Received: 21 April 2020 | Revised: 29 July 2020 | Accepted: 30 July 2020

DOI: 10.1111/trf.16049

DONOR INFECTIOUS DISEASE TESTING

TRANSFUSION

Development and evaluation of a Novel RT-PCR system for reliable and rapid SARS-CoV-2 screening of blood donations

Meng Li[1]# | Yuwei Zhao[1]# | Ying Li[1]# | Xue Chen[1]# | Dongxia Luo[2] | Mei Luo[3] | Jue Hou[1] | Juan Liu[4] | Humin Liu[1] | Huan Wang[1] | Yufang Dong[1] | Lanjiang Zhang[1] | Maosheng Ji[1] | Xin Zhao[1] | Caibing Wei[1] | Wen Li[1] | Jialiang Gao[1] | Hua Shan[5] | Xuemei Fu[1]

[1]Blood Research Laboratory, Chengdu Blood Center, Chengdu, China

[2]Teaching & Research Department, Public Health and Clinical Center of Chengdu, Chengdu, China

[3]Infectious Disease Research Laboratory, Public Health and Clinical Center of Chengdu, Chengdu, China

[4]Productresearch & Development Department, Sansure Biotechnology Company, Changsha, China

[5]Stanford University School of Medicine, Stanford, California

Correspondence
Xuemei Fu, Blood Research Laboratory, Chengdu Blood Center, South Renmin Road, 3 Yujie East Street, Chengdu, Sichuan Province 610041, China.
Email: xuemeifucdbc@163.com

Hua Shan, Stanford University School of Medicine, Stanford, CA 94304, USA.
Email: hshan@stanford.edu

Funding information
Research and Development, Grant/Award Number: YF05-00128-SN

Abstract

Background: The ongoing outbreak of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has caused great global concerns. In contrast to SARS, some SARS-CoV-2–infected people can be asymptomatic or have only mild nonspecific symptoms. Furthermore, there is evidence that SARS-CoV-2 may be infectious during an asymptomatic incubation period. With the discovery that SARS-CoV-2 can be detected in plasma or serum, blood safety is worthy of consideration.

Study Design and Methods: We developed a nucleic acid test (NAT) screening system for SARS-CoV-2 targeting nucleocapsid protein (N) and open reading frame 1ab (ORF 1ab) gene that could screen 5076 samples every 24 hours. The 2019 novel coronavirus RNA standard was used to evaluate linearity of standard curves. Diagnostic sensitivity and reproducibility were evaluated using artificial SARS-CoV-2. Specificity was evaluated with 61 other respiratory pathogens. Diagnostic performance was evaluated by testing two sputum and nine oropharyngeal swab specimens. The reverse transcription polymerase chain reaction (RT-PCR) assay was used to screen SARS-CoV-2 RNA in blood donor specimens collected during the outbreak of SARS-CoV-2 in Chengdu.

Results: Limits of detection of the SARS-CoV-2 RT-PCR assay for N and ORF 1ab gene were 12.5 and 27.58 copies/mL, respectively. Intra-assay and interassay for the SARS-CoV-2 RT-PCR assay based on cycle threshold were acceptably low. No cross-reactivity was observed with other respiratory virus and bacterial isolates. The overall agreement value between the SARS-CoV-2 RT-PCR assay and clinical diagnostic results was 100%. A total of 16 287 blood specimens collected from blood donors during SARS-CoV-2 surveillance were tested negative.

Abbreviations: 2019-nCoV, 2019 novel coronavirus; Ct, cycle threshold; CV, coefficient of variation; LOD, limit of detection; N, nucleocapsid protein; NAT, nucleic acid test; ORF 1ab, open reading frame 1ab; PCR, polymerase chain reaction; RT-PCR, reverse transcription polymerase chain reaction; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; WHO, World Health Organization.

# These authors contributed to this work equally.

Conclusions: A high-throughput NAT screening system was developed for SARS-CoV-2 screening of blood donations during the outbreak of SARS-CoV-2.

1 | INTRODUCTION

With a cluster of cases with pneumonia of unknown etiology in Wuhan City, Hubei Province, China, in December 2019, a new coronavirus was identified as the causative pathogen, which was subsequently named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).[1,2]Due to its rapid spread, high infectivity, and high fatality,[3–5] on 31 January 2020, the World Health Organization (WHO) declared the outbreak of coronavirus 2019 (COVID-19) in China as a public health emergency of international concern.[6] On 11 March, the WHO further declared COVID-19 as a pandemic.[7] According to data provided by the WHO, by 29 July 2020, there were 16 558 289 confirmed cases of COVID-19 since the beginning of the epidemic. At that time, a total of 656 093 patients had died of the SARS-CoV-2 infection.[8]

SARS-CoV-2 was first isolated from human airway epithelial cells and characterized by next-generation sequencing.[9,10] The genome sequence was released on 10 January (Wuhan-Hu-1, GenBank accession number MN908947).[11,12] SARS-CoV-2 belongs to beta coronaviruses that are a variety of enveloped, positive-stranded RNA viruses with a nucleocapsid.[13,14] The genome sequence of SARS-CoV-2 had about 80% nucleotide identity with that of human SARS-CoV.[2,14]

In contrast to SARS, the clinical spectrum of COVID-19 varies from asymptomatic or limited mild nonspecific flulike symptoms to respiratory failure and multiple organ dysfunctions.[15–17] It was established that there were about 81% of SARS-CoV-2 cases with nonpneumonia and mild pneumonia.[18,19] Chan et al[20] reported an asymptomatic carrier in a family cluster of COVID-19. SARS-CoV-2 might be infectious during an asymptomatic incubation period.[21–25] According to the investigations conducted by the Chinese Center for Disease Control, the incubation time is generally 3 to 7 days, with a mean incubation period of 5.2 days (95% confidence interval [CI], 4.1-7.0) and a 95th percentile of the distribution of 12.5 days.[26,27] The incubation period of SARS-CoV-2 is longer than that of SARS-CoV.[26,28,29] These factors increase the challenge for prevention and control of SARS-CoV-2 infections.

