SARS-CoV-2 Surveillance and Variant Analysis

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The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

cdc.gov/coronavirus
CDC SARS-CoV-2 Surveillance

- **Goals:**
  - Identify and track variants circulating nationally and globally
  - Conduct risk assessment through genomics and virological characterization under appropriate biosafety
  - Reporting data internally and externally
  - Use the data to inform SARS-CoV-2 Interagency Group (SIG) recommendations to leadership for decision making

- **Surveillance systems in use (genotypic and phenotypic)**
  - Contract sequencing established to expand our data and data publicly available
  - NS3 and enhanced surveillance generates sequence data and provides representative specimens needed for characterization (key for genotype to phenotype analysis)
  - Continual analysis of viral genomic sequence (GISAID/GenBank)

- **Solution to a limitation**
  - Occasionally, variants are identified by sequence analysis, but there are no specimens available in the U.S. and it may take a long time to obtain representatives
  - Our solution is to engineer recombinant SARS-CoV-2 variants by reverse genetics
Daily Trends in Number of COVID-19 Cases, United States

Predominating variants indicated above peaks

S-D614G
Delta
Alpha
Omicron

National SARS-CoV-2 Genomic Surveillance

Three primary data sources (tens of thousands sequences weekly)
- Leading the National SARS-CoV-2 Strain Surveillance (NS3) system
- Partnering with commercial sequencing laboratories — Most have close ties to diagnostic laboratories
- Supporting state, territorial, local, and tribal health departments

Additional data sources
- Partnering with universities
- Leading the SARS-CoV-2 Sequencing for Public Health Emergencies Response, Epidemiology, and Surveillance (SPHERES) Consortium
- Have established a tagging system to identify specimens that would be considered to meet surveillance criteria.

Changing Landscape of Circulating Variants

FIGURE 1. National weekly proportion estimates* of SARS-CoV-2 variants† — United States, January 2, 2021—January 22, 2022

Estimates of SARS-CoV-2 Lineages Are Publicly Disseminated (CDC COVID Data Tracker)

Nowcast estimates (as of 04/16/2022)

- A tool to compensate for the time needed for next generation sequencing
  - Most data available 2-3 weeks after swab is collected
- A multinomial logistic regression model
  - Shows predicted proportion and 95% prediction interval
- Omicron is estimated at 100%
  - BA.2 (except BA.2.12.1) -> 75% (68-80% PI)
  - BA.2.12.1 -> 19% (13-26% PI)
  - BA.1.1 -> 6% (5-7% PI)

https://covid.cdc.gov/covid-data-tracker/#variant-proportions
Coronavirus Spike (S)

- “Corona” appearance
- Receptor binding (S1, RBD)
  - Host range
  - Transmission
  - Pathogenesis
- Membrane fusion (S2)
- Neutralizing antibodies
  - Induced by infection and/or vaccination

Coronavirus binding and entry
DE Wentworth, KV Holmes - 2007
Virus Characterization: Genotype to Phenotype Analysis (via NS3)

- Representative viruses selected
- Virus isolation and propagation
  - Sequencing of stock viruses
  - Titration of stock viruses
- Antigenic characterization
  - Virus neutralization assays
  - Comparative reactivity with antisera (community infected, vaccinated)
- Subsets used for additional phenotypic analysis
  - In vitro replication (various cell types)
  - In vivo studies
    - Transmission/pathogenesis
    - Immune protection
Specific Variants Obtained from Swabs or by Developing Recombinant SARS-CoV-2 Viruses

- **in vitro transcription**
- **electroporation**
- **30 kb viral RNA**
- **CDC made cells:**
  - VeroE6/Nucleocapsid
  - VeroE6/TMPRSS2

Characterization:
- Plaque assay (WT & reporter viruses)
- Antigenicity (e.g. PRNT, FRNT)
- Replication fitness?
- Inhibition of SARS-CoV-2 by a DSR developed mAb
- Pathogenicity & transmissibility?
Antigenic Ranking of rSARS-CoV-2 Variants Generated by Reverse Genetics Prior to Omicron

Fold change compared to reference progenitor virus (ratio = variant FRNT50 titer / 614D FRNT50 titer)

Graph is only for comparing variants to 614D reference virus (set as 1-fold). If comparing between two variants, don’t be misled by the linear scale of the axis, e.g., the FRNT50 ratio of B.1.351/B.1.621 is only 1.2-fold, very small difference.

AY.4.2 likely to fall in this range

614D
614G
B.1.1.7

B.1.526 (E484K)
B.1.617.3
B.1.525
B.1.617.2
B.1.427/429
B.1.526 (L452R)

B.1.1.7 (E484K)
P.1
C.37
P.2

B.1.617.1
P.3

B.1.621
B.1.351

Susceptible to neutralization
Resistant to neutralization
In silico Analysis to Infer Impact Genetic Changes (e.g., Omicron spike)

Has substitutions, insertion, and deletions

- **S mutations likely** to increase transmission:
  - Increase binding to hACE2 (e.g., N501Y)
  - Fusion
    - S1/S2 cleavage site changes (H655Y, N679K, P681H)

- Changes in multiple epitopes (RBD and NTD), anticipate reduced neutralization by:
  - Therapeutic antibodies
  - Polyclonal antibodies induced by natural infection or vaccination (e.g., E484, G446, K417, and Q493)

- Insertion and deletions in S gene
  - Del 69/70 may lead to target failure in some rt-RTPCR assays
  - In/Del in NTD likely reduce neutralization

Preliminary Analysis
12/02/2021

N679 and P681 Not visible

CDC, SSEV Patterson
Omicron recombinant reporter virus shows significant escape from neutralization (38-fold) using Focus Reduction Neutralization Test (FRNT)
- Much lower than Beta (6-fold), the variant of concern (VOC) that previously had the greatest antigenic difference from the vaccine antigen

https://www.biorxiv.org/content/10.1101/2021.11.24.469906v2
Comparing Neutralization Profiles of Sera Collected pre- and post-boost (mRNA vaccines)

- Omicron (BA.1.1.529)
  - Pre-boost
    - Geometric mean titer (GMT) is very low (GMT=5) and 34-fold reduced compared to 614D (GMT=171)
  - Post-boost
    - GMT increases to 215 and is similar to 614D pre-boost (171)
    - 19-fold reduction compared to G614D post-boost (GMT=4,152)

Pre-Boost | Post-Boost
---|---
1.0 | 1.0
18 | 9.2
34 | 19

*Post-boost sera were collected 2-to-6 weeks post boost vaccination (5 Moderna and 5 Pfizer). The average fold changes of natural isolates relative to reference virus 614D (set as 1-fold)*

https://www.biorxiv.org/content/10.1101/2021.11.24.469906v2
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