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Classification of Omicron BA.1, BA.1.1, and BA.2 sublineages by TaqMan assay consistent with whole genome analysis data

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ABSTRACT

Objectives: Recently, the Omicron strain of SARS-CoV-2 has spread and replaced the previously dominant Delta strain. Several Omicron sublineages (BA.1, BA.1.1, and BA.2) have been identified, with *in vitro* and preclinical reports showing that the pathogenicity and therapeutic efficacy differs between BA.1 and BA.2. We sought to develop a TaqMan assay to identify these subvariants.

Methods: A TaqMan assay was constructed for rapid identification and genotyping of Omicron sublineages with 171 samples. We analyzed three characteristic mutations of the spike gene, $\Delta 69-70$, G339D, and Q493R, by TaqMan assay. The accuracy of the TaqMan assay was examined by comparing its results with the results of whole genome sequencing (WGS) analysis.

Results: A total of 171 SARS-CoV-2 positive samples were analyzed by WGS and TaqMan assay. The 127 samples determined as BA.1/BA.1.1 by WGS were all positive for $\Delta 69-70$, G339D and Q493R by TaqMan assay. A total of 42 samples, determined as BA.2 by WGS, were negative for $\Delta 69-70$ but positive for G339D and Q493R by TaqMan. Two samples with G339N were determined to be inconclusive by the TaqMan method. Except for these two samples, the concordance rate between WGS and the TaqMan assay was 100% (169/169).

Conclusion: TaqMan assays targeting characteristic mutations are useful for identification and discrimination of Omicron sublineages.

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Introduction

SARS-CoV-2 has mutated continuously, which affected the transmissibility of the virus and the efficacy of antiviral drugs and vaccines. To control the emergence of new strains, it is important

to continuously monitor viral evolution through genomic epidemiologic analysis (Munnink *et al.*, 2021).

Recently, a new strain, designated as B.1.1.529, was detected and isolated in Gauteng Province, South Africa (World Health Organization, 2021). The World Health Organization designated the B.1.1.529 lineage as a variant of concern (VOC) on November 26, 2021 (World Health Organization, 2021), naming it Omicron, in line with the naming convention adopted for previous VOCs using the Greek alphabet, and it has since spread to a number of countries to become a globally dominant strain (Desingu and Nagarajan, 2022). Omicron contains more than 30 nonsynonymous mutations in the

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spike protein; therefore, Omicron viruses may reduce the efficacy of antibody therapy and evade vaccine-induced immunity.

Omicron viruses are classified into several sublineages, including BA.1, BA.1.1, BA.2, and BA.3, which have been observed worldwide. In the early period after Omicron emergence, BA.1 was the dominant sublineage; however, in Denmark, the United Kingdom, India, the Philippines, and South Africa, BA.2 became dominant (Hodcroft, 2021; Fonager et al., 2022; Yamasoba et al., 2022), with BA.2 reported to be more transmissible than BA.1 (Ito et al., 2022).

Omicron contains numerous mutations compared with other VOCs (Gangavarapu, et al. 2022; Saxena et al., 2022; Wang and Cheng, 2022). A total of 33 mutations have been identified in the spike protein in BA.1, 34 in BA.1.1, and 29 in BA.2. BA.1 and BA.1.1 share 33 mutations, but BA.1.1 has an additional mutation in R346K compared with BA.1. Compared with BA.1 and BA.1.1, the spike protein mutations in BA.2 are very different. Unique mutations in BA.1/BA.1.1 are A67V, Δ 69–70, T95I, Δ 143–145, N211I, Δ 212, S371L, G466S, G496S, T547K, N856K, and L981F. Conversely, unique mutations in BA.2 are T19I, L24S, Δ 25–27, V213G, S371F, T376A, D405N, and R408S.

In this study, we present a TaqMan assay to discriminate between BA.1/BA.1.1 and BA.2. This method is feasible for any laboratory equipped to perform quantitative real-time polymerase chain reaction (PCR).

Methods

Samples

The study includes patients who are SARS-CoV-2 positive ($n = 294$) who were collected from all areas of Yamanashi Prefecture in Japan and tested positive at our hospital. The sample collection period was from January 12 to March 10, 2022. During this period, 294 positive samples were subjected to whole genome analysis (Hirotsu et al., 2022), and 171 of them were randomly selected to evaluate the performance of the TaqMan assay.