Although coronaviruses usually infect the upper and lower respiratory tract, viral shedding in blood is common.[3,30,31] It has been demonstrated that 15% to 40% of SARS-CoV-2–infected individuals have detectable RNAemia.[16,30,32] Furthermore, there were no differences between intensive care unit patients and patients with mild symptoms on median polymerase chain reaction (PCR) cycle threshold (Ct) value.[16] Data are limited about SARS-CoV-2 viremia at different stages of the infection. Screening of blood donors from Wuhan during the height of the epidemic found four asymptomatic blood donors with positive SARS-CoV-2 RNA in blood samples.[33] SARS-CoV-2 can infect a variety of tissue culture cells and organoids.[34] With limited available information, a theoretical risk of SARS-CoV-2 transfusion-transmitted infection could not be excluded.[3,35]

To protect transfusion recipients from transfusion-transmitted infection of SARS-CoV-2 during the epidemic period, Chinese blood collection facilities implemented additional predonation screening measures, including checking body temperature before blood donation and new deferral criteria. The new deferral criteria defer all blood donors for 28 days after the resolution of any COVID-19 symptoms, after a history of residence or travel to Hubei Province and close contact with individuals diagnosed with or suspected of having COVID-19. Even with these enhanced predonation donor screening measures, some asymptomatically infected donors may still donate blood. To prevent potential transfusion-transmitted SARS-CoV-2 infection and address public concerns about the safety of the blood supply during the COVID-19 epidemic, we developed a SARS-CoV-2 RNA donor screening assay and conducted donor screening testing with use of blood specimens from blood donors.

Based on the genome sequence of SARS-CoV-2, real-time reverse transcription polymerase chain reaction (RT-PCR) detection assays have been quickly developed to detect SARS-CoV-2 in sputum, throat swabs, and nasal swabs. However, there are no validated accurate NAT screening systems for detecting SARS-CoV-2 RNA in blood samples to date. Furthermore, available assays have limited sensitivity and are low throughput, precluding rapid screening of large numbers of samples.

In this study, we developed a high-throughput (5076 tests per 24 hours), high-sensitivity, and accurate NAT screening system for the SARS-CoV-2 RNA detection of blood donations in collaboration with Sansure Biotechnology Company. After evaluating its analytical performance and clinical applicability, the assay was used for screening SARS-CoV-2 RNA in blood specimens collected

from blood donors during the height of SARS-CoV-2 epidemic in Chengdu.

2 | MATERIALS AND METHODS

2.1 | Virus and clinical specimens

Two sputum and nine oropharyngeal swab specimens were used for clinical evaluation of the SARS-CoV-2 assay, which were collected from 10 patients with confirmed/suspected COVID-19 in the Public Health and Clinical Center of Chengdu. The specimens were deactivated by heating at 65°C for 30 minutes and previously tested with a nucleic acid isolation kit (Da'an Gene Corporation, Guangzhou, China; Cat: DA0630) and SARS-CoV-2 RT-PCR reagent (Da'an Gene Corporation; Cat: DA0930). The specimens were stored at 2 to 8°C for no more than 24 hours before detecting. Total nucleic acid of specimens was simultaneously extracted individually with a nucleic acid extraction and purification kit (Sansure Biotechnology Company, Hunan, China) and then immediately delivered to Chengdu Blood Center on dry ice. The nucleic acid was diluted 6-fold by Sansure eluting buffer, and then 20 μL of diluted nucleic acid was used as template for amplification. A total of 16 287 blood specimens collected from blood donors between 27 January 2020 and 29 February 2020 were tested at Chengdu Blood Center.

2.2 | Primers and probes

Primer sets for 2019 novel coronavirus (2019-nCoV) were designed using Primer 5 based on the sequence (Wuhan-Hu-1, GenBank accession number MN908947) targeting both the nucleocapsid protein (N) and open reading frame 1ab (ORF 1ab). The β-globin gene was amplified simultaneously as a heterologous endogenous internal control. Primer/probe sets were predicted on Basic Local Alignment Search Tool analysis to specifically amplify 2019-nCoV with no major combined homologies with other coronaviruses or human microflora. All primers and probes were produced by standard phosphoramidite chemical techniques at a biotechnology facility.

2.3 | Pooling, extraction, and amplification

Plasma specimens were pooled and total nucleic acids were extracted on an automatic workstation. The total input volume of each pool was 240 μL consisting of 40-μL equal aliquots from each donation (6 × 40). Samples were added to 96-deep-well plate (Axygen Biotech, Hangzhou, China; Cat: P-DW-20-C-S). Extraction of total nucleic acids was performed with a nucleic acid extraction and purification kit (Sansure Biotechnology Company, Hunan, China) according to the manufacturer's instructions. The nucleic acid was eluted with 50 μL elution buffer. Each PCR reaction was performed in a total volume of 50 μL (20 μL of template nucleic acid, 26 μL of RT-PCR buffer and 4 μL of enzyme mix). Primers and probes at 240 nM were used for one reaction. Reverse transcription was performed at 50°C for 30 minutes. PCR cycling was done at 95°C for 1 minute and 45 cycles of 95°C for 15 seconds, 60°C for 30 seconds and 25°C for 10 seconds. All RT-PCR reactions were performed with a real-time PCR system (7500 Fast Real Time PCR System, ABI7500, Applied Biosystems, Foster City, CA). Positive and negative controls were included in each run. A positive test result was defined as a well-defined exponential fluorescence curve that crossed the threshold of 40 cycles or less for the ORF 1ab, N, and β-globin genes.

