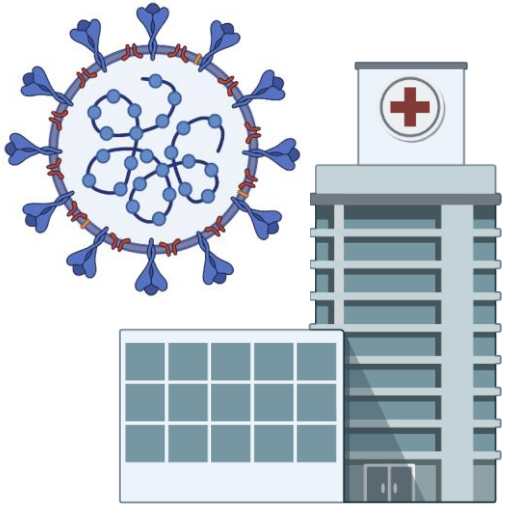
Diagnostic & Clinical Impacts of SARS-CoV-2 Variants



Adam Lauring, MD, PhD
Department of Medicine, Infectious Diseases
Department of Microbiology and Immunology
University of Michigan

Disclosures

- Paid consultant on antiviral drugs for Sanofi

- Paid member of Steering Committee for Roche clinical trial, ongoing CENTERSTONE: a global phase IIIb, randomised, double-blind, placebo-controlled clinical efficacy study of baloxavir marboxil for the reduction of direct transmission of influenza from otherwise healthy patients to household contacts

Learning Objectives

- Distinguish between Variants of Concern and Variants of Interest
- Identify variant attributes that could affect diagnostic testing
- Summarize the latest data on variants and vaccine efficacy

A variant consumers survival guide

Houston finds every known virus variant — and wonders what, if anything, it means.

Some critics, including Dr. Eric Topol, the founder and director of the Scripps Research Translational Institute, have said that the attention given to the succession of new variants — "scariants," he has called them — has done little more than frighten the public.

Dr. Musser agreed, referring to such reports as "mutant porn." Highlighting the existence of variants without indicating whether they make any functional difference to real-world patients was no more enlightening than collecting stamps or identifying the birds flying overhead, he said: "'There's a bird. There's another bird.'"

He added: "I think the crucial thing in all of this is that it is extraordinarily difficult for both the medical and lay public to really sort through all this noise about variants. At the end of the day, does any of this mean a hill of beans to anyone?"

"The big issue is to try to get things toned down."

