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**February 20, 2021**

SUBJECT: SARS-COV2 SEQUENCING RESULTS

Cumulative Summary of Sequencing Efforts:

| | | |
|---|---|---|
| Samples successfully sequenced to date | XXX | |
| Samples in current run | XX/XX successful (XX samples failed) | |
| Variant analysis | Current Run | Cumulative Total |
| UK Variant<br>20I/501Y.V1, Lineage B.1.1.7 | X | X |
| South African Variant<br>20H/501Y.V2 Lineage B.1.351 | X | X |
| Japanese/Brazilian Variant<br>20J/501Y.V3 Lineage B.1.1.28/B.1.1.248/P.1 | X | X |
| Rio de Janeiro/Brazilian Variant<br>20B Lineage B.1.1.28(E484K)/P.2 | X | X |
| Information related to L452R variant | Cumulative Total | |
| Number of samples classified as clade 20C | X | |
| Number of samples with Spike L452R mutation | X | |
| Number of samples with 5-site mutations (Lineage B.1.429 - Spike L452R, Spike S13I, Spike W152C, ORF1a I4205V, and ORF1b D1183Y) | X | |

*See Appendix for details.

**Current Report:**

LACPHL successfully performed SARS-CoV-2 whole genome sequencing (WGS) on XX respiratory samples consisting of specimens submitted to LACPHL for COVID-19 testing, specimen referred from clinical laboratories for sequencing, and specimens collected as part of an outbreak investigation at XXX.

Details of each specimen are described in the table below.

**Table 1** Details on specimens included in this report

| Sample ID | PHL Accession# | Collection Date (YYYY-MM-DD) | Submitter |
|---|---|---|---|
| **1** | XXXXX | YYYY-MM-DD | X |
| **2** | XXXXX | YYYY-MM-DD | X |
| **3** | XXXXX | YYYY-MM-DD | X |
| **4** | XXXXX | YYYY-MM-DD | X |
| **5** | XXXXX | YYYY-MM-DD | X |
| **6** | XXXXX | YYYY-MM-DD | X |
| **7** | XXXXX | YYYY-MM-DD | X |
| **8** | XXXXX | YYYY-MM-DD | X |
| **9** | XXXXX | YYYY-MM-DD | X |
| **10** | XXXXX | YYYY-MM-DD | X |
| **11** | XXXXX | YYYY-MM-DD | X |
| **12** | XXXXX | YYYY-MM-DD | X |
| **13** | XXXXX | YYYY-MM-DD | X |
| **14** | XXXXX | YYYY-MM-DD | X |
| **15** | XXXXX | YYYY-MM-DD | X |
| **16** | XXXXX | YYYY-MM-DD | X |
| **17** | XXXXX | YYYY-MM-DD | X |
| **18** | XXXXX | YYYY-MM-DD | X |
| **19** | XXXXX | YYYY-MM-DD | X |
| **20** | XXXXX | YYYY-MM-DD | X |

**Whole-Genome Sequencing and Data Analysis:**
Total nucleic acid from each remnant specimen was used as a template for cDNA synthesis and tiling PCR using ARTIC primers version 3 (https://artic.network/resources/ncov/ncov-amplicon-v3.pdf). PCR products were used as input for sequencing library preparation using Illumina DNA Prep Kit and IDT for Illumina DNA/RNA UD Indexes Set A or B. Libraries were pooled and sequenced on Illumina MiSeq platform using MiSeq Sequencing Reagent V2 (2 x 250). A Human Specimen Control (prepared from cultured human cell line) was included as a negative control throughout the process. Raw data was processed using Monroe v1.0.0 pipeline (https://staph-b.github.io/staphb_toolkit/workflow_docs/monroe/). SARS-CoV-2 clade determination and mutation identification was determined using NextClade (https://clades.nextstrain.org/). Lineage analysis was performed using Pangolin v2.1.7 (https://pangolin.cog-uk.io/) with the most updated lineage database at the time of analysis (database dated 2/18/21).

**Criteria for sequence passing QC:**

- Percent genome coverage > 90%
- Mean sequencing depth > 1000
- Mean base quality > 30
- Mean mapping quality > 30

Results are described in the following pages.