2.4 | Analytical performance evaluation

For validation, linearity of standard curves was verified by 2019-nCoV RNA standard (National Institute of Metrology, Beijing, China; Cat: GBW[E]091099), which is purified RNA containing 6.89×10^2 copies/μL ORF 1ab gene and 1.36×10^3 copies/μL N gene. Sensitivity and reliability were verified with use of artificial SARS-CoV-2 virus (MS2 bacteriophage-like particles packing SARS-CoV-2 ORF 1ab and N gene, Zeesan Biotech, Xiamen, China; Cat: JBD 279), which was obtained by armored RNA technology and quantified by 2019-nCoV RNA standard.

To determine linearity of the standard curve, 2019-nCoV RNA standards were diluted to six concentrations by tenfold serial dilutions with N gene from 1.36×10^5 to 1.36 copies/mL and ORF 1ab gene from 6.89×10^4 to 0.689 copies/mL using RNase free water, with 10 replicates per dilution. The PCR efficiency (E) was calculated using the standard curve slope according the following formula: $E = 10^{-1/\text{slope}}\text{-}1$. The correlation coefficient (R^2) was also calculated.

The limit of detection (LOD) and reliability was determined by testing individually using artificial SARS-CoV-2 virus by twofold serial dilutions with N gene from 800 copies/mL to 1.56 copies/mL and ORF 1ab gene from 882.48 to 1.72 copies/mL using SARS-CoV-2–negative plasma. Twenty replicates per dilution. The LOD was defined as the lowest concentration at which a fluorescent signal could be detected in all reactions.

Subsequently, diagnostic sensitivity of the assay was verified using 20 replicates of mock specimens with artificial SARS-CoV-2 virus containing 75 copies/mL N gene and 82.73 copies/mL ORF 1ab gene tested with equal volumes of five SARS-CoV-2–negative plasma in pools of six.

Reproducibility was evaluated with artificial SARS-CoV-2 virus of low, moderate, or high concentrations (N gene 25, 62.5 and 123 copies/mL; ORF 1ab gene 27.58, 68.94 and 137.89 copies/mL). For intra-assay variability evaluated, 20 replicates of three different concentrations were tested within the same run. Interassay variability was evaluated by testing three dilutions on 5 consecutive days in 20 replicates. Reproducibility (inter- and intra-assay variance) was assessed using the coefficient value calculated on the basis of Ct values.

Analytical specificity was evaluated using 61 other respiratory virus and bacterial isolates. Artificial Middle East respiratory syndrome and severe acute respiratory syndrome virus were obtained from GenePharma Co., Ltd. (Shanghai, China). Influenza virus A, *Klebsiella* pneumonia, *Haemophilus influenzae, Staphylococcus aureus*, *Pseudomonas aeruginosa, Legionella pneumophila*, and *Streptococcus pneumoniae* were purchased from National Institutes for Food and Drug Control of China. Other isolates were obtained from Sanway Clinical Laboratories Inc (Changsha, China).

2.5 | Interference study

The influence of hemoglobin and lipids on COVID-19 was separately evaluated in SARS-CoV-2–negative plasma sample spiked with increasing concentrations of hemoglobin or lipids. The concentrations of hemoglobin were 50 000, 40 000, 30 000, 20 000, 10 000, 5000, 2500, and 1250 mg/L. The triglyceride concentrations were 8000, 4000, and 2000 mg/L. The simulated plasma samples were tested for SARS-CoV-2 with or without about 3 × LOD artificial SARS-CoV-2 virus (ORF 1ab 41.37 copies/mL, N 37.5 copies/mL), with 10 replicates per dilution.

2.6 | Diagnostic performance

The diagnostic performance of the assay was evaluated by testing 11 clinical specimens (two sputum and nine oropharyngeal swab specimens) from 10 patients with confirmed/suspected COVID-19. The positive percent agreement and negative percent agreement values and kappa coefficients were calculated.

2.7 | Statistical analysis

Values were log10-transformed before analysis with computer software (Excel, Microsoft, Redmond, WA). The agreement between the Sansure SARS-CoV-2 RT-PCR assay and previously clinical SARS-CoV-2 RT-PCR assay was assessed by the kappa coefficient. Statistical analyses were performed by using computer software (SPSS 11, SPSS, Chicago, IL). A P value of $<.05$ was considered statistically significant.

2.8 | Ethical statement

All specimens were collected for diagnostic evaluation, and diagnostic purposes added no extra burden to patients and blood donors. This study was approved by the Ethics Committee of Chengdu Blood Center.

3 | RESULTS

Pooling samples and extraction of total nucleic acids were performed on an automated workstation (Sansure Biotechnology Company), which reduced the hands-on time and technical errors. This high-throughput, efficient, and accurate NAT system is useful for screening SARS-CoV-2 in large numbers of samples from blood donors.

We evaluated the NAT system using mock specimens and clinical specimens from COVID-19 patients. The calculated analytical detection limit of the assay (12.5 copies/mL) appears to be low. The low percentage of coefficient value of the cycle number demonstrates the low intra-assay variation. Stable reaction properties are also reflected in good quantification results (N gene linear range between 2.72×10^3 and 2.72 copies/reaction; ORF 1ab gene linear range between 1.378×10^3 and 1.378 copies/reaction). No cross reactivity was obtained with other respiratory isolates demonstrating high specificity for SARS-CoV-2. The NAT system demonstrates the feasibility of screening blood donors for SARS-CoV-2 in the setting of routine blood center operations.