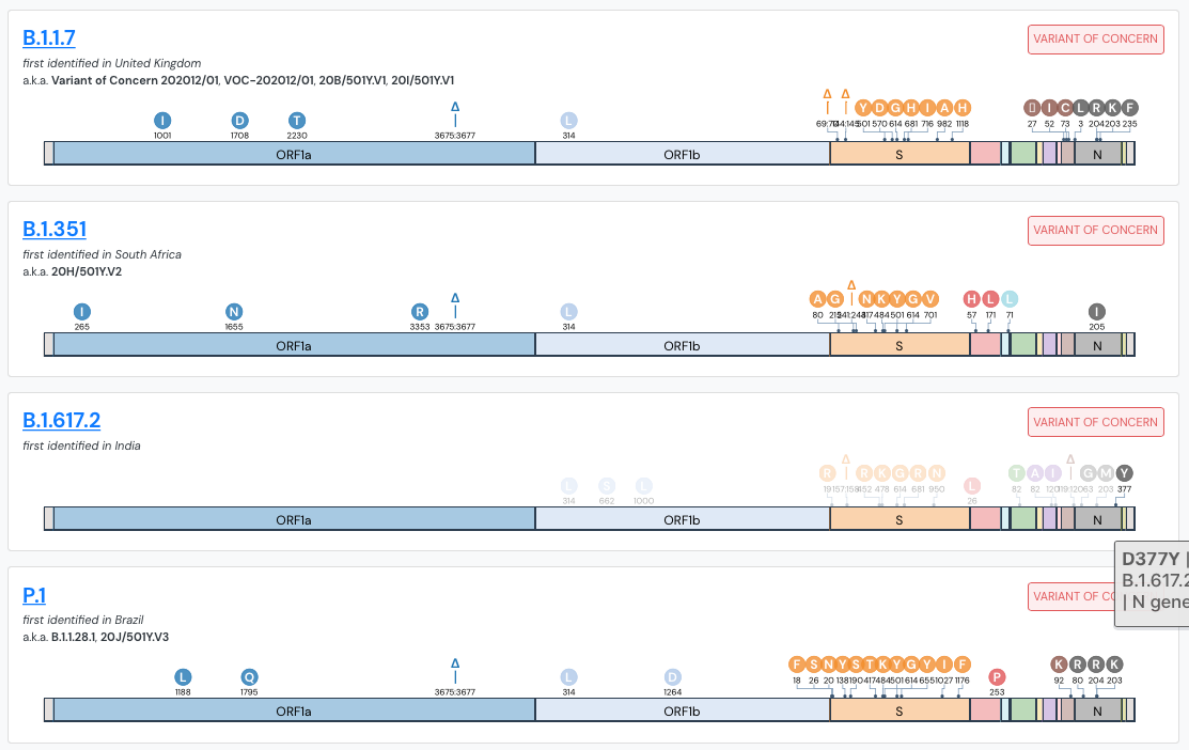
— *Gina Kolata*

1. Terminology, some definitions
2. Key variants of concern
3. Diagnostics
4. Monoclonal antibodies
5. Vaccines

Terminology

- Mutation – an actual change in the nucleic acid or amino acid sequence (e.g. N501Y, E484K)
- Variant – two sequences that are different
- Lineage – a variant and its descendants (as in a phylogenetic tree)
- Strain – technically a variant that is phenotypically different, but basically a garbage term these days

Variants of concern…of interest…under investigation



outbreak.info/situation-reports

Variants and mutations of interest

Variants		*Mutations*
B.1.427/429	“California”	E484K
B.1.526/526.1/526.2	“New York”	L452R
B.1.617/617.1/617.3	“India”	N501Y
P.2	“Brazil”	N501Y + E484K
		S131I + L452R

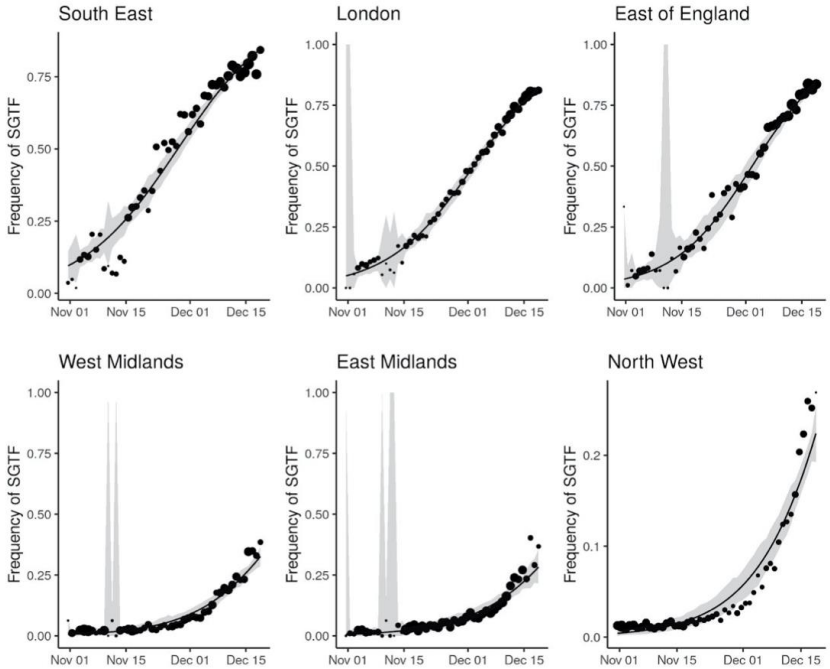
outbreak.info/situation-reports

Lineage B.1.1.7 (aka “the UK variant”)

S	DEL69/70.0
S	DEL144/144.0
S	N501Y
S	A570D
S	D614G
S	P681H
S	T716I
S	S982A
S	D1118H

- It arose as a long branch (immunocompromised host?)
- It spreads more rapidly, so is more difficult to contain
- Does not appear to vary with age (early speculation)
- Newer data suggest that it may cause more severe disease
    - Hard studies to do, lots of caveats

Lineage B.1.1.7 (aka “the UK variant”)



NERVETAG Report 12/31/2020

B.1.351 (aka “the South African Variant”)

S	K417N
S	E484K
S	N501Y
S	D614G
S	A701V

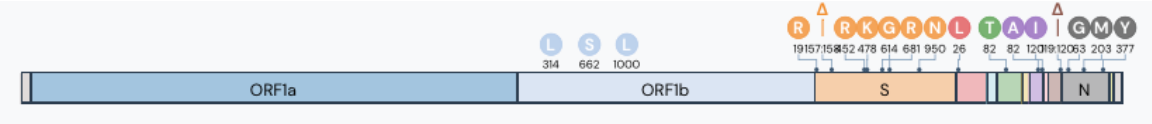
- It appears to spread rapidly
  - Staying at low level in US thus far (can’t compete with B.1.1.7?)
- Concerns that it is less neutralizable by sera (vax, convalescent)
- E484K!