SARS-CoV-2 diagnostic testing

Nasopharyngeal swab samples were collected using cotton swabs and placed in viral transport media (Copan Diagnostics, Murrieta, CA, USA). Multiple molecular diagnostic testing platforms, including SARS-CoV-2 quantitative reverse transcriptase-PCR in accordance with the protocol developed by the National Institute of Infectious Diseases in Japan (Shirato et al., 2020), FilmArray Respiratory Panel 2.1 with the FilmArray Torch system (bioMérieux, Marcy-l'Étoile, France) (Hirotsu et al., 2020a), Xpert Xpress SARS-CoV-2 test using Cepheid GeneXpert (Cepheid, Sunnyvale, CA, USA) (Hirotsu et al., 2022), and Lumipulse antigen test with the LUMIPULSE G600II system (Fujirebio, Inc., Tokyo, Japan) were used to identify positive samples for this study (Hirotsu et al., 2021; Hirotsu et al., 2020b).

Whole genome sequencing (WGS)

WGS analysis was performed as previously described (Hirotsu and Omata, 2021a; Hirotsu and Omata, 2021b; Hirotsu and Omata, 2021c). In brief, SARS-CoV-2 genomic RNA was reverse transcribed into cDNA and amplified by using the Ion AmpliSeq SARS-CoV-2 Insight Research Assay (Thermo Fisher Scientific, Waltham, MA, USA) on the Ion Torrent Genexus System. Sequencing reads and quality were processed using Genexus software with SARS-CoV-2 plugins. The sequencing reads were mapped and aligned using the torrent mapping alignment program. Assembly was performed with the Iterative Refinement Meta-Assembler (Shepard et al., 2016).

Table 1

Comparison of results between WGS and TaqMan assay.

Lineage	WGS n	TaqMan assay		
		Spike Δ 69-70	Spike G339D	Spike Q493R
BA.1/BA.1.1	127	Pos / Neg / Inc 127 / 0 / 0	Pos / Neg / Inc 127 / 0 / 0	Pos / Neg / Inc 127 / 0 / 0
BA.2	44	0 / 44 / 0	42 / 0 / 2 ^a	44 / 0 / 0

Inc, inconclusive; Neg, negative; Pos, positive; WGS, whole genome sequencing.

^a Two samples with spike G339N were included, and a TaqMan probe targeting spike G339D showed only low amplification in Figure 2. These samples were determined to be inconclusive.

To determine the viral clade and lineage classifications, the consensus FASTA files were downloaded and processed through Nextstrain (Hadfield et al., 2018) and Phylogenetic Assignment of Named Global Outbreak Lineages (PANGOLIN) (Rambaut et al., 2020). The FASTA files were deposited in the Global Initiative on Sharing Avian Influenza Data (GISAID) EpiCoV database (Shu and McCauley, 2017). All GISAID Accession IDs are noted in Table S1.

TaqMan assay

To distinguish BA.1/BA.1.1 from BA.2, we constructed a TaqMan assay system to analyze the spike protein mutations that characterize the Omicron sublineages. Thus, we used a predesigned TaqMan SARS-CoV-2 Mutation Panel (Thermo Fisher Scientific) to distinguish Δ 69–70, G339D, and Q493R. The TaqMan probe detected both wild-type and variant sequences of SARS-CoV-2, with the TaqMan minor groove binder probe for the wild-type allele labeled with VIC dye and the variant allele with FAM dye fluorescence. TaqPath 1-Step RT-qPCR Master Mix CG was used as master mix and the qPCR was performed on the Step-One Plus Real-Time PCR System (Thermo Fisher Scientific). The allelic discrimination software (Thermo Fisher Scientific) was used to analyze data and identify variant and wild-type alleles. The amplification curves of each data set were visually confirmed.