3.1 | Optimization of PCR reaction condition

Optimization of the PCR reaction components and cycling conditions were undertaken using the RNA standard including N and ORF 1ab gene of the SARS-CoV-2 virus. The optimum concentrations of primers, annealing, and data acquisition temperatures were evaluated. The optimum conditions were defined as those that

gave the maximum fluorescence and lowest Ct values without the presence of primer dimers or nonspecific amplification. The standard curves of N and ORF 1ab were plotted with slopes of −3.0579 and −3.0848, respectively.

3.2 | Linearity of standard curves

The dynamic range and efficiency of the assay were evaluated using duplicate 10-fold serial dilutions of RNA standard including N and ORF 1ab gene. As shown in Figure 1, standard curves were constructed in terms of mean Ct value and log10(copy numbers). There was a strong linear correlation ($R^2 = 99.91\%$ for N gene and 99.76% for ORF 1ab gene) between Ct values and the corresponding amount of copy numbers. The PCR efficiency of N gene and ORF 1ab gene was 112.34% and 110.95%, respectively.

3.3 | Analytical sensitivity

The LOD of the assay was determined using artificial SARS-CoV-2 virus. Serial twofold dilutions (N gene from 800 copies/mL to 1.56 copies/mL and ORF 1ab gene from 882.48 to 1.72 copies/mL) of artificial SARS-CoV-2 virus were prepared in SARS-CoV-2–negative plasma and were tested with each assay signature in 20 replicates for positive amplification; 240 μL of each sample was subjected to nucleic acids extraction with an elution volume of 50 μL. Then 20 μL of each elution was added as the template of amplification assay. The highest dilution of transcript at which all replicates were positive was defined as the LOD for each assay. As shown in Table 1, the LOD value of the assay was 12.5 copies/mL. The LODs for N and ORF 1ab gene were 12.5 and 27.58 copies/mL. Furthermore, 20 replicates of mock specimens using artificial SARS-CoV-2 virus containing 75 copies/mL N gene and 82.73 copies/mL ORF 1ab gene were tested when mixed with the other five SARS-CoV-2–negative plasmas in minipools of six. The positive detection rate was 90%. Probit regression analysis in SPSS was used to determine the 95% cutoff value. According to the probit regression analysis (as shown in Figure S1), the N has a 95% cutoff value of 11.47 copies/mL (95% confidence interval [CI]: from 8.49 to 21.14 copies/mL). The ORF 1ab assay detects 16.36 copies/mL with 95% probability (95% CI, 12.78-25.06 copies/mL).

3.4 | Reproducibility studies

Variability was evaluated using artificial SARS-CoV-2 virus of high, moderate, or low concentrations (N gene 20, 10 and 5 copies/mL; ORF 1ab gene 27.58, 68.94 and 137.89 copies/mL). The mean, SD, and coefficient of variation (CV) of the Ct value were determined. As shown in Table 2, The CV values of intra- and interassay for the N gene were 0.90-2.34 and 0.56-0.85. The CV values of intra- and interassay for ORF 1ab were 0.73-2.24 and 0.80-1.58. Overall, these results show that the assay is reliable, with different Ct values.

3.5 | Analytical specificity

The specificity of SARS-CoV-2 RT-PCR assay was evaluated by using 61 other respiratory pathogens that were tested in SARS-CoV-2 negative plasma. These

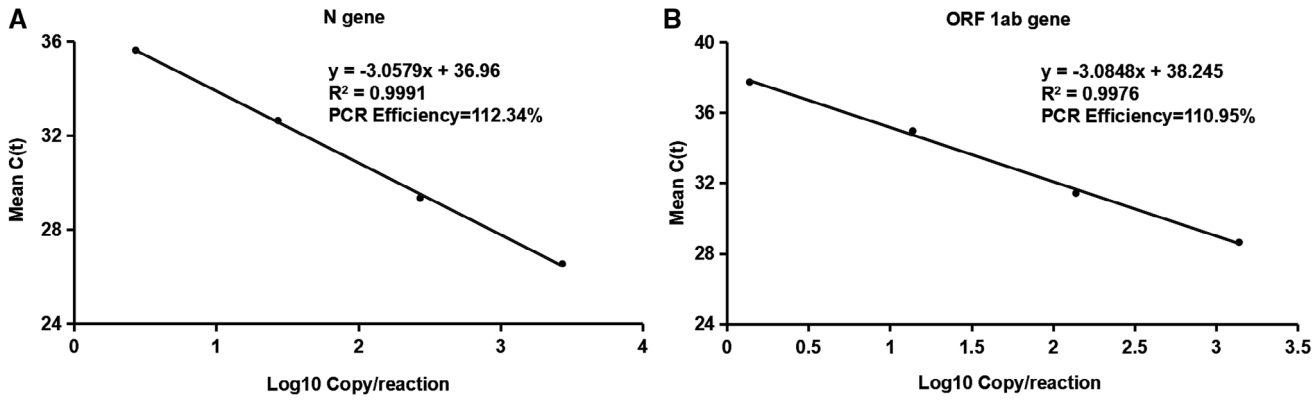

FIGURE 1 Standard curves of the SARS-CoV-2 RT-PCR assay. Plots of serial 10-fold dilutions of 2019-nCoV RNA standard analyzed by the SARS-CoV-2 RT-PCR assay with N gene from 1.36×10^5 to 1.36 copies/mL and ORF 1ab gene from 6.89×10^4 to 0.689 copies/mL using RNase free water. Linear correlation coefficients (R^2) and amplification efficiency for each assay were indicated. A, Plot inserts show mean Ct values of N gene vs Log10 (Lg) input copies/reaction; B, plot inserts show mean Ct values of ORF 1ab gene vsLg input copies/reaction