P.1 (aka “the Brazilian Variant”)

S	L18F
S	T20N
S	P26S
S	D138Y
S	R190S
S	K417T
S	E484K
S	N501Y
S	D614G
S	H655Y
S	T1027I

- Explosive outbreaks in Manaus (reinfection?)
- Has spread fairly quickly
- Troika of mutations also in B.1351

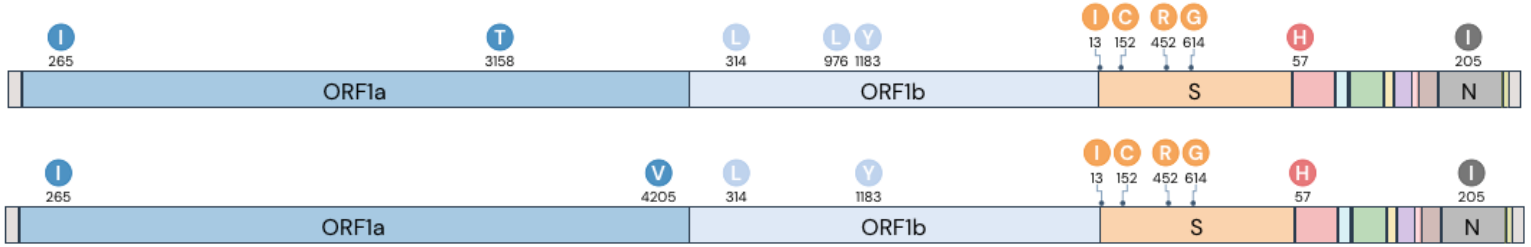
B.1.617.2 (aka “the Indian variant”)

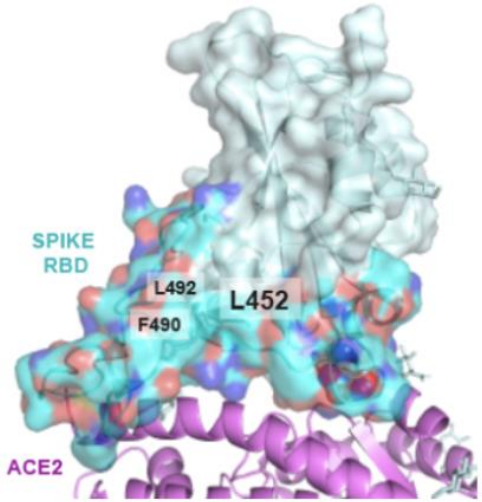


S	T19R
S	del157/158
S	L452R
S	T478K
S	D614G
S	P681R
S	D950N

- Co-incident with surge in India
- Relative transmissibility is unclear
- L452R and P681R
- Other ones have E484Q

