



Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.



Development and evaluation of an RT-qPCR for the identification of the SARS-CoV-2 Omicron variant

Correspondence

The Omicron (B.1.1.529/BA.1) variant of concern (VOC) emerged in Southern Africa in November 2021, and rapidly overtook Delta (B.1.617.2) as the predominant SARS-CoV-2 variant globally [1]. The mutations in the Omicron spike rendered ineffective the monoclonal antibody therapies casirivimab/imdevimab (REGEN—COV, Regeneron) and bamlanivimab/etesevimab (Lilly) [2]. In addition, the surge in positive cases overwhelmed genomic sequencing capacity, resulting in delayed variant reporting. To inform monoclonal antibody selection and support epidemiologic surveillance, we developed a reverse-transcription quantitative PCR (RT-qPCR) for the sensitive and specific detection of Omicron VOC.

This assay targets an Omicron-specific Spike (S) insertion-deletion mutation (indel_{211–214}) found in the B.1.1.529/BA.1 lineage and BA.1.1 sublineage, accounting for 99.9% of Omicron sequences in the U.S., and 96.6% of sequences worldwide as of 29 January 2022 [3]. The forward primer covers the deletion at amino acid position 211 (NL211I), while the probe interrogates the insertion at amino acid position 214 (ins214EPE) [4]. This indel_{211–214} assay was combined in multiplex with envelope (E) primers/probe as internal control (Table 1) [5]. A limitation of this RT-qPCR is that the BA.2 and BA.3 Omicron lineages do not have indel_{211–214} and only the E target would be detected. Though as of this writing, BA.2 and BA.3 account for ~0.1% of Omicron sequences in the U.S., BA.2 increased substantially in other countries during the last three weeks of January 2022. Notably, 72.5% (18,030/24,863) of global BA.2 sequences are currently submitted from Denmark [3]. Depending on local/regional prevalence, as well as the potential for further spread, the addition of primers/probes targeting BA.2 and/or BA.3 lineage-specific mutations may be warranted [6].

Each 20 μ L reaction using SuperScript III Platinum One-Step qRT-PCR Kit (Invitrogen) contained 10 μ L of 2X reaction mix, 0.4 μ L enzyme mix, 1 μ L primer-probe mix, 3.6 μ L nuclease-free water, and 5 μ L nucleic acid eluate. All experiments were conducted on a QuantStudio7 Pro real-time PCR instrument (Applied Biosystems). Cycling conditions were: 52 °C for 15:00, 94 °C for 2:00, and then 45 cycles of 94 °C for 00:15, 55.0 °C for 00:40, and 68 °C for 00:20. Fluorescence thresholds were manually set at 2000 Δ Rn for S:indel_{211–214} (CY5) and 5000 for E (FAM). Thresholds were selected to fall in the middle of the exponential-phase of the amplification curve, though values may be adjusted to account for variation in baseline or maximum fluorescence.

To determine analytical sensitivity, single-stranded DNA comprised of either the Omicron S or E target sequences (Table 2) were diluted to 10, 5, 4, 3, and 1 copies/ μ L in buffer AVE (Qiagen). Twenty replicates at each dilution were tested. Probit regression analysis determined the 95% Lower Limit of Detection was 1.3 copies/ μ L [95% confidence interval (CI) 1.0 - 1.6] for S:indel_{211–214} and 4.3 copies/ μ L (95% CI 3.8 - 5.1) for E.

To determine clinical performance, total nucleic acids were extracted from 94 SARS-CoV-2 positive upper respiratory specimens in 300 μ L transport media using the Chemagic Viral DNA/RNA 300 Kit automated on the Janus G3 Primary Sample Reformatter and Chemagic 360 extraction instrument (PerkinElmer). Specimens were collected December 8–23, 2021, consisting of a convenience set of 47 Omicron and 47 Delta variants confirmed by SARS-CoV-2 whole genome sequencing (WGS) (Supplemental Table 1) [7]. RT-qPCR was set-up using the Janus G3 PCR Workstation (PerkinElmer).

The Omicron-specific RT-qPCR detected S:indel_{211–214} and E in 100% (47/47; 95%CI: 95.1–100) of Omicron specimens. Similarly, S:indel_{211–214} was not detected in 100% (47/47; 95%CI: 95.1–100) of Delta specimens. Initially, one Delta failed to amplify E, but amplification was observed upon repeat testing of the original eluate, as well as the re-extracted specimen.

In summary, we describe an accurate RT-qPCR for rapid identification of the Omicron VOC (B.1.1.529/BA.1, BA.1.1), suitable for clinical decision-making, near real-time variant surveillance, and triage of samples for WGS.

Table 1
Primer and Probe Sequences.

Name	Sequence (5' to 3')	Final concentration
Omicron F Primer	TTCTAAGCACACGCCTATTATAGTG	300 nM
Omicron R Primer	GGCAAATCTACCAATGGTTCTA	300 nM
Omicron Probe	CY5-CGTGAGCCAGAAGATCTCCCTCAG-BHQ2	100 nM
E gene F Primer	ACAGGTACGTTAATAGTTAATAGCGT	300 nM
E gene R Primer	ATATTGCAGCAGTACGCACACA	300 nM
E gene Probe	FAM-ACACTAGCCATCCTTACTGCGCTTCG-BHQ1	100 nM

E, Envelope; F, Forward; R, Reverse; Cy5, Cyanine-5; FAM, 6-Carboxyfluorescein; BHQ, Black Hole Quencher.

<https://doi.org/10.1016/j.jcv.2022.105101>

Received 21 January 2022; Received in revised form 2 February 2022; Accepted 4 February 2022

Available online 5 February 2022

1386-6532/© 2022 Elsevier B.V. All rights reserved.