## 1. WGS Sequence Quality and Clade/Lineage Analysis

Table 2 Summary of SARS-CoV-2 WGS Sequence Quality and Clade/Lineage

| Sample ID | PHL Accession# | Percent Coverage | Mean Seq Depth | Mean Base Quality | Mean Mapping Quality | Clade | Lineage | L452R Mutation | 5-site Mutation* |
|---|---|---|---|---|---|---|---|---|---|
| **1** | **XXXXX** | **99.5084** | **4059.43** | **38.2** | **51.2** | **20I/501Y.V1** | **B.1.1.7** | **-** | **-** |
| **2** | XXXXX | 99.5051 | 3083.51 | 38.2 | 51.1 | 20C | B.1 | - | - |
| **3** | XXXXX | 99.1673 | 3842.79 | 38.2 | 51.2 | 20C | B.1.429 | + | + |
| **4** | XXXXX | 99.5719 | 4369.95 | 38.2 | 52.2 | 20C | B.1.429 | + | + |
| **5** | XXXXX | 99.7994 | 1867.66 | 38.2 | 51.4 | 20C | B.1.429 | + | + |
| **6** | XXXXX | 99.5586 | 4286.85 | 38.2 | 51.4 | 20C | B.1.429 | + | + |
| **7** | XXXXX | 99.6054 | 3434.04 | 38.2 | 51.1 | 20C | B.1.429 | + | + |
| **8** | XXXXX | 99.5184 | 2599.83 | 38.2 | 51.4 | 20C | B.1.427 | + | - |
| **9** | XXXXX | 99.6321 | 3550.51 | 38.2 | 51.5 | 20C | B.1.427 | + | - |
| **10** | XXXXX | 99.7224 | 3610.66 | 38.2 | 51.2 | 20C | B.1.427 | + | - |
| **11** | XXXXX | 99.2609 | 1267.53 | 38.2 | 51.3 | 20C | B.1.429 | + | + |
| **12** | XXXXX | 99.8361 | 4598.37 | 38.3 | 56.1 | 20C | B.1.429 | + | + |
| **13** | XXXXX | 99.3178 | 1642.89 | 38.2 | 51.1 | 20C | B.1.429 | + | + |
| **14** | XXXXX | 99.3914 | 1710.45 | 38.2 | 51.6 | 20C | B.1.429 | + | + |
| **15** | XXXXX | 99.388 | 2146.21 | 38.2 | 51 | 20C | B.1.429 | + | + |
| **16** | XXXXX | 99.9431 | 2131.05 | 38.2 | 51.2 | 20C | B.1.427 | + | - |
| **17** | XXXXX | 99.6489 | 1653.49 | 38.2 | 51.9 | 20C | B.1.429 | + | + |
| **18** | XXXXX | 95.89 | 1555.11 | 38.2 | 51.4 | 20C | B.1.429 | + | + |
| **19** | XXXXX | 99.4181 | 3116.74 | 38.2 | 51.5 | 20C | B.1.429 | + | + |
| **20** | XXXXX | 98.7125 | 1683.19 | 38.2 | 51.1 | 20C | B.1.429 | + | + |

* Spike L452R, Spike S13I, Spike W152C, ORF1a I4205V, and ORF1b D1183Y

Note: UK VOC-202012/01 Variant is of Clade 20I/501Y.V1 and Lineage B.1.1.7.

South African Variant is of Clade 20H/501Y.V2 and Lineage B.1.351

Japanese/Brazilian Variant is of Clade 20J/501Y.V3 and Lineage B.1.1.248 or P.1

Rio de Janeiro/Brazilian Variant is of Clade 20B Lineage B.1.1.28(E484K) or P.2

## 2. Mutational Analysis of Samples

Table 3 List of genetic alterations in each sequenced sample (comparison to Wuhan-Hu-1 strain GenBank: MN908947.3)