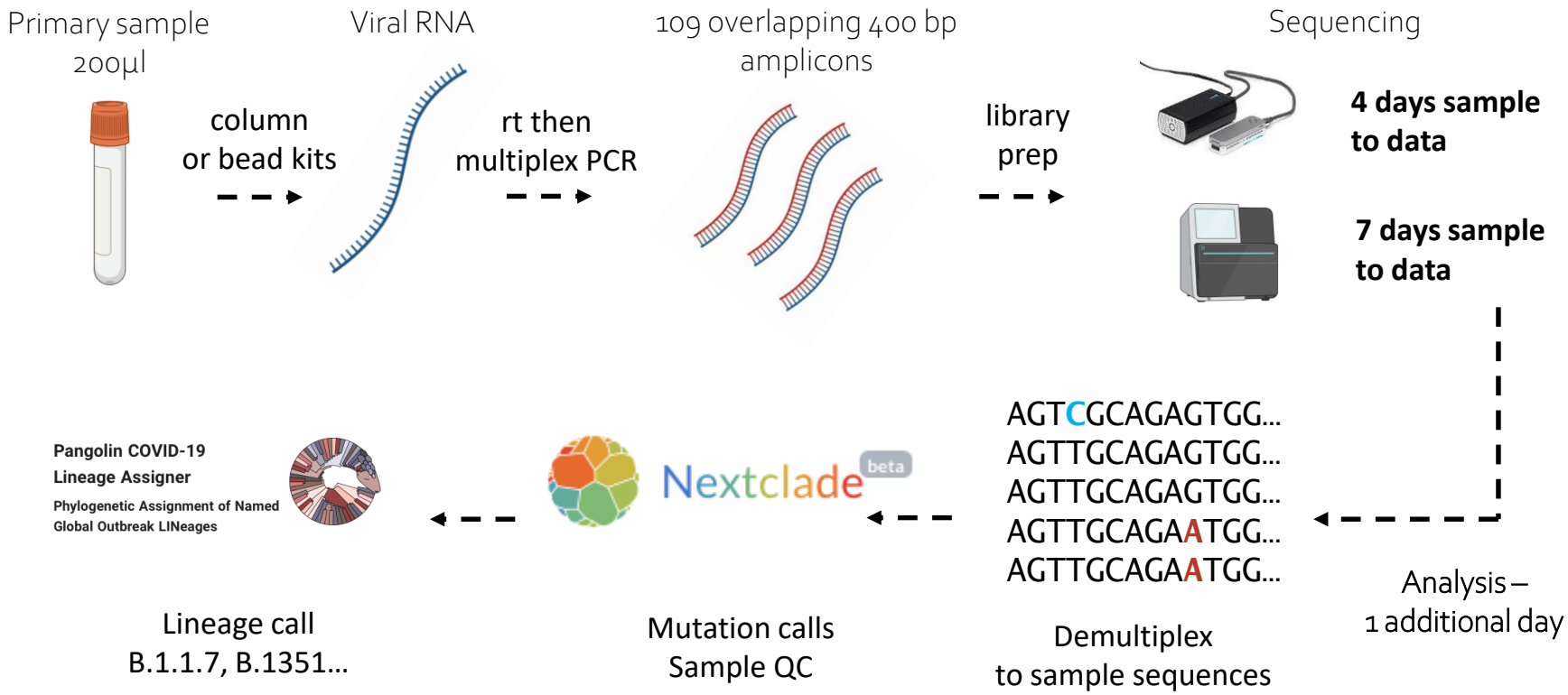
B.1.427 and B.1.429



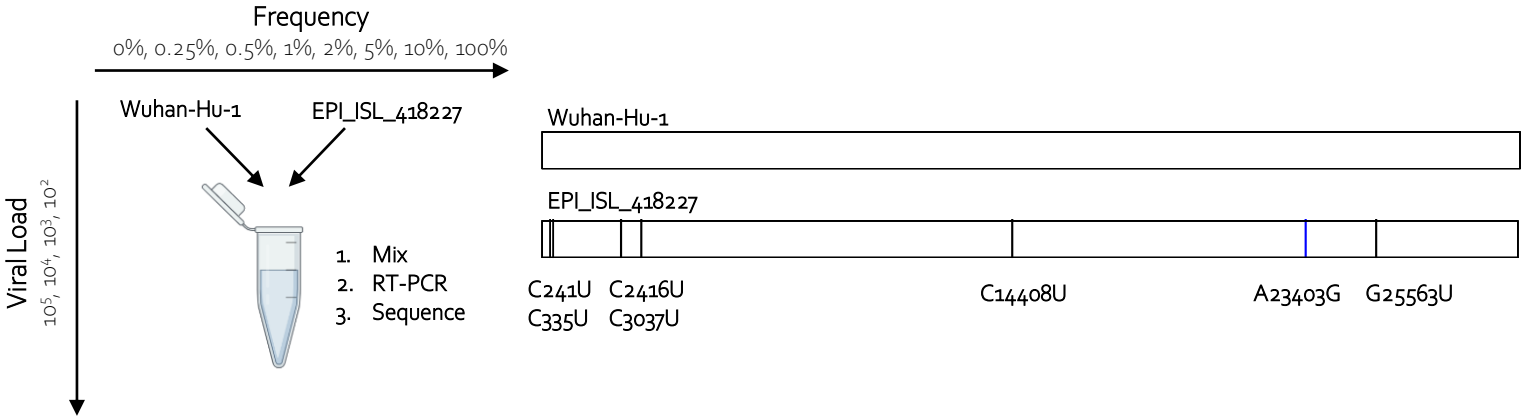


Deng et al. medRxiv 2021

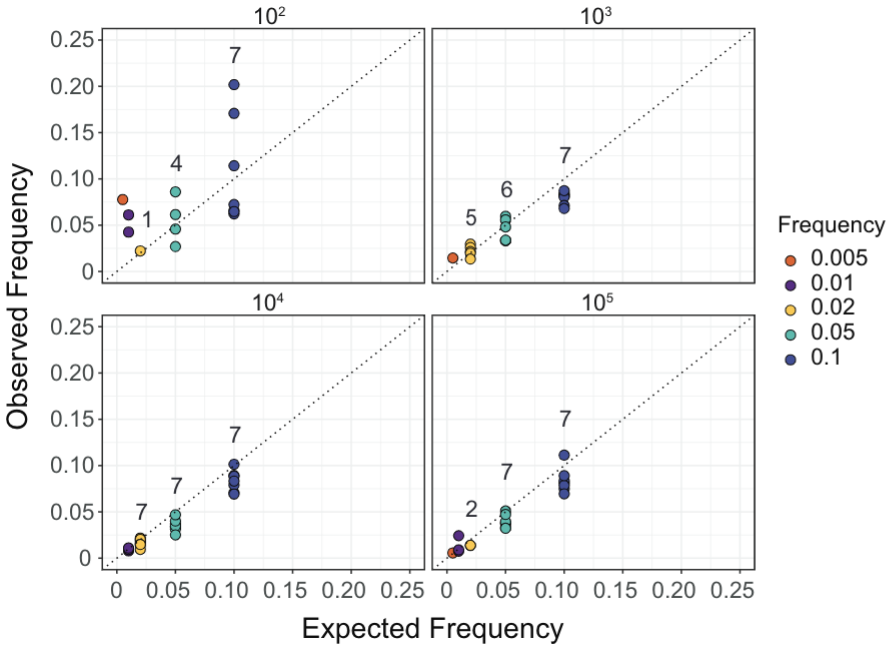
Sequencing

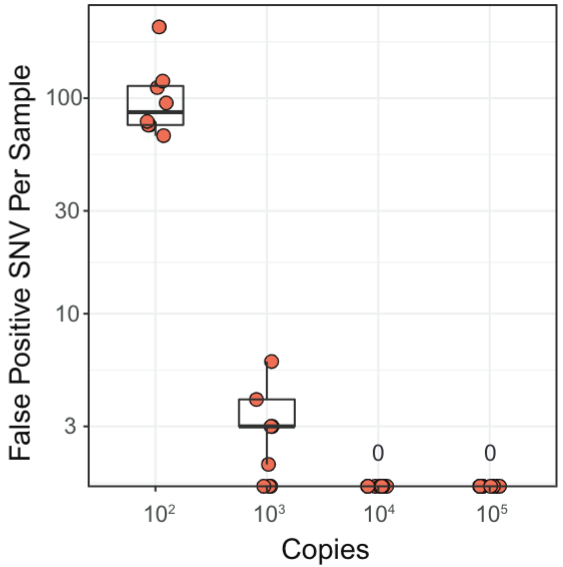

Primary sample
200µl
column
or bead kits
Viral RNA
rt then
multiplex PCR
109 overlapping 400 bp
amplicons
library
prep
Sequencing
4 days sample
to data
7 days sample
to data
Analysis –
1 additional day
AGTCGCAGAGTGG...
AGTTGCAGAGTGG...
AGTTGCAGAGTGG...
AGTTGCAGAATGG...