Table 2
Single-Stranded DNA Oligonucleotides.

Name	Sequence (5' to 3')
Omicron ssDNA	TTCTAAGCACAGCCTATTATAGTGC GTGAGCCAGAAGATCTCCCTCAGGG TTTTTCGGCTTTAGAACCATTGGTA GATTTGCC
E gene ssDNA	TTCGGAAGAGACAGGTACGTTAA TAGTTAATAGCGTACTTCTTTTCTTG CTTTCGTGGTATTCTTGCTAGTTACACT AGCCATCCTTACTGCGCTTCGATTGT GTGCGTACTGCTGCAATATTGTTAACGTG

E, envelope; ssDNA, single-stranded DNA.

Declaration of Competing Interest

The authors declare no conflicts of interest.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.jcv.2022.105101](https://doi.org/10.1016/j.jcv.2022.105101).

References

- [1] R. Viana, S. Moyo, D.G. Amoako, H. Tegally, C. Scheepers, C.L. Althaus, U.J. Anyaneji, P.A. Bester, M.F. Boni, M. Chand, W.T. Choga, R. Colquhoun, M. Davids, K. Deforche, D. Doolabh, S. Engelbrecht, J. Everatt, J. Giandhari, M. Giovanetti, D. Hardie, V. Hill, N.-Y. Hsiao, A. Iranzadeh, A. Ismail, C. Joseph, R. Joseph, L. Koopile, S.L.K. Pond, M. U. Kraemer, L. Kuate-Lere, O. Laguda-Akingba, O. Lesetedi-Mafoko, R.J. Lessells, S. Lockman, A.G. Lucaci, A. Maharaj, B. Mahlangu, T. Maponga, K. Mahlakwane, Z. Makatini, G. Marais, D. Maruapula, K. Masupu, M. Matshaba, S. Mayaphi, N. Mbhele, M.B. Mbulawa, A. Mendes, K. Mlisana, A. Mnguni, et al., Rapid epidemic expansion of the SARS-CoV-2 Omicron variant in southern Africa, medRxiv (2021), <https://doi.org/10.1101/2021.12.19.21268028>.
- [2] D. Planas, N. Saunders, P. Maes, F. Guivel-Benhassine, C. Planchais, J. Buchrieser, W.-H. Bolland, F. Porrot, I. Staropoli, F. Lemoine, H. Péré, D. Veyer, J. Puech, J. Rodary, G. Baele, S. Dellicour, J. Raymenants, S. Gorissen, C. Geenen, B. Vanmechelen, T. Wawina -Bokalanga, J. Martí-Carreras, L. Cuyppers, A. Sève, L. Hocqueloux, T. Prazuck, F. Rey, E. Simon-Lorière, T. Bruel, H. Mouquet, E. André, O. Schwartz, Considerable escape of SARS-CoV-2 Omicron to antibody neutralization, Nature (2021), <https://doi.org/10.1038/s41586-021-04389-z>.
- [3] A.A. Latif, J.L. Mullen, M. Alkuzweny, G. Tsueng, M. Cano, E. Haag, J. Zhou, M. Zeller, E. Hufbauer, N. Matteson, C. Wu, K.G. Andersen, A.I. Su, K. Gangavarapu, L.D. Hughes, The center for viral systems biology, Omicron Variant Rep. (2022) <https://outbreak.info/situation-reports/omicron>. Accessed 29 January.
- [4] M. Gerdol, K. Dishnica, A. Giorgetti, Emergence of a recurrent insertion in the N-terminal domain of the SARS-CoV-2 spike glycoprotein, Virus Res. 310 (2022), 198674.
- [5] V.M. Corman, O. Landt, M. Kaiser, R. Molenkamp, A. Meijer, D.K. Chu, T. Bleicker, S. Brunink, J. Schneider, M.L. Schmidt, D.G. Mulders, B.L. Haagmans, B. van der Veer, S. van den Brink, L. Wijsman, G. Goderski, J.L. Romette, J. Ellis, M. Zambon, M. Peiris, H. Goossens, C. Reusken, M.P. Koopmans, C. Drosten, Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR, Euro Surveill (2020) 25.
- [6] Bacterial and Viral Bioinformatics Resource Center. Real-time tracking and early warning system for SARS-CoV-2 variants and lineages of concern. <https://www.bv-brc.org/view/VariantLineage>. Accessed 27 January 2022.
- [7] H. Wang, J.A. Miller, M. Verghese, M. Sibai, D. Solis, K.O. Mfuh, B. Jiang, N. Iwai, M. Mar, C. Huang, F. Yamamoto, M.K. Sahoo, J. Zehnder, B.A. Pinsky, Multiplex SARS-CoV-2 genotyping reverse transcriptase PCR for population-level variant screening and epidemiologic surveillance, J. Clin. Microbiol. 59 (2021) e0085921.

Mamdouh Sibai^a, Hannah Wang^a, Priscilla S-W Yeung^a, Malaya K. Sahoo^a, Daniel Solis^a, Kenji O. Mfuh^b, ChunHong Huang^a, Fumiko Yamamoto^a, Benjamin A. Pinsky^{a,b,c,*}

^a Department of Pathology, Stanford University School of Medicine, Stanford, CA, USA

^b Clinical Virology Laboratory, Stanford Health Care, Stanford, CA, USA

^c Division of Infectious Diseases and Geographic Medicine, Department of Medicine, Stanford University School of Medicine, Stanford, CA, USA

* Corresponding author.

E-mail address: bpinsky@stanford.edu (B.A. Pinsky).