| Sample ID | PHL Accession# | Genetic Alteration* (Gene Name:Alteration) | | |
|---|---|---|---|---|
| | | **Substitutions** | **Deletions** | **Insertions** |
| **1** | XXXXX | N:M1X,**N:D3L**,**N:R203K**,**N:G204R**,**N:S235F**,**ORF1a:T1001I**,**ORF1a:A1708D**,ORF1a:K2143N,ORF1b:P314L,ORF1b:K1383R,ORF1b:E1871G,ORF3a:A99T,**ORF8:Q27***,**ORF8:R52I**,**ORF8:Y73C**,**S:N501Y**,**S:A570D**,**S:D614G**,**S:P681H**,**S:T716I**,**S:S982A**,**S:D1118H** | **ORF1a:S3675-F3677-**,**S:H69-V70-**,**S:Y144-** | None |
| **2** | XXXXX | N:T205I,ORF1a:T265I,ORF1a:P885L,ORF1a:A1473V,ORF1a:L3951F,ORF1a:T4249I,ORF1b:H73Y,ORF1b:P314L,ORF1b:A1291S,ORF1b:R1737L,ORF1b:S2258N,ORF3a:Q57H,ORF3a:L85F,ORF8:S24L,ORF8:P70L,ORF8:Q72H,**S:D614G** | None | None |
| **3** | XXXXX | N:T205I,**N:M234I**,ORF1a:T265I,ORF1a:T814I,**ORF1a:I4205V**,ORF1a:D4218N,ORF1b:P314L,**ORF1b:D1183Y**,ORF3a:Q57H,ORF8:V100L,**S:S13I**,S:V62F,**S:W152C**,**S:L452R**,**S:D614G** | None | None |
| **4** | XXXXX | N:T205I,ORF1a:T265I,ORF1a:T2978I,ORF1a:M3780V,**ORF1a:I4205V**,ORF1b:P314L,**ORF1b:D1183Y**,ORF3a:Q57H,**S:S13I**,**S:W152C**,**S:L452R**,**S:D614G** | None | None |
| **5** | XXXXX | N:T205I,**N:M234I**,ORF1a:T265I,ORF1a:L3210F,ORF1a:K3215R,ORF1a:N3243K,**ORF1a:I4205V**,ORF1b:P314L,**ORF1b:D1183Y**,ORF3a:Q57H,ORF8:V100L,**S:S13I**,**S:W152C**,**S:L452R**,**S:D614G** | None | None |
| **6** | XXXXX | N:T205I,ORF1a:T265I,ORF1a:M2606I,ORF1a:A2994V,**ORF1a:I4205V**,ORF1b:P314L,ORF1b:G1129C,**ORF1b:D1183Y**,ORF3a:Q57H,ORF8:E110*,**S:S13I**,**S:W152C**,**S:L452R**,**S:D614G** | None | None |
| **7** | XXXXX | N:T205I,ORF1a:T265I,ORF1a:S376L,ORF1a:K1280N,**ORF1a:I4205V**,ORF1b:P314L,**ORF1b:D1183Y**,ORF1b:Q1878R,ORF3a:Q57H,**S:S13I**,**S:W152C**,**S:L452R**,**S:D614G** | ORF1a:F1028-S1029- | None |
| **8** | XXXXX | N:T205I,ORF1a:T265I,ORF1a:P1472S,ORF1a:T2877I,ORF1a:S3158T,ORF1b:P314L,ORF1b:P976L,**ORF1b:D1183Y**,ORF3a:Q57H,ORF8:A65V,ORF8:I74V,**S:S13I**,S:W258L,**S:L452R**,**S:D614G** | None | None |
| **9** | XXXXX | N:T205I,ORF1a:T265I,ORF1a:T2877I,ORF1a:S3158T,ORF1b:P314L,ORF1b:P976L,**ORF1b:D1183Y**,ORF3a:Q57H,ORF8:A65V,**S:S13I**,**S:W152C**,S:W258L,**S:L452R**,**S:D614G** | None | None |
| **10** | XXXXX | N:T205I,ORF1a:T265I,ORF1a:T2877I,ORF1a:S3158T,ORF1b:P314L,ORF1b:P976L,**ORF1b:D1183Y**,ORF3a:Q57H,ORF8:A65V,**S:S13I**,S:W258L,**S:L452R**,**S:D614G** | None | None |
| **11** | XXXXX | N:T205I,ORF1a:T265I,ORF1a:M2606I,ORF1a:A2994V,**ORF1a:I4205V**,ORF1b:P314L,ORF1b:G1129C,**ORF1b:D1183Y**,ORF3a:Q57H,ORF8:E110*,**S:S13I**,**S:W152C**,**S:L452R**,**S:D614G** | None | None |
| **12** | XXXXX | N:T205I,ORF1a:T265I,ORF1a:M2606I,ORF1a:A2994V,**ORF1a:I4205V**,ORF1b:P314L,ORF1b:G1129C,**ORF1b:D1183Y**,ORF3a:Q57H,ORF8:E110*,**S:S13I**,**S:W152C**,**S:L452R**,**S:D614G** | None | None |
| **13** | XXXXX | N:T205I,ORF1a:T265I,ORF1a:T3459M,**ORF1a:I4205V**,ORF1b:P314L,**ORF1b:D1183Y**,ORF3a:Q57H,ORF8:D35Y,**S:S13I**,**S:W152C**,**S:L452R**,S:S494P,**S:D614G** | None | None |
| **14** | XXXXX | N:T205I,**N:M234I**,ORF1a:T265I,ORF1a:T1474I,**ORF1a:I4205V**,ORF1b:P314L,**ORF1b:D1183Y**,ORF3a:Q57H,ORF8:W45S,ORF8:V100L,**S:S13I**,**S:W152C**,**S:L452R**,**S:D614G** | None | None |
| **15** | XXXXX | N:T205I,**N:M234I**,ORF1a:T265I,ORF1a:L3210F,ORF1a:K3215R,ORF1a:N3243K,**ORF1a:I4205V**,ORF1b:P314L,**ORF1b:D1183Y**,ORF3a:Q57H,ORF8:V100L,**S:S13I**,**S:W152C**,**S:L452R**,**S:D614G** | None | None |
| **16** | XXXXX | N:T205I,ORF1a:T265I,ORF1a:S3158T,ORF1b:P314L,ORF1b:P976L,**ORF1b:D1183Y**,ORF3a:Q57H,**S:S13I**,**S:W152C**,**S:L452R**,**S:D614G** | None | None |
| **17** | XXXXX | N:T205I,N:G238C,N:D348H,ORF1a:T265I,ORF1a:I1128V,ORF1a:T3459M,**ORF1a:I4205V**,ORF1b:P314L,**ORF1b:D1183Y**,ORF3a:K21R,ORF3a:Q57H,**S:S13I**,**S:W152C**,S:G181A,**S:L452R**,**S:D614G** | None | None |