AGTTGCAGAATGG...
Demultiplex
to sample sequences
Nextclade
beta
Mutation calls
Sample QC
Pangolin COVID-19
Lineage Assigner
Phylogenetic Assignment of Named
Global Outbreak LINeages
Lineage call
B.1.1.7, B.1351...

Quality control by sequencing defined templates

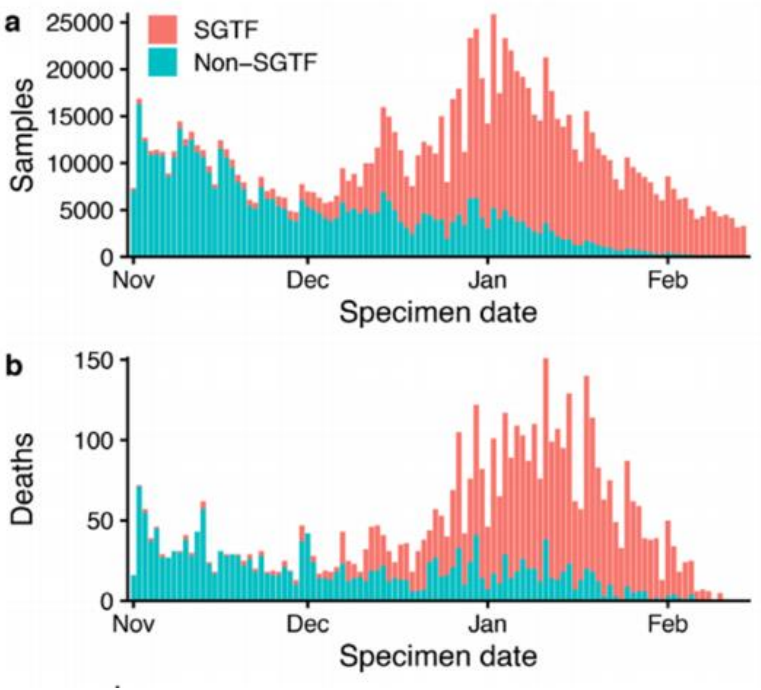


Quality control by sequencing defined templates





Non-sequence diagnostics



ThermoFisher TaqPath
N, ORF1ab, S

"S gene target failure"

CDC.gov

Other variants have SGTF (del 69-70)

