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

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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 μL reaction using SuperScript III Platinum One-Step qRT-PCR Kit (Invitrogen) contained 10μL of 2X reaction mix, 0.4μL enzyme mix, 1μL primer-probe mix, 3.6μL nuclease-free water, and 5μL 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 ΔRn 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/μL 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/μL [95% confidence interval (CI) 1.0 - 1.6] for S:indel_211–214 and 4.3 copies/μL (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 μL 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.

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Table 2
Single-Stranded DNA Oligonucleotides.

Name	Sequence (5′ to 3′)
Omicron ssDNA	TTCTAAGCACACGCCTATTATAGTGC GTGAGCCAGAAGATCTCCCTCAGGG TTTTTCGGCTTTAGAACCATTGGTA GATTTGCC
E gene ssDNA	TTCGGAAGAGACAGGTACGTTAA TAGTTAATAGCGTACTTCTTTTTCTTG 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.

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