Genomic Sequencing of SARS-CoV-2 E484K Variant B.1.243.1, Arizona, USA

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Genomic surveillance can provide early insights into new circulating severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variants. While conducting genomic surveillance (1,663 cases) from December 2020–April 2021 in Arizona, USA, we detected an emergent E484K-harboring variant, B.1.243.1. This finding demonstrates the importance of real-time SARS-CoV-2 surveillance to better inform public health responses.

Genomic sequencing surveillance tracks the evolution of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and can provide early-warning insight of new variants circulating in communities. SARS-CoV-2 continues to acquire mutations in its genome as it spreads around the world. Although many mutations have little or no consequence on virus fitness, some mutations affect receptor binding or reduce antibody neutralization (*1*,*2*). Other mutations have been associated with increased transmission and clinical disease severity (*3*; Y. Liu et al., unpub. data, https://doi.org/10.1101/2021.03.08.434499). As of July 2021, the US SARS-CoV-2 Interagency Group has designated 4 variants of concern (VOC) and 7 variants of interest (VOI) in the United States based on the combination of mutations and associated attributes (*5*). Several of these VOCs and VOIs (e.g., Beta/B.1.351, Gamma/P.1, Delta/B.1.617.2) harbor the E484K mutation in the spike glycoprotein gene (*4*). Studies have demonstrated that the E484K mutation reduces antibody neutralization (*2*,*5*,*6*). E484K variants have also been identified in reinfection cases, suggesting a role in breakthrough infections (*2*,*5*–*7*); these findings indicate the need to monitor for SARS-CoV-2 variants in real time.

In an effort to provide statewide genomic surveillance, we sequenced the SARS-CoV-2 genome from 1,663 positive samples collected December 28, 2020–April 12, 2021 in Arizona, United States. Samples were primarily from Maricopa (56.9%), Coconino (26.4%), and Pima (8.5%) Counties. Study participants were 53.8% male, 46.2% female; age range was 5–81 years (median of 25 years). We successfully sequenced 1,538 (92.5%) high-quality complete genomes and found VOCs Alpha/B.1.1.7 (n = 336, 21.8%), Gamma/P.1 (n = 5, 0.33%), Beta/B.1.351 (n = 1, 0.07%), and Delta/B.1.617.2 (n = 1, 0.07%) and VOIs Epsilon/B.1.427/B.1.429 (n = 416, 27.0%), Iota/B.1.526 (n = 7, 0.5%), and Zeta/P.2 (n = 8, 0.5%) (Appendix Table 1, https://wwwnc.cdc.gov/EID/article/27/10/21-1189-App1.pdf). We detected 8 genomes associated with a common B.1.243 variant that had acquired an E484K mutation in the spike protein. The novel variant had 11 lineage-defining mutations, including V213G and E484K in the spike gene, a 9-nt deletion in open reading frame (ORF) 1ab (ΔSGF3675–77), a 3-nt insertion in the noncoding intergenic region upstream of the N gene, and other synonymous substitutions (Appendix Table 2, Figure 1). These 11 conserved mutations are distinct from the mutations associated with the parent lineage, B.1.243. The parent B.1.243 lineage is a common variant circulating in the United States that was observed in March 2020, early in the pandemic (Figure, panels A–C). The B.1.243 parent lineage encodes the spike gene D614G substitution but none of the other concerning mutations (Appendix Table 3, Figure 1). This new E484K-harboring variant has been officially designated as B.1.243.1 using the pangolin nomenclature system (*8*).

We examined the GISAID repository (https://www.gisaid.org) for additional B.1.243.1 genomes to determine its prevalence and geographic distribution. We found that B.1.243.1 is predominantly established in Arizona. Of 24 cases of B.1.243.1 sequenced during February 1–April 14, a total of 21 cases were from Arizona (Figure, panel C; Appendix Table 4). Two cases were sequenced from samples collected in Texas on February 24 and March 20 and another from a sample collected in New Mexico on March 8, suggesting that B.1.243.1 had spread to other states. We also identified 2 instances in which the parent B.1.243 lineage independently acquired the E484K mutation. However, both genomes lacked the other B.1.243.1 lineage-defining mutations and appear to be dead-end transmission events. Phylogenetic analyses indicate that the B.1.243.1 sequences form a monophyletic clade within the B.1.243 clade (Appendix Figure 2). Multiple internal branching observed in the B.1.243.1 clade indicates continued diversification of the lineage sequences, which suggests that B.1.243.1 was being established in circulation within Arizona. In contrast, the 2 additional