Brito et al. and Larsen and Worobey, Virological.org

FDA Guidance

SARS-CoV-2 Viral Mutations: Impact on COVID-19 Tests

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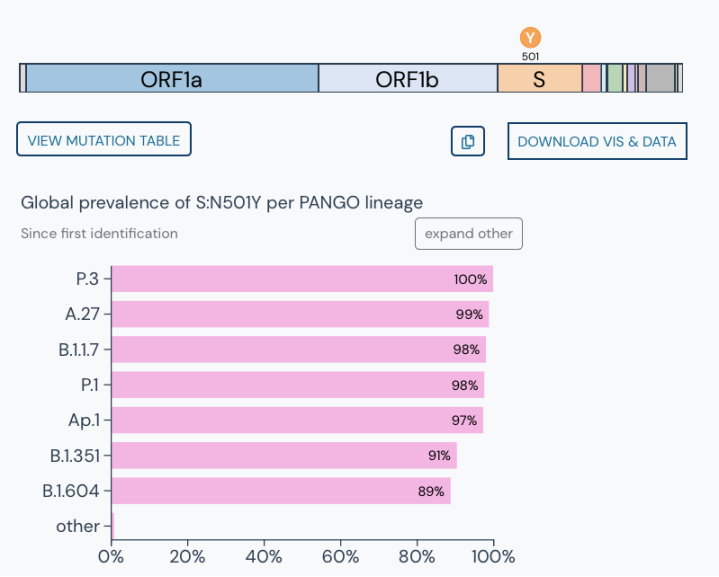
The SARS-CoV-2 virus has mutated over time, resulting in genetic variation in the population of circulating viral strains over the course of the COVID-19 pandemic. Molecular, antigen, and serology tests are affected by viral mutations differently due to the inherent design differences of each test.

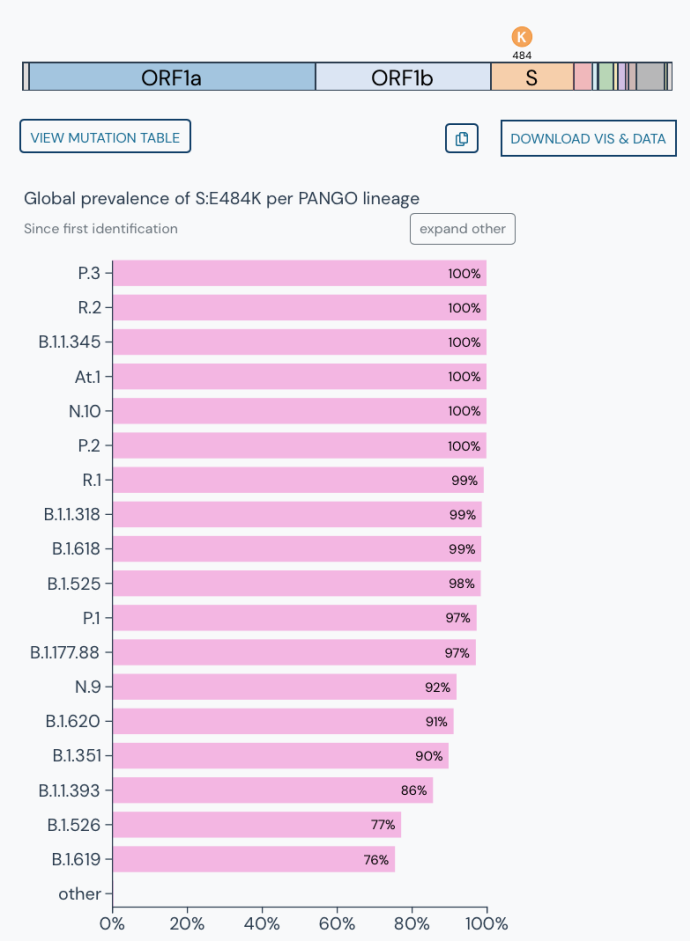
This page provides information regarding the impact of viral mutations on COVID-19 tests, recommendations for clinical laboratory staff and health care providers, and information about certain tests for which the FDA has identified potential impacts on performance due to SARS-CoV-2 genetic mutations. The FDA will update this page as significant new information becomes available.

On this page:

