

About the Author

Mr. Lu is deputy chief of the Department of Control and Prevention for Infectious Disease at the Guangzhou Center for Disease Control and Prevention. His research interests are the surveillance, control, and prevention of respiratory infectious diseases, including influenza, avian influenza, and scarlet fever.

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Address for correspondence: Zhicong Yang, Guangzhou Center for Disease Control and Prevention, Guangzhou, Guangdong Province 510440, China; email: yangzc@gzcdc.org.cn; Bin Xu, Guangzhou Yuexiu District Center for Disease Control and Prevention, Guangzhou, Guangdong Province 510100, China; email: xubin6710@163.com

Severe Acute Respiratory Syndrome Coronavirus 2 RNA Detected in Blood Donations

Le Chang,¹ Lei Zhao,¹ Huafei Gong, Lunan Wang, Lan Wang

Author affiliations: National Center for Clinical Laboratories, Beijing Hospital, National Center of Gerontology; Institute of Geriatric Medicine, Chinese Academy of Medical Sciences, Beijing, China (L. Chang, Lunan Wang); Wuhan Blood Center, Wuhan, China (L. Zhao, Lan Wang); Shanghai Haoyuan Biotech Co., Ltd, Shanghai, China (H. Gong); Peking Union Medical College Graduate School, Chinese Academy of Medical Sciences, Beijing (Lunan Wang)

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Because of high rates of 2019 novel coronavirus disease in Wuhan, China, Wuhan Blood Center began screening for severe acute respiratory syndrome coronavirus 2 RNA on January 25, 2020. We screened donations in real-time and retrospectively and found plasma samples positive for viral RNA from 4 asymptomatic donors.

Because of the rapid increase of cases of 2019 novel coronavirus disease (COVID-19; 1) and detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) RNA in plasma (2,3), the safety of China's blood supply became a major concern (4). Most blood centers and blood banks in China began taking measures to ensure blood safety (5); on January 25, 2020, we began screening all donations collected at the Wuhan Blood Center.

We performed real-time reverse transcription PCR (RT-PCR) for SARS-CoV-2 RNA by using MultiScreen Pro RT-PCR assay (SYM-BIO LifeScience, <https://www.sym-bio.com.cn>). We performed pool testing by mixing plasma from 6–8 samples or individual testing by using 1.6 mL of plasma. We eluted 100 μ L of nucleic acid template and added 40 μ L of it to the RT-PCR mix.

By March 4, we had screened 2,430 donations in real-time, including 1,656 platelet and 774 whole blood donations. We identified the first positive donor in our center in a positive pool with a weak amplification of the open reading frame 1ab gene. The donor gave 2 units of platelets on January 28, which were included in the pool. However, the donor's prior donations collected on December 12 and 26 and January 13 were negative for viral RNA. Hubei Province Center for Disease Control and Prevention performed follow-up

¹These authors contributed equally to this article.

tests on plasma on February 2, which showed a weak positive result near the limit of detection; a throat swab specimen collected from the donor on February 10 also was positive, indicating an extremely low viral load in plasma. The donor reported no symptoms and was quarantined in a cabin hospital in Wuhan until 2 consecutive negative throat swab results on February 23 and February 25 (Figure).

We also performed retrospective testing of 4,995 donations collected during December 21, 2019–January 22, 2020, by using retained nucleic acid template after routine pool testing. On February 10, we found a positive result in a nucleic acid template derived from donations collected on January 19. We individually tested samples that were in storage at 2°C–8°C for 23 days because no plasma samples stored at -20°C were available. We identified another positive donor of whole blood. We tested plasma products from his donation twice and noted similar results, which suggests that viral RNA is relatively stable in plasma (Appendix Table, <https://wwwnc.cdc.gov/EID/article/26/7/20-0839-App1.pdf>). We immediately traced all blood products produced from donor 2’s whole blood, and they had not been used. Telephone follow-ups on February 15 and 25 showed donor 2 remained asymptomatic and quarantined at home.

In telephone follow-ups with donors who gave blood during January and February, we identified 33 donors who developed a fever after donation; all of their donations were removed from circulation. We performed retrospective individual screening on frozen plasma products from 17 donors and tested the retained nucleic acid templates after routine pool testing of the other 16 donors. We found 2 more positive donors who donated whole blood on January 20. Both had weak positive results, and donors reported fever onset on January 21 (Figure; Appendix Table). Donor 3 treated patients infected with SARS-CoV-2 in a

Wuhan hospital. His temperature returned to normal 8 days after donation. Donor 4’s temperature also returned to normal 7 days after taking self-prescribed antipyretic medications.