According to the GISAID database, as of March 11, 2022, the frequency of mutations targeted in this study detected in each sublineage were as follows. Δ 69–70 in 96.1%, 95.7%, and 0.1% of BA.1, BA.1.1, and BA.2, respectively; G339D in 87.9%, 99.3%, and 96.3% of BA.1, BA.1.1, and BA.2, respectively; Q493R in 88.3%, 91.2%, and 92% of BA.1, BA.1.1, and BA.2, respectively (Gangavarapu, et al. 2022). The spike Δ 69–70 mutation was also used to distinguish sublineages because it was detected in BA.1/BA.1.1 but not in BA.2 (Supplemental Figure 1a). Spike G339D and Q493R mutations are detected in BA.1/BA.1.1 and BA.2 but not in other VOCs (Supplemental Figure 1b).

Results

Construction of a TaqMan assay to distinguish Omicron sublineages

When we analyzed using nucleic acids extracted from nasopharyngeal swabs, G339D and Q493R mutations were specifically detected in Omicron-infected patients (Figure 1a and 1b). In addition, the Δ 69–70 mutation was distinct between BA.1/BA.1.1 and BA.2 (Figure 1c). These results showed that TaqMan assays could detect Omicron strains and distinguish between Omicron sublineages.

Comparison of TaqMan assay and WGS data

To examine whether the TaqMan assay could accurately distinguish Omicron sublineages, we compared WGS data and TaqMan assay results using 171 SARS-CoV-2-positive samples (Table 1 and Table S1). WGS analysis determined 127 samples to be BA.1/BA.1.1