| | | | | |
|---|---|---|---|---|
| **18** | W44929 | N:T205I,ORF1a:T265I,ORF1a:A2279T,**ORF1a:I4205V**,ORF1b:P314L,**ORF1b:D1183Y**,ORF3a:Q57H,**S:S13I**,**S:P26S**,**S:W152C**,**S:L452R**,**S:D614G** | None | None |
| **19** | W44930 | N:T205I,N:S413I,ORF1a:T265I,**ORF1a:I4205V**,ORF1b:P314L,**ORF1b:D1183Y**,ORF3a:Q57H,ORF9b:S50L,**S:S13I**,**S:P26S**,**S:W152C**,**S:L452R**,**S:D614G** | None | None |
| **20** | W44934 | N:T205I,**N:M234I**,ORF1a:T265I,ORF1a:Q362R,ORF1a:D2299N,**ORF1a:I4205V**,ORF1b:P314L,**ORF1b:D1183Y**,ORF3a:Q57H,ORF8:V100L,**S:S13I**,**S:W152C**,**S:L452R**,**S:D614G** | None | None |

*In comparison to Wuhan-Hu-1 strain (GenBank: MN908947.3)

**Result Interpretation:**

1. We identified one additional sample containing the UK 20I/501Y.V1 Variant from XXXXX.
2. For all other samples in this run, we did not detect patterns of mutations indicative of the the South African 20H/501Y.V2 Variant, the Japanese/Brazilian 20J/501Y.V3/P.1 Variant, and the Rio de Janeiro/Brazilian 20B/B.1.1.28(E484K)/P.2 Variant.

**Cumulative Summary:**

For this sequencing run, we identified one additional sample containing the UK 20I/501Y.V1 Variant. We have not identified samples containing the South African 20H/501Y.V2 Variant, the Japanese/Brazilian 20J/501Y.V3 Variant, or the Rio de Janeiro/Brazilian 20B/B.1.1.28(E484K)/P.2 Variant.

**Disclaimer:**
Interpretations are provided as heuristic guidelines only, based on review of literature, SNP, and phylogenetic analysis. Relationships among strains should be determined in conjunction with clinical, microbiologic, and epidemiologic information. This report may be used for investigational purposes and outbreak investigations only, where an outbreak has been determined by ACD program epidemiologists and is to be distinguished from a surveillance report. This analysis may not be used as a replacement for a thorough epidemiological investigation. This report may not be used for individual patient diagnostic purposes.

