



Stability of SARS-CoV-2 Spike G614 Variant Surpasses That of the D614 Variant after Cold Storage

 Sheng-Yu Huang,^{a,b} Yu-An Kung,^{a,b} Peng-Nien Huang,^{a,b,c} Sheng-Yun Chang,^{a,d}  Yu-Nong Gong,^{a,e} Yi-Ju Han,^{a,f} Huan-Jung Chiang,^{a,b} Kuan-Ting Liu,^{a,f}  Kuo-Ming Lee,^a Chia-Yu Chang,^{g,h} Chia-Ching Chang,^{g,h,i,j} Chung-Guei Huang,^{b,e}  Shin-Ru Shih^{a,b,e,k,l,m}

^aResearch Center for Emerging Viral Infections, College of Medicine, Chang Gung University, Taoyuan, Taiwan

^bDepartment of Medical Biotechnology and Laboratory Science, College of Medicine, Chang Gung University, Taoyuan, Taiwan

^cDivision of Infectious Diseases, Department of Pediatrics, Linkou Chang Gung Memorial Hospital, Taoyuan, Taiwan

^dBachelor Program in Artificial Intelligence, College of Engineering, Chang Gung University, Taoyuan, Taiwan

^eDepartment of Laboratory Medicine, Linkou Chang Gung Memorial Hospital, Taoyuan, Taiwan

^fGraduate Institute of Biomedical Science, Division of Biotechnology, College of Medicine, Chang Gung University, Taoyuan, Taiwan

^gDepartment of Biological Science and Technology, College of Biological Science and Technology, National Yang Ming Chiao Tung University, Hsinchu, Taiwan

^hCenter for Intelligent Drug Systems and Smart Bio-devices (IDS2B), National Yang Ming Chiao Tung University, Hsinchu, Taiwan

ⁱDepartment of Electrophysics, National Yang Ming Chiao Tung University, Hsinchu, Taiwan

^jInstitute of Physics, Academia Sinica, Nankang, Taipei, Taiwan

^kResearch Center for Chinese Herbal Medicine, Chang Gung University of Science and Technology, Taoyuan, Taiwan

^lResearch Center for Food and Cosmetic Safety, Chang Gung University of Science and Technology, Taoyuan, Taiwan

^mGraduate Institute of Health Industry Technology, College of Human Ecology, Chang Gung University of Science and Technology, Taoyuan, Taiwan

Sheng-Yu Huang, Yu-An Kung, and Peng-Nien Huang contributed equally to this work; author order was decided by drawing straws.

ABSTRACT Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) carrying the D614G mutation on the spike protein is the predominant circulating variant and is associated with enhanced infectivity. However, whether this dominant variant can potentially spread through the cold chain and whether the spike protein affects virus stability after cold storage remain unclear. To compare the infectivity of two SARS-CoV-2 variants, namely, SARS-CoV-2 variants with spike protein with the D614 mutation (S-D614) and G614 mutation (S-G614), after different periods of refrigeration (4°C) and freezing (−20°C). We also determined the integrity of the viral RNA and the ability of the spike protein to bind angiotensin-converting enzyme 2 (ACE2) after storage at these conditions. The results showed that SARS-CoV-2 was more stable and infectious after storage at −20°C than at 4°C. Particularly, the S-G614 variant was found to be more stable than the S-D614 variant. The spike protein of the S-G614 variant had better binding ability with the ACE2 receptor than that of the S-D614 variant after storage at −20°C for up to 30 days. Our findings revealed that SARS-CoV-2 remains stable and infectious after refrigeration or freezing, and their stability and infectivity up to 30 days depends on the spike variant. Stability and infectivity are related to each other, and the higher stability of S-G614 compared to that of S-D614 may contribute to rapid viral spread of the S-G614 variant.

IMPORTANCE It has been observed that variants of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) are more stable and infectious after storage at −20°C than at 4°C. A SARS-CoV-2 S-D614G variant is currently the most dominant variant in circulation and is associated with enhanced infectivity. We compared the stability of two SARS-CoV-2 variants: the early S-D614 variant carrying the D614 spike protein and the new S-G614 variant carrying the G614 spike protein, stored at both 4°C and −20°C for different periods. We observed that SARS-CoV-2 remains stable and infectious after refrigeration or freezing, which further depends on the spike variant, that

Citation Huang S-Y, Kung Y-A, Huang P-N, Chang S-Y, Gong Y-N, Han Y-J, Chiang H-J, Liu K-T, Lee K-M, Chang C-Y, Chang C-C, Huang C-G, Shih S-R. 2021. Stability of SARS-CoV-2 spike G614 variant surpasses that of the D614 variant after cold storage. *mSphere* 6:e00104-21. <https://doi.org/10.1128/mSphere.00104-21>.

Editor Helene F. Rosenberg, National Institute of Allergy and Infectious Diseases

Copyright © 2021 Huang et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution 4.0 International license](https://creativecommons.org/licenses/by/4.0/).

Address correspondence to Shin-Ru Shih, srshih@mail.cgu.edu.tw.

Received 3 February 2021

Accepted 10 March 2021

Published 31 March 2021