By March 4, we identified 4 blood donors in Wuhan whose plasma samples tested positive for SARS-CoV-2 RNA (Figure; Appendix Table). Samples from these donors were further tested for specific IgG and IgM against SARS-CoV-2 by ELISA; results were negative, indicating the possibility of infection in the early stage and the need to follow-up with these donors.

We found SARS-CoV-2 RNA in plasma during routine screening of blood donors, considered a healthy population. We tested the 4 donors multiple times, using different sample sources, including sample tubes, retained nucleic acid templates, or blood products, indicating the accuracy and validity of our results (Appendix Table). One limitation of our study is that we did not have more detailed information on donors 2, 3, and 4. Although we could not confirm virions in blood or whether the virus could be transmitted in blood products, the potential risk should not be neglected. However, detectable RNA might not signify infectivity. Further studies, such as virus culture, should be done to explore the possibility of viremia and follow-up of donors also is essential.

Of note, the donors all donated in late January, and we did not detect SARS-CoV-2 in plasma samples after then, indicating the strict containment measures taken by the government of China were effective. In China, donors are screened for related symptoms and asked if they feel healthy when they donate blood. Having donors call the blood donation center if they have any symptoms after donating is essential to avoid the risk of donation during the COVID-19 incubation period. Moreover, as more asymptomatic cases occur, screening donors

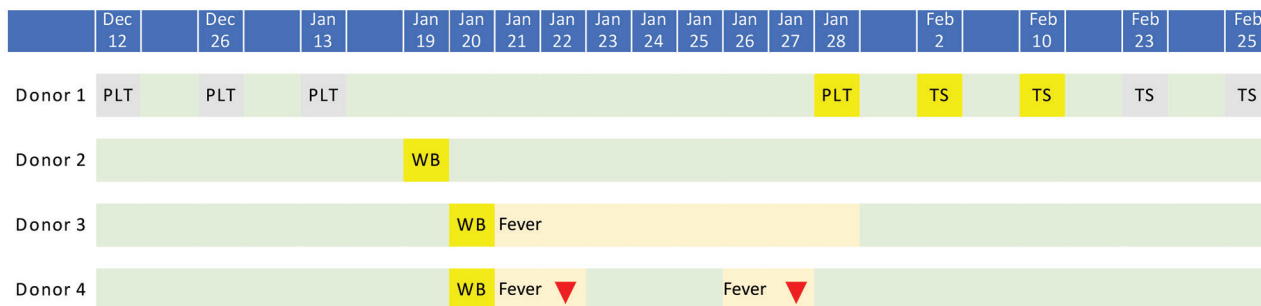


Figure. Timeline of donations and symptom onset of 2019 novel coronavirus disease from 4 blood donors, China. Gray indicates a negative result for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) RNA; yellow indicates a positive result. Green indicates the donor was asymptomatic or their temperature returned to normal; orange indicates fever; red triangle indicates the donor’s fever subsided after taking self-prescribed antipyretic medications. PLT, platelet; TS, throat swab; WB, whole blood.

for viral RNA with high-sensitivity assays, as we are doing in Hubei Province, will be critical to ensure blood safety.

H.G. is employed by Shanghai Haoyuan Biotech Co., Ltd. No other authors have disclosures to declare.

About the Author

Dr. Chang works as research assistant professor in National Center for Clinical Laboratories, Beijing Hospital, focusing on the detection of transfusion-transmitted infectious pathogens. Dr. Zhao is chief of the laboratory department of Wuhan Blood Center. His primary focus is detection of transfusion-transmitted infectious pathogens.