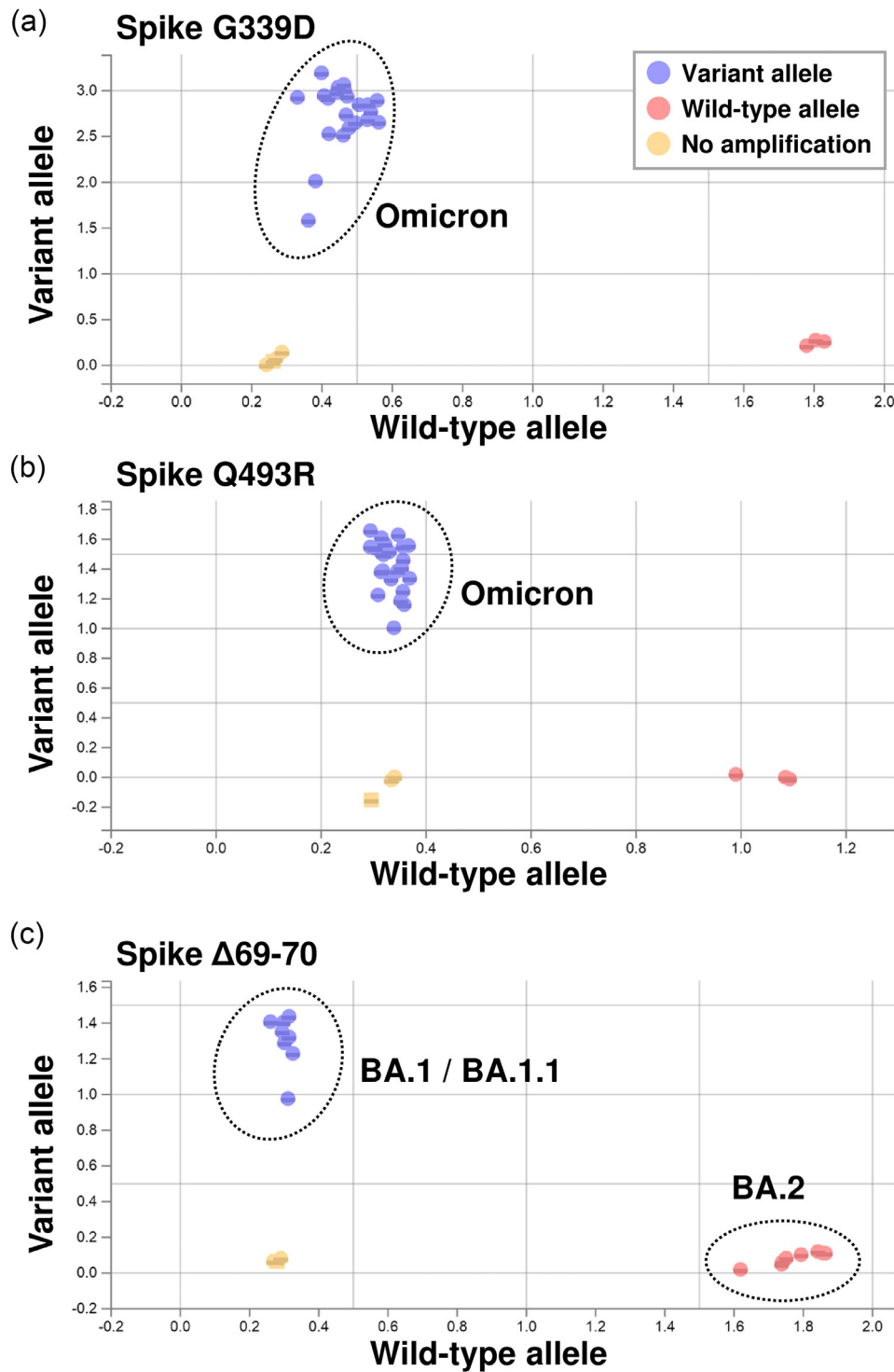


Figure 1. Genotyping of Omicron sublineages by TaqMan assay. (a-c) Samples were analyzed with a TaqMan assay that detects mutations in Omicron spike proteins. Spike protein mutations G339D (a), Q493R (b), and $\Delta 69-70$ (c) were targeted. G339D and Q493R indicated Omicron (including BA.1/BA.1.1 and BA.2), while $\Delta 69-70$ was used to distinguish BA.1/BA.1.1 from BA.2. Blue circles indicate variant alleles (FAM dye) and red circles indicate wild-type alleles (VIC dye). The results were plotted on a scatterplot of wild-type alleles (x axis) versus variant alleles (y axis) using the allelic discrimination software.

and 44 samples to be BA.2 (Table 1). In these samples, TaqMan assay analysis showed that all BA.1/BA.1.1 samples were positive for $\Delta 69-70$, G339D, and Q493R, whereas BA.2 samples were negative for $\Delta 69-70$ and positive for G339D and Q493R (Table 1). Two samples were determined to be inconclusive because of equivocal results in the G339D assay. Excluding these two samples, the TaqMan assay data were consistent with WGS data, demonstrating

100% (169/169) agreement. Therefore, the TaqMan assay represents a useful technique for distinguishing Omicron sublineages.

Mutations in TaqMan assay target sites

In the two samples determined to be inconclusive aforementioned, WGS analysis results revealed these samples had the spike