TABLE 1 Determination of limits of detection based on artificial SARS-CoV-2 virus

Concentration of target gene(copies/mL)		Number of detections /number of replicates (%)		Mean Ct	
N	ORF 1ab	N	ORF 1ab	N	ORF 1ab
800	882.48	20/20 (100)	20/20 (100)	29.24	31.80
400	441.24	20/20 (100)	20/20 (100)	29.84	31.90
200	220.62	20/20 (100)	20/20 (100)	30.94	33.40
100	110.31	20/20 (100)	20/20 (100)	31.76	34.23
50	55.15	20/20 (100)	20/20 (100)	32.94	35.55
25	27.58[a]	20/20 (100)	20/20 (100)	34.10	36.09
12.5[a]	13.79	20/20 (100)	17/20 (85)	35.04	36.62
6.25	6.89	16/20 (80)	13/20 (65)	35.16	36.75
3.125	3.45	13/20 (65)	8/20 (40)	35.85	37.74
1.5625	1.72	8/20 (40)	3/20 (15)	36.62	38.28

Abbreviations: Ct, threshold cycle; ORF 1ab, open reading frame 1ab; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.
[a]The highest dilution at which 100% of replicates were positive.

TABLE 2 Intra-assay and interassay of SARS-CoV-2 RT-PCR assay based on Ct

	Target	Concentration (copies/mL)	Replications	Mean Ct	SD	CV(%)
Interassay	N	25.00	20	34.07	0.19	0.56
		62.50	20	33.03	0.28	0.85
		125.00	20	31.90	0.23	0.72
	ORF 1ab	27.58	20	36.74	0.58	1.58
		68.94	20	35.15	0.52	1.48
		137.89	20	34.09	0.27	0.80
Intra-assay	N	25.00	20	34.12	0.80	2.34
		62.50	20	33.12	0.51	1.55
		125.00	20	31.85	0.29	0.90
	ORF 1ab	27.58	20	36.43	0.82	2.24
		68.94	20	35.19	0.49	1.40
		137.89	20	34.05	0.25	0.73

Abbreviations: Ct, cycle threshold; CV, coefficient of variation; ORF 1ab, open reading frame 1ab; RT-PCR, reverse transcription polymerase chain reaction; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

pathogens and their concentrations were listed in Table S1. Melting curve analysis of each sample showed that there was no evidence of amplification. No false-positive test results were obtained with any sample, demonstrating high specificity for the detection of SARS-CoV-2.

3.6 | Interference study

As shown in Table 3, high concentration of hemoglobin (≥20 000 mg/L) could affect the detection rate of SARS-CoV-2. The lipids interference study demonstrated that there was no detectable interference at the concentration of 8000, 4000, or 2000 mg/L (Table 4).

3.7 | Clinical studies

1. Performance of the RT-PCR assay with human clinical specimens from patients with confirmed/suspected COVID-19. Two sputum and nine oropharyngeal swab specimens collected from 10 patients with confirmed/suspected COVID-19 were evaluated for diagnostic performance. The clinical specimens were collected following the Guidance of Laboratory

TABLE 3 The hemoglobin interference study of SARS-CoV-2 RT-PCR assay

	Number of positive tests/number of replicates (%)		Mean Ct	
Hemoglobin concentration (mg/L)	ORF 1ab	N	ORF 1ab	N
20 000	9/10 (90)	10/10 (100)	37.01	34.48
10 000	10/10 (100)	10/10 (100)	36.08	33.13
5000	10/10 (100)	10/10 (100)	36.68	33.22
2500	10/10 (100)	10/10 (100)	34.28	32.98
1250	10/10 (100)	10/10 (100)	35.32	32.96

Abbreviations: Ct, cycle threshold; ORF 1ab, open reading frame 1ab; RT-PCR, reverse transcription polymerase chain reaction; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

TABLE 4 The lipids interference study of SARS-CoV-2 RT-PCR assay

	Number of positive tests/number of replicates (%)		Mean Ct	
Triglyceride concentration (mg/L)	ORF 1ab	N	ORF 1ab	N
8000	10/10 (100)	10/10 (100)	35.33	33.28
4000	10/10 (100)	10/10 (100)	35.57	33.09
2000	10/10 (100)	10/10 (100)	35.63	33.23

Abbreviations: Ct, cycle threshold; ORF 1ab, open reading frame 1ab; RT-PCR, reverse transcription polymerase chain reaction; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

TABLE 5 Diagnostic performance comparison with previous clinical NAT testing

			SARS-CoV-2 RT-PCR assay			Clinical NAT testing		
Number	Sample type	Clinical diagnosis	ORF 1ab Ct	N Ct	Result	ORF 1ab Ct	N Ct	Result
1	Sputum	Confirmed	38.25	33.23	Pos.	39.06	39.60	Pos.
	OP swab	Confirmed	...	33.55	Pos.	38.17	...	Pos.
2	Sputum	Confirmed	35.87	31.46	Pos.	38.22	38.99	Pos.
3	OP swab	Confirmed	29.41	28.51	Pos.	32.83	33.61	Pos.
4	OP swab	Confirmed	...	33.53	Pos.	...	39	Pos.
5	OP swab	Suspected	...	...	Neg.	...	...	Neg.
6	OP swab	Suspected	...	...	Neg.	...	...	Neg.
7	OP swab	Suspected	...	...	Neg.	...	...	Neg.
8	OP swab	Suspected	...	...	Neg.	...	...	Neg.
9	OP swab	Suspected	...	...	Neg.	...	...	Neg.
10	OP swab	Suspected	...	...	Neg.	...	...	Neg.