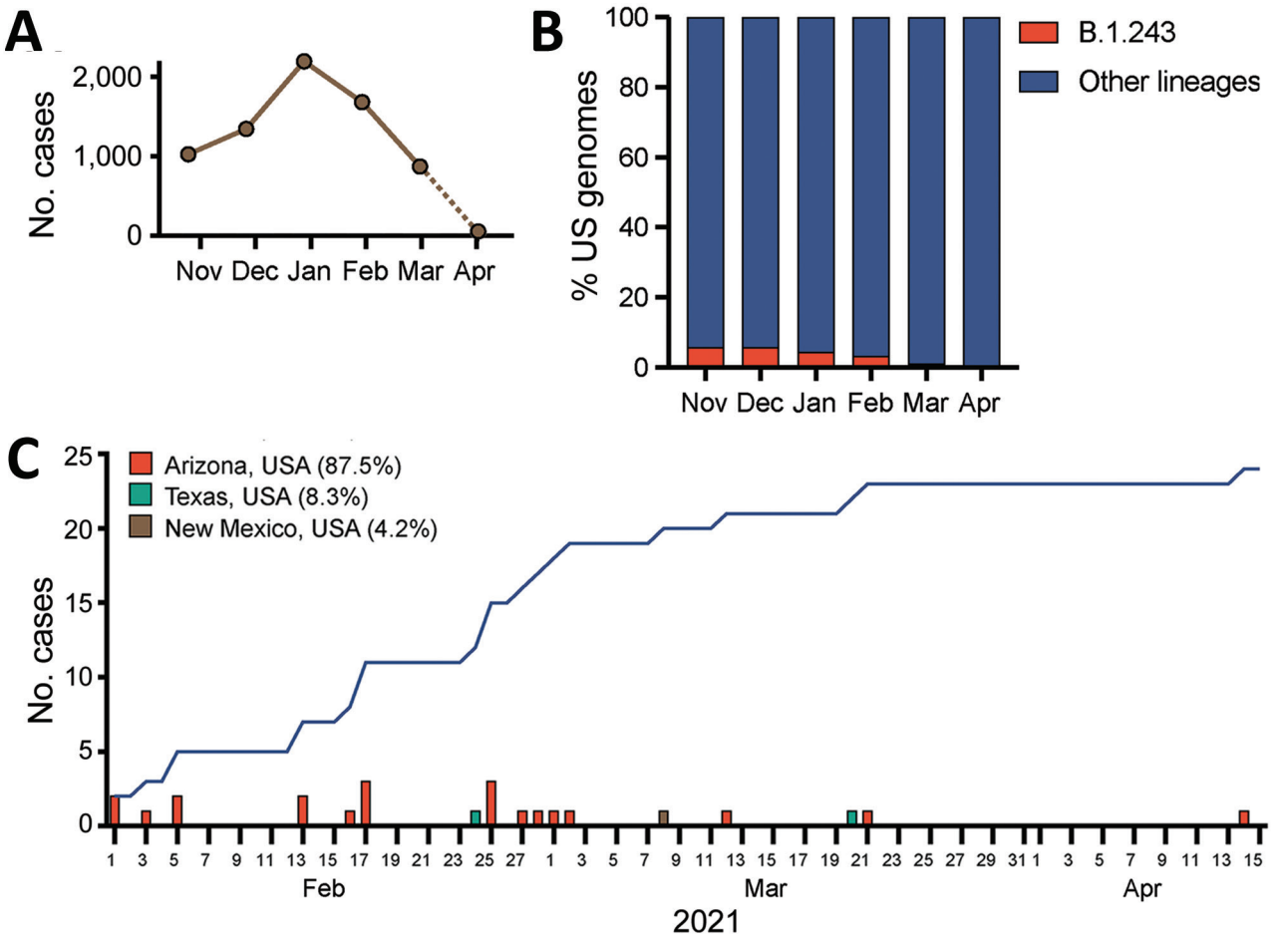

Figure. Emergence of E484K-harboring B.1.243.1 variant of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in Arizona, United States. A) Prevalence of B.1.243 parental lineage in the United States by number of cases per month, November 2020–April 2021. Dashed line indicates incomplete reporting of sequences from April 2021. B) Prevalence of B.1.243 parental lineage in the United States by proportion of sequenced genomes per month, November 2020–April 2021. C) Total B.1.243.1 cases reported February–April 2021. Blue curve indicates cumulative case incidence.

