.gov.cn/yzygj/s7653p/202002/8334a8326dd94d329df351d7da8aefc2.shtml (Accessed February 2020) [Chinese].
13. Burton JE, Easterbrook L, Pitman J, Anderson D, Roddy S, Bailey D, et al. The effect of a non-denaturing detergent and a guanidinium-based inactivation agent on the viability of Ebola virus in mock clinical serum samples. J Virol Methods 2017;250:34–40.
14. Wu F, Zhao S, Yu B, Chen YM, Wang W, Song ZG, et al. A new coronavirus associated with human respiratory disease in China. Nature 2020;579:265–9.
15. Hoffmann M, Kleine-Weber H, Krüger N, Müller M, Drosten C, Pöhlmann S. The novel coronavirus 2019 (2019-nCoV) uses the SARS-coronavirus receptor ACE2 and the cellular protease TMPRSS2 for entry into target cells. A version of this article is available as a preprint at https://www.biorxiv.org/content/10.1101/2020.01.31.929042v1 (2020).
16. Bermingham A, Heinen P, Iturriza-Gomara M, Gray J, Appleton H, Zambon MC. Laboratory diagnosis of SARS. Phil Trans R Soc Lond B 2004;359:1083–9.
17. Mackay IM, Arden KE. MERS coronavirus: diagnostics, epidemiology, and transmission. Virol J 2015;12:222.
18. Darnell ME, Subbarao K, Feinstone SM, Taylor DR. Inactivation of the coronavirus that induces severe acute respiratory syndrome, SARS-CoV. J Virol Methods 2004;121:85–91.
19. Leclercq I, Batéjat C, Burguière AM, Manuguerra J-C. Heat inactivation of the Middle East respiratory syndrome coronavirus. Influenza Other Respir Viruses 2014;8:585–6.
20. Chen PS, He YT, Huang YL, Chen YL, Huang H, Yu XG, et al. Inactivation of 2019 new coronary virus before quantitative real-time PCR testing. Chin J Lab Med 2020;43:E004. [Chinese].
21. Pan Y, Zhang D, Yang P, Poon LLM, Wang Q. Viral load of SARS-CoV-2 in clinical samples. Lancet Infect Dis 2020;20:411–2.
22. Rabenau HF, Cinatl J, Morgenstern B, Bauer G, Preiser W, Doerr HW. Stability and inactivation of SARS coronavirus. Med Microbiol Immunol 2005;194:1–6.
23. Yunoki M, Urayama T, Yamamoto I, Abe S, Ikuta K. Heat sensitivity of a SARS-associated coronavirus introduced into plasma products. Vox Sang 2004;87:302–3.
24. World Health Organization. Laboratory Biosafety Guidance Related to Coronavirus Disease 2019 (COVID-19). Geneva; 2020. https://apps.who.int/iris/bitstream/handle/10665/331138/WHO-WPE-GIH-2020.1-eng.pdf (Accessed March 2020).