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Addresses for correspondence: Lunan Wang, National Center for Clinical Laboratories, Beijing Hospital, No. 1 Dahua Rd, Beijing 100730, China; email: lunan99@163.com; Lan Wang, Wuhan Blood Center, No. 8 Baofengyi Rd, Wuhan 430030, China; email: wlan66@126.com

Triplex Real-Time RT-PCR for Severe Acute Respiratory Syndrome Coronavirus 2

Jesse J. Waggoner, Victoria Stittleburg, Renee Pond, Youssef Saklawi, Malaya K. Sahoo, Ahmed Babiker, Laila Hussaini, Colleen S. Kraft, Benjamin A. Pinsky, Evan J. Anderson, Nadine Roupael

Author affiliations: Emory University, Atlanta, Georgia, USA (J.J. Waggoner, V. Stittleburg, R. Pond, Y. Saklawi, A. Babiker, L. Hussaini, C.S. Kraft, E.J. Anderson, N. Roupael); Stanford University, Stanford, California, USA (M.K. Sahoo, B.A. Pinsky)

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Most reverse transcription PCR protocols for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) include 2–3 targets for detection. We developed a triplex, real-time reverse transcription PCR for SARS-CoV-2 that maintained clinical performance compared with singleplex assays. This protocol could streamline detection and decrease reagent use during current high SARS-CoV-2 testing demands.

Detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) typically relies on molecular testing of respiratory tract specimens, although viral RNA can be detected in other specimens (1). Real-time reverse transcription PCR (rRT-PCR) protocols have been described for SARS-CoV-2, but most involve testing with multiple, singleplex reactions (2–6). Such algorithms use large volumes of reagents and limit laboratory testing capacity, both of which have become crucial during the ongoing coronavirus disease pandemic (7). Multiplex assays are commercially available (8,9) but require specific platforms and are more expensive than laboratory-developed methods.

Our objective was to develop an internally controlled, triplex assay to detect SARS-CoV-2 RNA in clinical samples. We initially evaluated 6 individual rRT-PCRs, 3 published by the US Centers for Disease Control and Prevention (2) that target the nucleocapsid (N) gene, N1, N2, and N3; and 3 published by Corman, et al. (4) that target RNA-dependent RNA polymerase (RdRp), envelope (E), and N genes. We performed assays in 20 μ L reactions of the Luna Universal Probe One-Step RT-qPCR Kit (New England Biolabs, <https://www.neb.com>) on a Rotor-Gene Q (QIAGEN, <https://www.qiagen.com>) by using 5 μ L of eluate and our standard cycling protocol (10). We extracted total nucleic acids from samples on an

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Appendix

Appendix Table. Information and reverse transcription-PCR results on samples from asymptomatic blood donors tested for severe acute respiratory syndrome coronavirus 2, China*

Donor	Sex	Age	Donation date	Sample source†	Cycle threshold		Detection date
					ORF1ab	N	
1	M	53	2020 Jan 28	A	37.405	36.635	2020 Jan 28
				B	34.346	34.004	2020 Jan 28
				C	34.423	34.577	2020 Feb 7
2	M	37	2020 Jan 19	D	40.219	39.834	2020 Feb 10
				B‡	UD	38.715	2020 Feb 13
				E	38.254	37.655	2020 Feb 14
				E	38.495	37.189	2020 Feb 14
3	M	42	2020 Jan 20	E	UD	40.183	2020 Feb 15
				E	38.268	UD	2020 Feb 16
4	F	21	2020 Jan 20	E	37.607	UD	2020 Feb 7
				E	38.732	37.015	2020 Feb 8
				E	38.644	UD	2020 Feb 8

*The limit of detection of Pro RT-PCR assay (SYM-BIO LifeScience, <https://www.sym-bio.com.cn>) is 10 copies/mL in 1.6 mL of plasma. Clinical sensitivity and specificity were >99.99% based on donation screening data in Wuhan, China and no cross-reactivity was found with other human coronaviruses, influenza viruses, common human viruses, or transfusion-transmitted pathogens. Cycle threshold of a positive result is ≤ 42 for 1 region and ≤ 45 for the other region. Any other situations for any amplification of the 2 regions, such as only 1 region detected or cycle threshold of the 2 regions both were between 42 and 45, the specimen should be retested. N, nucleocapsid region; ORF, open reading frame; UD, undetected. †A, screening sample tube (pool testing); B, screening sample tube (individual testing); C, platelet product (individual testing); D, retained nucleic acid template after routine pool testing; E, frozen plasma product (individual testing). Pool testing was performed by mixing plasma from 6–8 samples. Individual testing used 1.6 mL of plasma samples. A 40 μ L volume of nucleic acid template from 100 μ L of nucleic acid eluted was added to the RT-PCR mix.

‡Because of limited sample volume, we diluted the sample 4-fold before testing.