Testing performed by: **XXX XXXXXXXXX, PHM**
**XXX XXXXXXXXX, CLS**

Report prepared by: **Peera Hemarajata, MD, PhD, D(ABMM)**

Report reviewed by: **Nicole M. Green, PhD, D(ABMM)**

**Appendix**

Variant-defining mutations for the UK 20I/501Y.V1 Variant, South African 20H/501Y.V2 Variant, and Japanese/Brazilian B.1.1.248/P.1 Variant

| Gene | Nucleotide | Amino acid | UK 20I/501Y.V1 | SA 20H/501Y.V2 | JP/BZ P.1 | BZ P.2 |
|---|---|---|---|---|---|---|
| ORF1ab | **C1059T** | **T265I** | | + | | |
| | **C3267T** | **T1001I** | + | | | |
| | **C3828T** | **S1188L** | | | + | |
| | **G28048T** | **K1655N** | | + | | |
| | **5230T** | **A1708D** | + | | | |
| | **A5648C** | **K1795Q** | | | + | |
| | **T6954C** | **I2230T** | + | | | |
| | **A10323G** | **K3353R** | | + | | |
| | **T10667G** | **L3468V** | | | | + |
| | **11288-11296 del** | **SGF 3675-3677 del** | + | | | |
| | **C12053T** | **L3930F** | | | | + |
| Spike | **C21614T** | **L18F** | | | + | |
| | **C21621A** | **T20N** | | | + | |
| | **C21638T** | **P26S** | | | + | |
| | **21765-21770 del** | **HV 69-70 del** | + | | | |
| | **A21801C** | **D80A** | | + | | |
| | **G21974T** | **D138Y** | | | | |
| | **21991-21993 del** | **Y144 del** | + | | | |
| | **G22132T** | **R190S** | | | + | |
| | **A22812C** | **K417T** | | | + | |
| | **G22813T** | **K417N** | | + | | |
| | **G23012A** | **E484K** | | + | + | + |
| | **A23063T** | **N501Y** | + | + | + | |
| | **C23271A** | **A570D** | + | | | |
| | **A23403G** | **D614G** | + | + | + | + |
| | **C23525T** | **H655Y** | | | + | |
| | **C23604A** | **P681H** | + | | | |
| | **C23664T** | **A701V** | | + | | |
| | **C23709T** | **T716I** | + | | | |
| | **T24506G** | **S982A** | + | | | |
| | **C24642T** | **T1027I** | | | + | |
| | **G24914C** | **D1118H** | + | | | |
| | **G25088T** | **V1176F** | | | | + |
| Orf8 | **C27972T** | **Q27stop** | + | | | |
| | **G28048T** | **R52I** | + | | | |
| | **A28111G** | **Y73C** | + | | | |
| | **G28167A** | **E92K** | | | + | |
| ORF3a | **G25563T** | **Q57H** | | + | | |
| | **C25904T** | **S171L** | | + | | |
| E | **C26456T** | **P71L** | | + | | |
| N | **28280 GAT->CTA** | **D3L** | + | | | |
| | **C26456T** | **P71L** | | + | | |
| | **C28512G** | **P80R** | | | + | |
| | **G28628T** | **A119S** | | | | + |
| | **G28882A** | **R203K** | | | | + |
| | **G28883C** | **G204R** | | | | + |
| | **G28975T** | **M234I** | | | | + |
| | **C28977T** | **S235F** | + | | | |

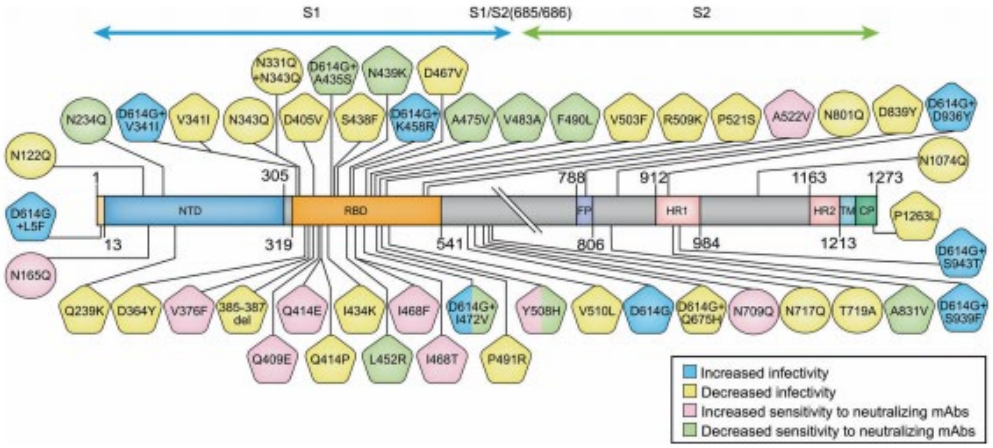
Table of mutations associated with phenotype – From Li et al. 2020. Cell. 182:1284–1294.