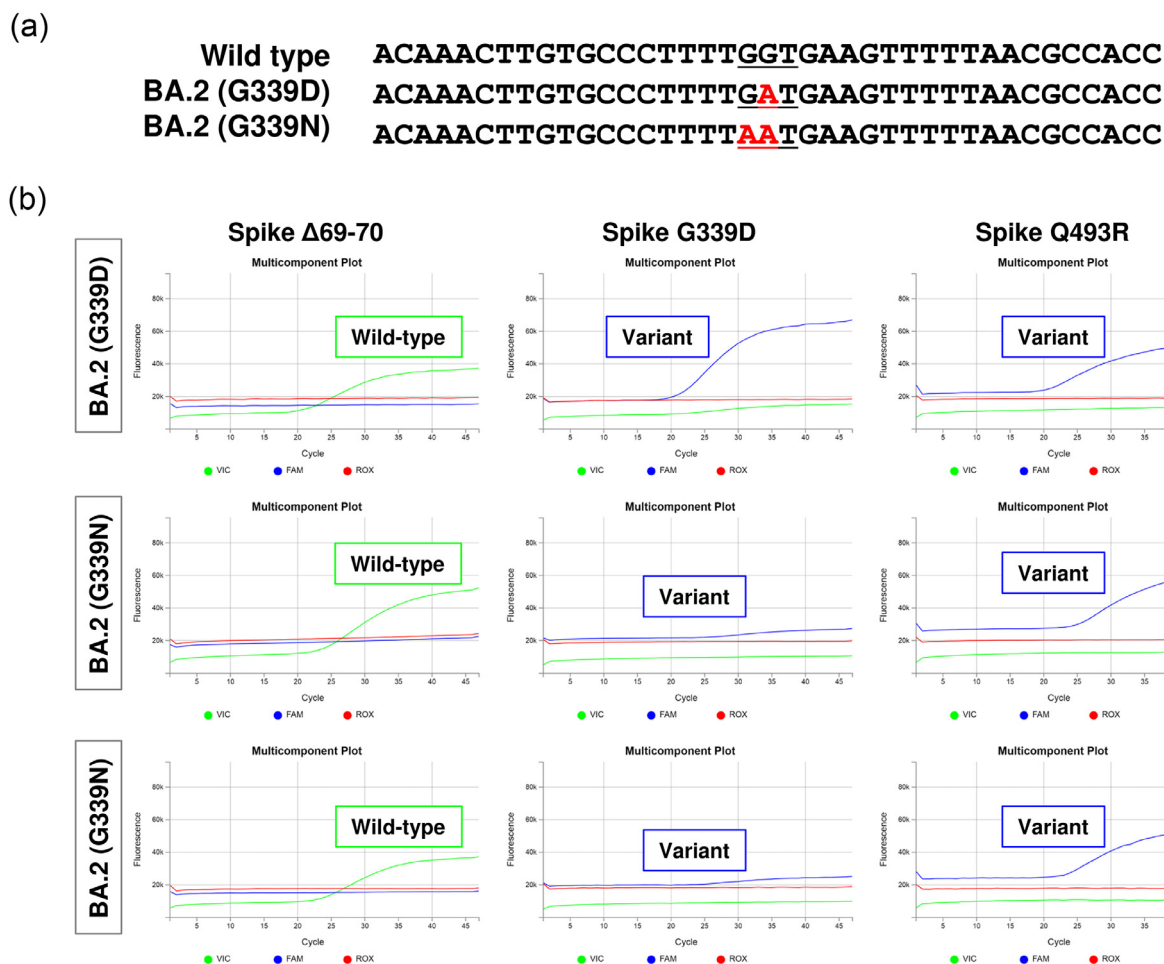


Figure 2. Analysis of samples with mutations in the TaqMan probe site. Two samples were classified by WGS analysis as BA.2 containing a G339N mutation. (a) The mutation site occurred at codon 339, from wild-type (GGT, glycine [G]) to the variant form (GAT, aspartic acid [D] or AAT, asparagine [N]). (b) Most BA.2 lineage viruses carry the G339D mutation, but two samples showed a G339N mutation by WGS. Compared with BA.2 G339D, the fluorescent signal (blue line) was lower in the BA.2 G339N samples.

G339N mutation (position 22577–22578: c.1015_1016delGGGinsAA) (Accession ID: EPI_ISL_11018144 and EPI_ISL_11018145) (Figure 2a). Analysis with PANGOLIN classified these samples as BA.2. The mutation that occurred at codon 339 overlapped with the target site of the TaqMan probe. Therefore, we examined whether the TaqMan probe targeting G339D could efficiently amplify the target in the samples with G339N. In both BA.2 G339D and BA.2 G339N samples, sufficient amplification signals were obtained for Δ69–70 and Q493R. However, compared with BA.2 G339D, BA.2 G339N showed a lower amplification efficiency of the variant allele-specific signal (blue line in Figure 2b). In conclusion, the TaqMan assay used in this study specifically detected characteristic mutations related to Omicron sublineages in clinical samples.

Discussion

In this study, we present data regarding the use of a TaqMan assay to distinguish between Omicron strains and their sublineages. WGS analysis is the most standard method to determine these sublineages. However, WGS analysis of all specimens is restricted by limited resources and is difficult to apply under conditions of rapid spread of infection. In this regard, we have established a TaqMan assay that more conveniently and rapidly identifies Omicron strains and distinguishes the sublineages. BA.1/BA.1.1 and BA.2 are reported to differ in transmissibility and treatment response and so, the World Health Organization recommends monitoring BA.2

as a separate sublineage (World Health Organization, 2022). Therefore, assay systems that distinguish these subtypes will be important in determining preventive measures, infection control, and treatment strategies.

Each viral lineage has its own characteristic mutations. By targeting these mutations, it is possible to distinguish mutant strains and sublineages. On the basis of the accumulating WGS data during surveillance, we consider the TaqMan assay to be a suitable approach to detect characteristic mutations that occur frequently among viral lineages. In this study, we subjected SARS-CoV-2-positive samples collected from all areas of Yamanashi Prefecture in Japan between January 12, 2022 and March 10, 2022. To evaluate the performance of the TaqMan assay, 171 randomly selected samples of the 294 samples that completed the WGS analysis were also analyzed by the TaqMan assay in this study. The main prevalent mutant strain during this period was the Omicron strain (Hirotsu et al., 2022), which was in the process of replacing BA.1/BA.1.1 with BA.2. The TaqMan assay and WGA results were consistent in almost all samples, thus TaqMan assay results reflected the viral genome epidemiology of the region at the time.