Abbreviations: Ct, cycle threshold; OP, oropharyngeal; ORF 1ab, open reading frame 1ab; N, nucleocapsid protein; NAT, nucleic acid test; Neg., negative; Pos., positive; RT-PCR, reverse transcription polymerase chain reaction; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

Testing for 2019 Novel Coronavirus issued by National Health Commission of the People's Republic of China. Oropharyngeal samples were collected as follows: Two oropharyngeal swabs were used simultaneously to rub the posterior wall of the oropharynx under direct visualization. Two oropharyngeal swabs were immediately placed into sterile tubes containing 2 mL of standard virus transport media, breaking the rod with one swift and controlled movement. The sputum sampling was easier. Patients forced out a deep cough and spit the sputum into sputum containers containing 2 mL of sampling solution. The clinical specimens were tested by our assay in a blinded manner. As shown in Table 5, all five specimens from four

patients previously confirmed positive for COVID-19 infection by RT-PCR assay at the Public Health and Clinical Center of Chengdu were positive and the other specimens were negative, consistent with clinical diagnosis. The kappa coefficient between the two qualitative results was 100% (Table S2). However, the testing results of ORF 1ab and N gene for oropharyngeal swab sample for one patient were different (Tables S3 and S4).

2. Performance of the RT-PCR assay with plasma specimens from blood donors during COVID-19 surveillance. A total of 16 287 blood specimens collected from donors between 27 January 2020 and 29 February 2020 in Chengdu were tested in minipools of six, and all results were negative. We also performed retrospective individual donation retesting of 15 stored samples from donors who notified us after blood donation that they had contacted patients with suspected COVID-19 or had fever, cough, or other suspected SARS-CoV-2 symptoms. All tested negative, and these donors have not become patients with COVID-19.

4 | DISCUSSION

SARS-CoV-2 RT-PCR is widely used for clinical diagnostic purpose. Many reports have described RT-PCR assays for detection of SARS-CoV-2 RNA targeting regions of ORF 1ab and N gene.[36–38] No RT-PCR assay for blood donor screening had been reported so far.

Although there has not been definitive evidence demonstrating that SARS-CoV-2 can be transmitted through blood, SARS-CoV-2 RNA has been detected in asymptomatic blood donors.[33] Some asymptomatic infected individuals may not be deferred by body temperature check, health history, travel/residence history, or contact history during predonation screening. NAT technology may be helpful to detect SARS-CoV-2 virus in these potential donors.[33,39–41] We urgently developed a NAT system to screen a blood donor's sample for SARS-CoV-2 with Sansure Biotechnology Company at the early stage of SARS-CoV-2 epidemic in Chengdu.

To address a potential risk of SARS-CoV-2 transmission through blood, our NAT donor screening system was used for screening blood donors in Chengdu during the epidemic period. A total of 16 287 consecutive donor blood specimens were tested. All results were negative. In contrast, a donor screening study in Wuhan found SARS-CoV-2 RNA in blood samples from four asymptomatic blood donors.[34] The difference between the results of these two studies most likely reflects the significant different level of infectious burden of the two populations. Comparing to Chengdu, Wuhan had a significantly higher population prevalence of COVID-19 during this time period (27 January to 29 February 2020). The reported population prevalence in Wuhan was 42.4 in 10000 (47 532 infections in a population of 11.2 million), comparing to 0.06 in 10000 (106 infections in a population of 16.6 million) in Chengdu. The additional precautionary predonation nontesting screening measures implemented at our blood center prior to the initiation of NAT testing may have also contributed to our result of no positive samples. The nontesting measures included (a) body temperature check; (b) new questions in donor screening questionnaire about history (within past 28 days) of having fever, cough, or other suspected SARS-CoV-2 symptoms; contact with patients with suspected/confirmed COVID-19 or traveling/residence to Hubei Province. Any "yes" answer would constitute a deferral for at least 28 days; and (c) blood donors were required to immediately notify the blood center if they were suspected or confirmed for COVID-19 within 1 month after their donation. Protecting the safety of blood supply presents particular challenge during the early stage of an emerging infection when sufficient data were not available to address all safety concerns. Public health concerns demand the timely implementation of additional security measures in protecting blood safety. Both nontesting and testing strategies based on available information should be considered under the principle of abundance of caution while additional scientific information is being collected. The additional donor deferral criteria may have helped to screen out SARS-CoV-2–infected potential donors in regions with mild and moderate epidemic such as Chengdu. On the other hand, for regions with high infection prevalence, additional measures such as donor screening with NAT may be necessity if future additional data indicate a transfusion transmission risk for SARS-CoV-2. Implementation of a sensitive and specific donor screening test can also offer the benefit of relaxing nontesting deferral criteria therefore reduce the loss of otherwise eligible donors. Donor availability can be a critical challenge for blood services during emerging epidemics when both the safety and the availability of blood are of significant concern. Moreover, timely screening of blood donors during an emerging infectious epidemic is a useful tool to obtain valuable epidemiological data in a convenient representative sample of the health population. Such data are helpful for public health officials in understanding how an infection spreads in the general population, in addition to assessing local blood safety risk.