B.1.243 cases bearing the E484K mutation alone were phylogenetically distinct from the B.1.243.1 clade, suggesting that those isolates had evolved independently.

Genomic sequencing surveillance can provide early warnings of emergent variants. Because phylogenetic evidence suggested that B.1.243.1 was beginning to circulate in Arizona, the Arizona Department of Health Services (ADHS) was notified on March 18, 2021, and contact tracing was performed for the early B.1.243.1 cases. Of the case-patients who were interviewed, none reported connection to other patients. At the time of reporting (May 2021), the most recent case of B.1.243.1 had been reported on April 14, 2021 (Appendix Table 4). The limited spread of B.1.243.1 coincides with competition from the rapid rise in transmission of the Alpha (B.1.1.7) variant in the United States (*9*).

A limitation of this study is that the sequencing surveillance represented 0.31% of 503,825 total SARS-CoV-2 cases in Arizona during the study period. Targeted sampling efforts, such as prescreening samples for the E484K mutation by PCR-based assays, would complement random sampling for genomic sequencing surveillance. Our study highlights the need for sustained genomic surveillance in public health strategies and responses.

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About the Author

Mr. Skidmore is a bioinformatician at Arizona State University under the supervision of Efrem Lim. His primary research interests include the role of the microbiome in health and disease and tracking the spread of infectious diseases.

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SARS-CoV-2 Neutralization Resistance Mutations in Patient with HIV/AIDS, California, USA

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We report persistent severe acute respiratory syndrome coronavirus 2 infection in a patient with HIV/AIDS; the virus developed spike N terminal domain and receptor binding domain neutralization resistance mutations. Our findings suggest that immunocompromised patients can harbor emerging variants of severe acute respiratory syndrome coronavirus 2.

In December 2020, a 61-year-old woman living with HIV/AIDS was tested for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection at a community testing center in California, USA; she produced an anterior nasal swab sample that tested positive by reverse transcription PCR (RT-PCR). At the time of sample collection, she had a 10-day history of nonproductive cough, and was not receiving antiretroviral therapy (Figure). Her CD4 count was

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Appendix

Materials and Methods

Study Population

As part of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) genomic surveillance efforts, saliva samples submitted for COVID-19 testing to the Arizona State University's Biodesign Clinical Testing Laboratory (ABCTL) that tested positive (TaqPath COVID-19 Combo Kit, Applied Biosystems, https://www.thermofisher.com) were randomly selected for next-generation sequencing (*1*). Samples covered a broad distribution from counties across Arizona, United States. The Arizona State University Institutional Review Board approved this study.

SARS-CoV-2 Sequencing

RNA was extracted from 250 µL of saliva sample (KingFisher Flex, Thermo Scientific) according to the manufacturer's guidelines. First-strand cDNA synthesis was performed using random hexamers (SuperScript III/IV reverse transcription; Life Technologies, https://www.thermofisher.com), followed by PCR amplification of tiled amplicons spanning the SARS-CoV-2 genome (Swift Normalase Amplicon Panel; Swift Biosciences, https://swiftbiosci.com) and library construction. Libraries were sequenced on the Illumina MiSeq (version 2, 2×150; https://www.illumina.com) and NextSeq 500 (version 2.5, 2×150, mid or high output).

Sequencing Analysis

Illumina sequencing reads were quality filtered to remove adaptors and low-quality bases using BBTools (https://jgi.doe.gov/data-and-tools/bbtools). High-quality-filtered reads were mapped to the SARS-CoV-2 Wuhan1 reference genome (NC_045512.2) using BWA-MEM (H. Li, unpub. data, https://arxiv.org/abs/1303.3997) and amplicon primers were trimmed using

Primerclip version 0.3.8 (*2*). Consensus sequences were called using iVar (version 1.0; parameters -q 20, -t 0.75, -m 20, -n N) (*3*). Lineages were assigned using pangolin version 2.3.8 (*4*). Sequence alignments were performed with MAFFT version 7.471 (*5*) and variant calling using Geneious Prime version 2021 (https://www.geneious.com). High-quality complete genomes were defined as genomes >29,000 bp in length with <50% ambiguities. Sequences used in phylogenetic analysis include the global nextregions sequences from GISAID (*6*) subset to 500 randomly selected sequences and a random subset of 100 B.1.243 sequences from all B.1.243 GISAID sequences using the augur filter command (–no-probabilistic-sampling) from NextStrain (https://docs.nextstrain.org), and the 24 B.1.243.1 lineage sequences. Phylogenetic reconstruction was performed with IQTree version 2.0.3 (*7*), iqtree -nt AUTO -bb 1000 -m MFP -mset GTR, and Augur version 11.3.0 (*8*).