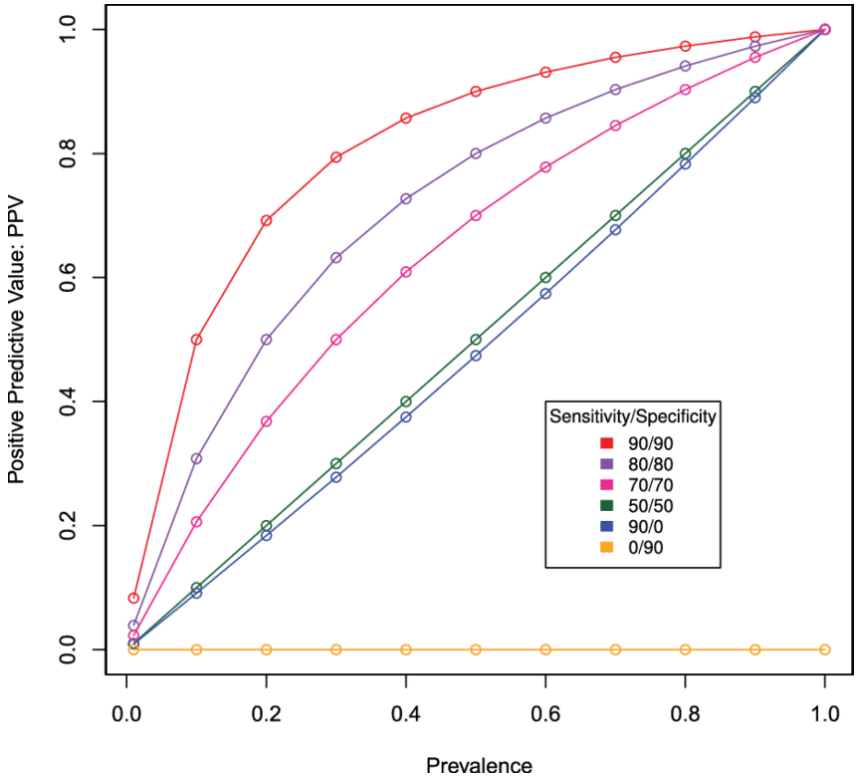
Newer primer/probe sets

IDT sets include:
Spike A570D, D80A, E484K, N501Y, S13I
W152C, L452R, 417T…N D3L





Importance of quality control



Research Gate, Menke

Key points in evaluating diagnostics

- Analytic sensitivity and specificity
  - Discrimination between alternate alleles (delta Ct)
  - Determine cut-off Ct, limit of detection
  - Synthetic materials often more readily available, more quantitative
- High accuracy necessary when mutations/variants are rare
- Matching mutations to variants is important

Filkins et al. Clinical Chemistry 2021

Monoclonal antibodies

Casirivimab + Imdevimab

Lineage with Spike Protein Substitution	Key Substitutions Tested	Fold Reduction in Susceptibility
B.1.1.7 (UK origin)	N501Y[a]	no change[c]
B.1.351 (South Africa origin)	K417N, E484K, N501Y[b]	no change[c]
P.1 (Brazil origin)	K417T + E484K	no change[c]
B.1.427/B.1.429 (California origin)	L452R	no change[c]
B.1.526 (New York origin)[d]	E484K	no change[c]

Bamlanivimab

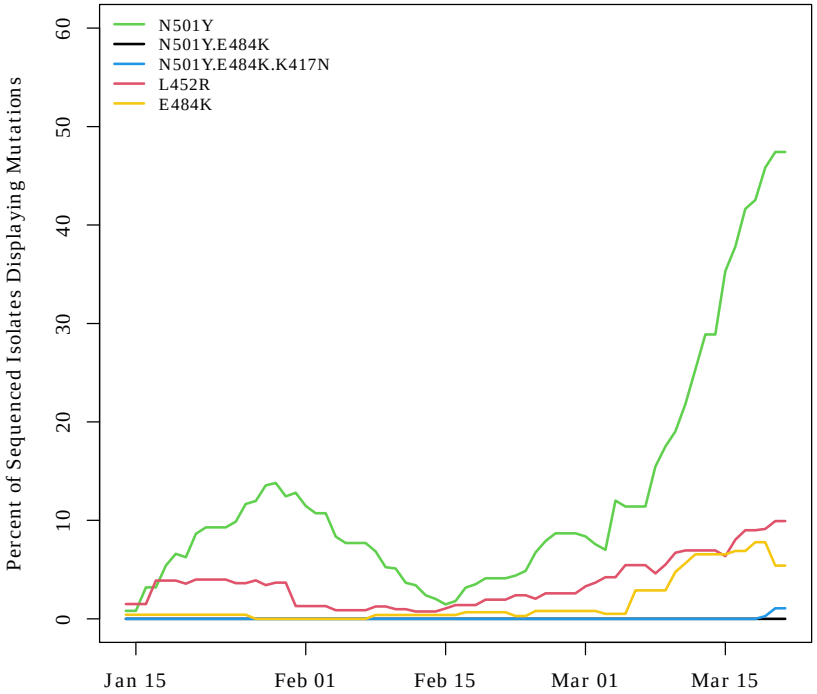
Lineage with Spike Protein Substitution	Key Substitutions Tested[a]	Fold Reduction in Susceptibility
B.1.1.7 (UK origin)	N501Y	no change[b]
B.1.351 (South Africa origin)	E484K	>2,360[c]
P.1 (Brazil origin)	E484K	>2,360[c]
B.1.427/B.1.429 (California origin)	L452R	>1,020[c]
B.1.526 (New York origin)[d]	E484K	>2,360[c]