This study has several limitations. First, the assay was validated with respiratory specimens from patients with

confirmed COVID-19. Up to now, data were limited on the viral load in plasma of asymptomatic carriers or individuals during the incubation period. Second, only 11 clinical specimens were used to compare assay performance across different NAT PCR platforms. Due to a relatively mild local epidemic of SARS-CoV-2 and strict population prevention measures, it was very difficult to get more clinical specimens during this study. Sansure Biotechnology Company provided the data about clinical evaluation between the Sansure Biotech Novel Coronavirus Nucleic Acid Diagnostic Kit (nucleic acid extraction and purification method was different with that used in this study but amplification method was the same) and the BGI Genomics comparator. Of 246 clinical samples, 52 specimens were tested positive. The results demonstrated a positive percent agreement of 94.34% and negative percent agreement of 98.96%. The data further demonstrate this assay's amplification ability of SARS-CoV-2. In conclusion, we developed a NAT screening system with demonstrated feasibility of screening large number of blood donors for SARS-CoV-2 on a routine basis if necessary.

ACKNOWLEDGMENTS

X.M.F. made significant contributions to the design of the study, supervised the research and finalized the manuscript. M.L., Y.W.Z., Y.L., and X.C. evaluated the diagnostic performance of the RT-PCR assay and drafted the manuscript. L.J. developed the RT-PCR assay. D.X.L. and M.L. performed clinical diagnosis and NAT testing for 11 specimens from patients with confirmed/suspected COVID-19 for clinical study. M.L., Y.W.Z., Y.L., X.C., W.L., H.M.L., H.W., Y.F.D., L.J.Z., M.S.J., X.Z., and C.B.W. performed the SARS-CoV-2 screening of 16 287 blood donations. M.L. and Y.W.Z. contributed to the analysis and interpretation of data. W.L. and J.L.G. reviewed and revised the manuscript. H.S. contributed to the study design and manuscript development. The work should be attributed to Chengdu Blood Center.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

ORCID

Hua Shan https://orcid.org/0000-0003-3764-1787
Xuemei Fu https://orcid.org/0000-0003-3371-6171

REFERENCES

1. Chen J. Pathogenicity and transmissibility of 2019-nCoV-A quick overview and comparison with other emerging viruses. Microbes Infect. 2020;22:69–71.
2. Lu R, Zhao X, Li J, et al. Genomic characterisation and epidemiology of 2019 novel coronavirus: Implications for virus origins and receptor binding. Lancet. 2020;395:565–574.
3. Chang L, Yan Y, Wang L. Coronavirus disease 2019: Coronaviruses and blood safety. Transfus Med Rev. 2020;34:75–80.
4. Fan C, Liu L, Guo W, et al. Prediction of epidemic spread of the 2019 Novel Coronavirus driven by spring festival transportation in China: A population-based study. Int J Environ Res Public Health. 2020;17:1679.
5. Adhikari SP, Meng S, Wu YJ, et al. Epidemiology, causes, clinical manifestation and diagnosis, prevention and control of coronavirus disease (COVID-19) during the early outbreak period: A scoping review. Infect Dis Poverty. 2020;9:29.
6. WHO. Statement on the second meeting of the International Health Regulations (2005) Emergency Committee regarding the outbreak of novel coronavirus (2019-nCoV) [Internet]. Geneva: World Health Organization, 2020. https://www.who.int/news-room/detail/30-01-2020-statement-on-the-second-meeting-of-the-international-health-regulations-(2005)-emergency-committee-regarding-the-outbreak-of-novel-coronavirus-(2019-ncov). Accessed January 30, 2020.
7. WHO. WHO director-General's opening remarks at the media briefing on COVID-19 - march 11, 2020 [internet]. Geneva: World Health Organization, 2020. https://www.who.int/dg/speeches/detail/who-director-general-s-opening-remarks-at-the-media-briefing-on-covid-19-March 11, 2020.
8. WHO. Coronavirus disease (COVID-2019) situation reports - 191 July 29, 2020 [internet]. Geneva: World Health Organization, 2020. https://www.who.int/docs/default-source/coronaviruse/situation-reports/20200729-covid-19-sitrep-191.pdf?sfvrsn=2c327e9e_2. Accessed July 29, 2020.
9. Zhu N, Zhang D, Wang W, et al. A novel coronavirus from patients with pneumonia in China, 2019. N Engl J Med. 2020;382:727–733.
10. Cleemput S, Dumon W, Fonseca V, et al. Genome detective Coronavirus typing tool for rapid identification and characterization of novel coronavirus genomes. Bioinformatics. 2020;36:3552–3555.
11. Corman VM, Landt O, Kaiser M, et al. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. Euro Surveill. 2020;25:2000045.
12. Y-Z. Z. Novel *2019 coronavirus genome.Virological. [internet]*. 2020. http://virological.org/t/novel-2019-coronavirus-genome/319. Accessed January 21, 2020.
13. Guo YR, Cao QD, Hong ZS, et al. The origin, transmission and clinical therapies on coronavirus disease 2019 (COVID-19) outbreak - an update on the status. Mil Med Res. 2020;7:11.
14. Chan JF, Kok KH, Zhu Z, et al. Genomic characterization of the 2019 novel human-pathogenic coronavirus isolated from a patient with atypical pneumonia after visiting Wuhan. Emerg Microb Infect. 2020;9:221–236.
15. Cascella M, Rajnik M, Cuomo A, Dulebohn SC, Di Napoli R. Features, evaluation and treatment Coronavirus (COVID-19) StatPearls. Treasure Island (FL). 2020.
16. Huang C, Wang Y, Li X, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet. 2020;395:497–506.
17. Wang Y, Wang Y, Chen Y, Qin Q. Unique epidemiological and clinical features of the emerging 2019 novel coronavirus pneumonia (COVID-19) implicate special control measures. J Med Virol. 2020;92:568–576.