Data Availability

Sequence data have been deposited in GISAID.

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Appendix Table 1. SARS-CoV-2 lineages found in general surveillance sequencing of 1,538 positive samples, Arizona*

Lineage	Count	Lineage	Count	Lineage	Count
A.2.4	1	B.1.177	1	B.1.429	289
A.2.5	1	B.1.189	3	B.1.517	1
B	2	B.1.2	327	B.1.526	7
B.1	86	B.1.232	3	B.1.526.1	2
B.1.1	11	B.1.234	13	B.1.526.2	4
B.1.1.1	3	B.1.239	8	B.1.551	12
B.1.1.142	1	B.1.240	2	B.1.561	12
B.1.1.207	1	B.1.241	1	B.1.564	1
B.1.1.222	12	B.1.243	50	B.1.567	1
B.1.1.231	1	B.1.243.1	1	B.1.568	3
B.1.1.239	1	B.1.265	1	B.1.575	1
B.1.1.28	1	B.1.311	11	B.1.577	1
B.1.1.316	6	B.1.336	2	B.1.582	1
B.1.1.318	2	B.1.346	1	B.1.595	3
B.1.1.322	1	B.1.351	1	B.1.596	49
B.1.1.348	3	B.1.369	2	B.1.609	11
B.1.1.416	1	B.1.375	2	B.1.612	1
B.1.1.432	3	B.1.378	1	B.1.617.2	1
B.1.1.519	64	B.1.396	2	B.1.81	1
B.1.1.7	336	B.1.400	6	C.13	1
B.1.111	1	B.1.404	12	P.1	5
B.1.153	2	B.1.423	1	P.2	8
B.1.160	1	B.1.427	127	R.1	4

*SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

Appendix Table 2. B.1.243.1 lineage–defining mutations in SARS-CoV-2 samples, Arizona*

Gene	Nucleotide change	Amino acid change	Number of B.1.243.1 genomes with mutation
ORF1ab	C4321T	Synonymous	24/24
ORF1ab	11288–11296 deletion	SGF 3675–3677 deletion	24/24
ORF1ab	C17999T	T5912I	24/24
ORF1ab	G19962T	Synonymous	24/24
S	T22200G	V213G	23/23†
S	G23012A	E484K	22/22†
M	C26873T	Synonymous	24/24
M	G27065A	Synonymous	24/24
Noncoding, upstream of N	28266 GCC insertion	Non-coding	24/24
N	C28603T	Synonymous	24/24
3′ UTR	29750–29761 deletion	Non-coding	19/19†

* All genome positions in reference to the SARS-CoV-2 Wuhan-1 sequence (NC_045512.2). ORF, open reading frame; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.
†1 or more sequences omitted due to ambiguous (N) nucleotides or low coverage.

Appendix Table 3. Lineage-defining mutations in SARS-CoV-2 samples of parental B.1.243 lineage, Arizona

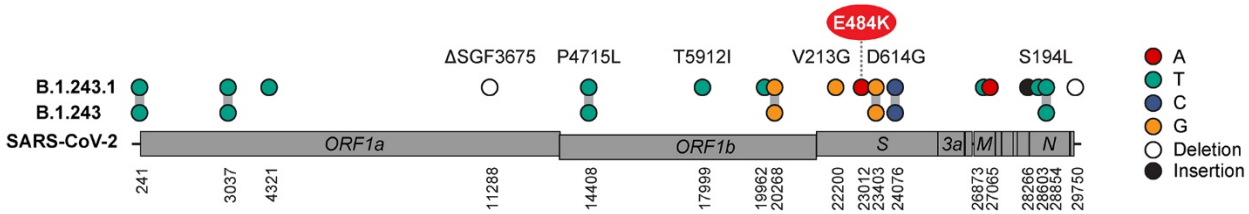
Gene	Nucleotide change	Amino acid change	% of B.1.243 genomes with mutation
5′ UTR	C241T	Non-coding	98.0
ORF1ab	C3037T	Synonymous	99.4
ORF1ab	C14408T	P4715L	98.6
ORF1ab	A20268G	Synonymous	95.5
S	A23403G	D614G	100
S	T24076C	Synonymous	98.9
N	C28854T	S194L	97.9