**Table 1. Characteristics of Variants and Mutants**

| | Group A | Group B | Group C |
|---|---|---|---|
| Number of variants or mutants | 29 | 51 | 26 |
| Increased infectivity | D614G, D614G+L5F, D614G+D936Y, D614G+S939F, D614G+S943T | D614G+V341I, D614G+K458R, D614G+I472V[a] | none |
| Decreased infectivity | Q239K, D839Y, P1263L, D614G+Q675H | V341I, D364Y, 385-387del, D405V, Q414P, I434K, S438F, D467V, P491R, V503F, R509K, V510L, P521S | N122Q, N343Q, N717Q, T719A, N801Q, N1074Q, N331Q+N343Q |
| Increased sensitivity to neutralizing mAbs | none | V367F, Q409E, Q414E, I468F, I468T, Y508H, A522V | N165Q, N709Q |
| Decreased sensitivity to neutralizing mAbs | A831V | N439K, L452R, A475V, V483A, F490L, Y508H, D614G+A435S, D614G+I472V[a] | N234Q |
| Increased sensitivity to convalescent sera | V615L | F338L, V367F, I468F, I468T | N149H, N149Q, N165Q, N331Q, N354D, N709Q, N1173Q |
| Decreased sensitivity to convalescent sera | Y145del, A831V, D614G+A831V, D614G+A879S, D614G+M1237I | Q414E, N439K, G446V, K458N, I472V, A475V, T478I, V483I, F490L, H519P, D614G+Q321L, D614G+I472V[a] | none |

[a]D614G+I472V is the only variant with increased infectivity and decreased sensitivity to neutralizing mAb and convalescent sera. It is of note only one sequence is recorded in GISAID.

Diagram of mutations associated with phenotype – From Wang et al. 2020. Signal Transduction and Targeted Therapy. 5:185.



## Summary of Variants of Concern (VOCs) In Specimens Collected from LAC Residents

| # | Date Reported or Confirmed by DPH | Identified By | Patient Name | DOB | Notes | Clade | Lineage | Acc# or GISAID ID | Collection Date |
|---|---|---|---|---|---|---|---|---|---|
| **1** | MM/DD/YY | XXXXX | XXXXX | MM/DD/YY | XXXXX | 20I/501Y.V1 | B.1.1.7 | XXXXX | MM/DD/YY |
| **2** | MM/DD/YY | XXXXX | XXXXX | MM/DD/YY | XXXXX | 20I/501Y.V1 | B.1.1.7 | XXXXX | MM/DD/YY |
| **3** | MM/DD/YY | XXXXX | XXXXX | MM/DD/YY | XXXXX | 20I/501Y.V1 | B.1.1.7 | XXXXX | MM/DD/YY |
| **4** | MM/DD/YY | XXXXX | XXXXX | MM/DD/YY | XXXXX | 20I/501Y.V1 | B.1.1.7 | XXXXX | MM/DD/YY |
| **5** | MM/DD/YY | XXXXX | XXXXX | MM/DD/YY | XXXXX | 20I/501Y.V1 | B.1.1.7 | XXXXX | MM/DD/YY |
| **6** | MM/DD/YY | XXXXX | XXXXX | MM/DD/YY | XXXXX | 20I/501Y.V1 | B.1.1.7 | XXXXX | MM/DD/YY |
| **7** | MM/DD/YY | XXXXX | XXXXX | MM/DD/YY | XXXXX | 20I/501Y.V1 | B.1.1.7 | XXXXX | MM/DD/YY |
| **8** | MM/DD/YY | XXXXX | XXXXX | MM/DD/YY | XXXXX | 20I/501Y.V1 | B.1.1.7 | XXXXX | MM/DD/YY |

## References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