Of the total 171 tested samples, two samples harbored the D339N mutation, which was revealed by WGS. These two samples were determined to be negative when the results of a TaqMan probe targeting D339D were analyzed using automated assay software. However, a weak but low signal amplification was observed by visual confirmation; therefore, these samples were considered

as inconclusive in this study for convenience. On the basis of these results, it should be noted that if a mutation occurs in the TaqMan probe sequence, it will not be amplified efficiently due to insufficient annealing. Mutations in primer sequences can also cause similar problems. Therefore, visual examinations of the results may help to avoid missing subtle changes caused by mutations around the primers and TaqMan probe.

To determine each lineage, it is efficient to know which mutant strain is prevalent at a given time and analyze the characteristic mutations of that strain. In this analysis, spike $\Delta 69-70$ was used to distinguish between BA.1/BA.1.1 and BA.2. There are other mutations that are also useful for distinguishing Omicron strains (Erster et al., 2022; Lee et al., 2022). For example, Q493R is a useful site to distinguish BA.1, BA.2, and BA.3 from BA.4 and BA.5. Q493R is found in BA.1, BA.2, and BA.3 but wild-type in BA.4 and BA.5 (Gangavarapu, et al. 2022). On the other hand, BA.4 and BA.5 have spike L452R and F486V mutations, which can be analyzed to distinguish them from BA.1, BA.2, and BA.3. The TaqMan method could be adapted to identify any such newly emerging mutant strains. Analysis of multiple characteristic mutations is expected to improve the accuracy of classification of virus lineage.

The TaqMan assay has a shorter turnaround time and lower cost than WGS. TaqMan assays require 2–3 hours until results are available. However, WGS generally requires 1–2 days due to the labor-intensive procedures, such as reverse transcription reactions from RNA to cDNA, library preparation, purification, and library quantification. In addition, facilities and laboratories with limited analytical equipment and resources cannot perform WGS analysis. Alternatively, TaqMan assays are useful to distinguish VOCs and their sublineages quickly and easily in any laboratory with qualitative PCR facilities. In addition, the TaqMan assay costs only a few thousand Japanese yen (JPY) per specimen, whereas WGS costs tens of thousands of JPY. Therefore, the TaqMan assay is considered useful for rapid screening of other samples.

There are, however, several limitations. TaqMan assays do not always accurately determine the sublineages in some situations. WGS analysis may be necessary when the characteristic mutations of newly emerging strains or sublineages remain under investigation. It should also be noted that if mutations occur at the TaqMan probe or primer locations, the PCR amplification efficiency will be reduced and the signal will be attenuated, which may make interpretation difficult (Figure 2b).

Our study showed that the TaqMan assay can be used to specifically detect Omicron viruses and classify subvariants. By applying this method to SARS-CoV-2-positive specimens, it is possible to analyze multiple specimens rapidly. Although accumulated data are still needed (Iketani et al., 2022; Takashita et al., 2022; Yamasoba et al., 2022; Zhou et al., 2022), BA.1 and BA.2 show different susceptibilities to antibody and antiviral therapy (Takashita et al., 2022). Rapid classification of Omicron sublineages may be clinically critical in providing appropriate treatment to patients with COVID-19.

Data availability

The sequences of SARS-CoV-2 genomes are available on GISAID (www.gisaid.org). We have provided the accession numbers in the Supplementary Table 1. Source data are provided with this report.

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Conflict of interest

The authors have no competing interests to declare.

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Ethical Approval statement

The institutional review board of the Clinical Research and Genome Research Committee at Yamanashi Central Hospital approved this study and the use of an opt-out consent method (Approval No. C2019-30). The requirement for written informed consent was waived owing to the observational nature of this study and the urgent need to collect COVID-19 data.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:[10.1016/j.ijid.2022.06.039](https://doi.org/10.1016/j.ijid.2022.06.039).

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