*Based on 7,211 global B.1.243 genomes downloaded from GISAID on March 20, 2021. ORF, open reading frame; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

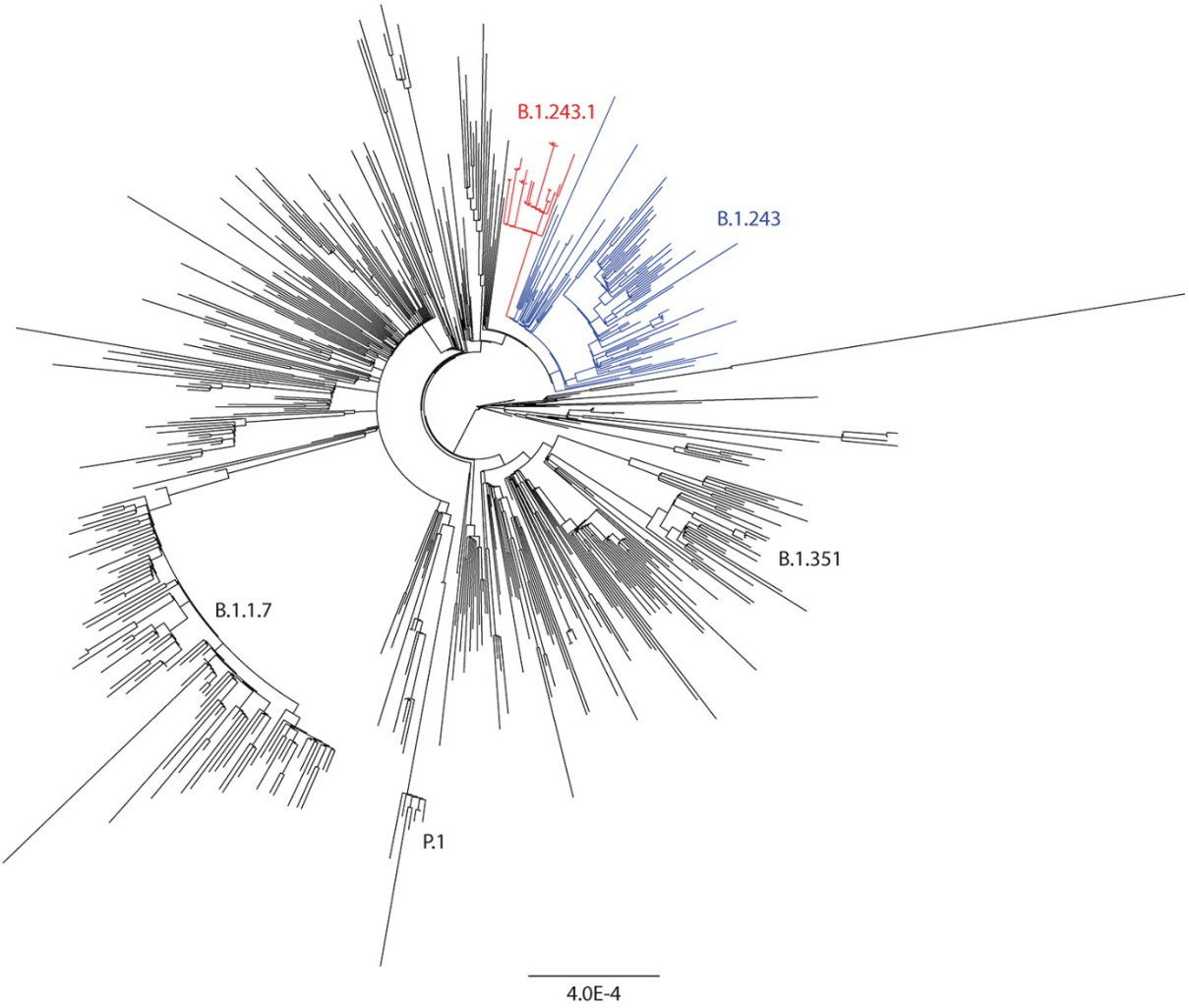
Appendix Table 4. B.1.243.1 sequences reported in study of SARS-CoV-2 mutations, Arizona*