18. Wu Z, McGoogan JM. Characteristics of and important lessons from the coronavirus disease 2019 (COVID-19) outbreak in China: summary of a report of 72 314 cases from the Chinese Center for Disease Control and Prevention. JAMA. 2020;323:1239.
19. Tian S, Hu N, Lou J, et al. Characteristics of COVID-19 infection in Beijing. J Infect. 2020;80:401–406.
20. Chan JF, Yuan S, Kok KH, et al. A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: a study of a family cluster. Lancet. 2020;395:514–523.
21. Yu P, Zhu J, Zhang Z, Han Y, Huang L. A familial cluster of infection associated with the 2019 novel coronavirus indicating potential person-to-person transmission during the incubation period. J Infect Dis. 2020;221:1757–1761.
22. Pung R, Chiew CJ, Young BE, et al. Singapore Novel Coronavirus outbreak Research Team. Investigation of three clusters of COVID-19 in Singapore: Implications for surveillance and response measures. Lancet. 2020;395:1039–1046.
23. Tong ZD, Tang A, Li KF, et al. Potential presymptomatic transmission of SARS-CoV-2, Zhejiang Province, China, 2020. Emerg Infect Dis. 2020;26:1052–1054.
24. Li P, Fu JB, Li KF, et al. Transmission of COVID-19 in the terminal stage of incubation period: A familial cluster. Int J Infect Dis. 2020;96:452–453.
25. WHO. Coronavirus disease 2019 (COVID-19) situation report −73 [internet]. Geneva: World Health Organization, 2020. https://www.who.int/docs/default-source/coronaviruse/situation-reports/20200402-sitrep-73-covid-19.pdf?sfvrsn=5ae25bc7_2. Accessed April 4, 2020.
26. Lauer SA, Grantz KH, Bi Q, et al. The incubation period of Coronavirus disease 2019 (COVID-19) from publicly reported confirmed cases: Estimation and application. Ann Intern Med. 2020;172:577–582.
27. Li Q, Guan X, Wu P, et al. Early transmission dynamics in Wuhan, China, of novel coronavirus-infected pneumonia. N Engl J Med. 2020;382:1199–1207.
28. Yang HY, Duan GC. Analysis on the epidemic factors for the Corona virus disease. Zhonghua Yu Fang Yi Xue Za Zhi. 2020;54:E021.
29. Xie M, Chen Q. Insight into 2019 novel coronavirus - an updated interim review and lessons from SARS-CoV and MERS-CoV. Int J Infect Dis. 2020;94:119–124.
30. Zhang W, Du RH, Li B, et al. Molecular and serological investigation of 2019-nCoV infected patients: Implication of multiple shedding routes. Emerg Microb Infect. 2020;9:386–389.
31. Chen W, Lan Y, Yuan X, et al. Detectable 2019-nCoV viral RNA in blood is a strong indicator for the further clinical severity. Emerg Microb Infect. 2020;9:469–473.
32. Wang W, Xu Y, Gao R, et al. Detection of SARS-CoV-2 in different types of clinical specimens. JAMA. 2020;323:1843–1844.
33. Chang L, Zhao L, Gong H, Wang L, Wang L. Severe acute respiratory syndrome coronavirus 2 RNA detected in blood donations. Emerg Infect Dis. 2020;26:1631–1633.
34. Monteil V, Kwon H, Prado P, et al. Inhibition of SARS-CoV-2 infections in engineered human tissues using clinical-grade soluble human ACE2. Cell. 2020;181:905–913. e7.
35. Eurosurveillance editorial team. Updated rapid risk assessment from ECDC on the novel coronavirus disease 2019 (COVID-19) pandemic: Increased transmission in the EU/EEA and the UK. Euro Surveill. 2020;25:2003121.
36. Liu R, Han H, Liu F, et al. Positive rate of RT-PCR detection of SARS-CoV-2 infection in 4880 cases from one hospital in Wuhan, China, from Jan to Feb 2020. Clin Chim Acta. 2020;505:172–175.
37. Yu F, Yan L, Wang N, et al. Quantitative detection and viral load analysis of SARS-CoV-2 in infected patients. Clin Infect Dis. 2020;71:793–798.
38. Wang J, Cai K, Zhang R, et al. Novel one-step single-tube nested quantitative real-time PCR assay for highly sensitive detection of SARS-CoV-2. Anal Chem. 2020;92:9399–9404.
39. Drosten C, Gunther S, Preiser W, et al. Identification of a novel coronavirus in patients with severe acute respiratory syndrome. N Engl J Med. 2003;348:1967–1976.
40. Mizumoto K, Kagaya K, Zarebski A, Chowell G. Estimating the asymptomatic proportion of coronavirus disease 2019 (COVID-19) cases on board the diamond princess cruise ship, Yokohama, Japan, 2020. Euro Surveill. 2020;25:2000180.
41. Hu Z, Song C, Xu C, et al. Clinical characteristics of 24 asymptomatic infections with COVID-19 screened among close contacts in Nanjing, China. Sci China Life Sci. 2020;63:706–711.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

How to cite this article: Li M, Zhao Y, Li Y, et al. Development and evaluation of a Novel RT-PCR system for reliable and rapid SARS-CoV-2 screening of blood donations. *Transfusion*. 2020;60:2952–2961. https://doi.org/10.1111/trf.16049