Name	GISAID accession no.	State	County	Collection date	ORF1ab C_t	S C_t	N C_t
hCoV-19/USA/AZ-ASU2621/2021	EPI_ISL_1364812	Arizona	Pima	2021 Feb 1	26.2	26.3	26.8
hCoV-19/USA/AZ-ASU2625/2021	EPI_ISL_1364644	Arizona	Pima	2021 Feb 1	27.2	27.4	27.8
hCoV-19/USA/AZ-ASU2857/2021	EPI_ISL_1364649	Arizona	Pima	2021 Feb 3	23.1	23.1	22.7
hCoV-19/USA/AZ-CDC-21801839/2021	EPI_ISL_1090700	Arizona	NA	2021 Feb 5	NA	NA	NA
hCoV-19/USA/AZ-CDC-21802041/2021	EPI_ISL_1090853	Arizona	NA	2021 Feb 5	NA	NA	NA
hCoV-19/USA/AZ-CDC-22062741/2021	EPI_ISL_1139766	Arizona	NA	2021 Feb 13	NA	NA	NA
hCoV-19/USA/AZ-ASU2754/2021	EPI_ISL_1364775	Arizona	Maricopa	2021 Feb 13	14.7	14.8	14.9
hCoV-19/USA/AZ-ASU3758/2021	EPI_ISL_1592344	Arizona	Maricopa	2021 Feb 16	28.9	30	28.1
hCoV-19/USA/AZ-ASU3132/2021	EPI_ISL_1365543	Arizona	Maricopa	2021 Feb 17	20.7	20	21.2
hCoV-19/USA/AZ-ASU2925/2021	EPI_ISL_1365483	Arizona	Maricopa	2021 Feb 17	28	26.8	28
hCoV-19/USA/AZ-ASU3099/2021	EPI_ISL_1365622	Arizona	Maricopa	2021 Feb 17	24.1	23.2	24
hCoV-19/USA/TX-HMH-MCoV-29140/2021	EPI_ISL_1303700	Texas	Harris	2021 Feb 24	NA	NA	NA
hCoV-19/USA/AZ-ASU2540/2021	EPI_ISL_1291671	Arizona	Maricopa	2021 Feb 25	26	26.4	27
hCoV-19/USA/AZ-TG758899/2021	EPI_ISL_1292269	Arizona	Maricopa	2021 Feb 25	NA	NA	NA
hCoV-19/USA/AZ-TG758666/2021	EPI_ISL_1292117	Arizona	Maricopa	2021 Feb 25	NA	NA	NA
hCoV-19/USA/AZ-CDC-22555310/2021	EPI_ISL_1290985	Arizona	NA	2021 Feb 27	NA	NA	NA
hCoV-19/USA/AZ-TG759060/2021	EPI_ISL_1292381	Arizona	Maricopa	2021 Feb 28	NA	NA	NA
hCoV-19/USA/AZ-CDC-22554229/2021	EPI_ISL_1290992	Arizona	NA	2021 Mar 1	NA	NA	NA
hCoV-19/USA/AZ-TG761699/2021	EPI_ISL_1296905	Arizona	NA	2021 Mar 2	NA	NA	NA
hCoV-19/USA/NMDOH-2021075279/2021	EPI_ISL_1340909	New Mexico	NA	2021 Mar 8	NA	NA	NA
hCoV-19/USA/AZ-TG787352/2021	EPI_ISL_1464762	Arizona	Maricopa	2021 Mar 12	NA	NA	NA
hCoV-19/USA/TX-CDC-QDX23213780	EPI_ISL_1479977	Texas	NA	2021 Mar 20	NA	NA	NA
hCoV-19/USA/AZ-CDC-QDX23313079/2021	EPI_ISL_1525953	Arizona	NA	2021 Mar 21	NA	NA	NA
hCoV-19/USA/AZ-CDC-ASC210070999/2021	EPI_ISL_1999732	Arizona	NA	2021 Apr 14	NA	NA	NA

*C_t, cycle threshold; NA, not available; ORF, open reading frame; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.



Appendix Figure 1. B.1.243.1 lineage-defining mutations on the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) genome. Mutations are shown in reference to the SARS-CoV-2 Wuhan-1 genome position (NC_045512.2). ORF, open reading frame.



Appendix Figure 2. Maximum-likelihood phylogeny of diverse SARS-CoV-2 sequences including 500 representative global sequences, 100 B.1.243 parent lineage sequences, and the 24 B.1.243.1 sequences we identified. The novel B.1.243.1 lineage is indicated in red branches (clade bootstrap support: 100), and the parental B.1.241 lineage in blue. Scale bar represents number of nucleotide substitutions per site.