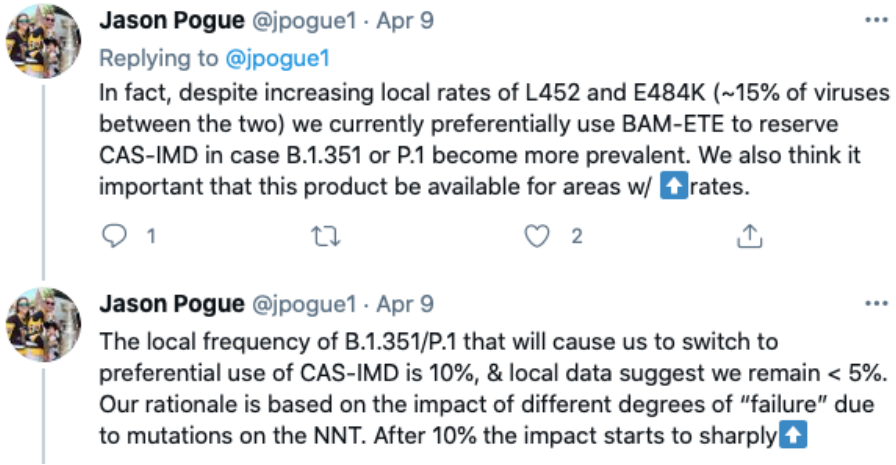
Bamlanivimab + Etesevimab

Lineage with Spike Protein Substitution	Key Substitutions Tested[a]	Fold Reduction in Susceptibility
B.1.1.7 (UK origin)	N501Y	no change[b]
B.1.351 (South Africa origin)	K417N + E484K + N501Y	>45[c]
P.1 (Brazil origin)	K417T + E484K + N501Y	>511[c]
B.1.427/B.1.429 (California origin)	L452R	7.4
B.1.526 (New York origin)[d]	E484K	17

FDA EUA Fact Sheets
h/t Jason Pogue, Dan Kaul

Surveillance informing empiric therapy





h/t Jason Pogue, Tejal Gandhi, Lindsay Petty

What about vaccines?

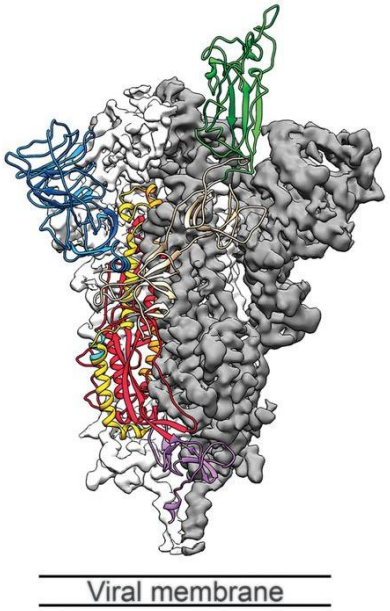
DAILY COMMENT

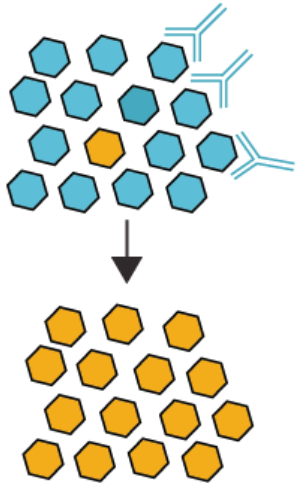
CAN THE COVID-19 VACCINE BEAT THE PROLIFERATION OF NEW VIRUS MUTATIONS?

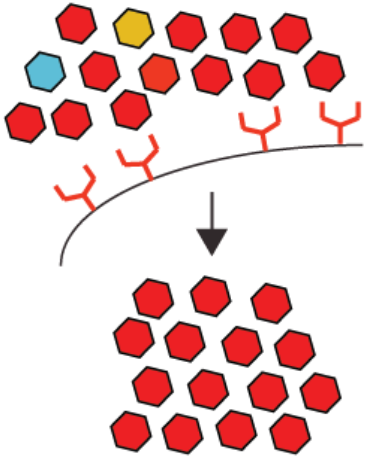
By Lawrence Wright

January 21, 2021

Spike is *the* antigen

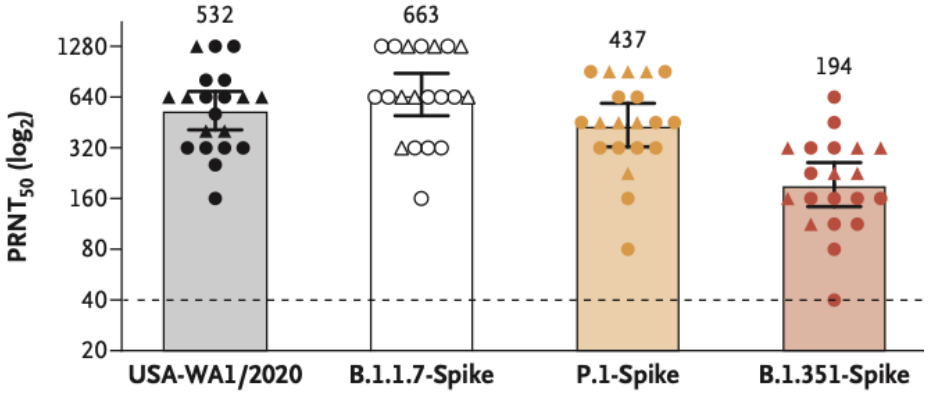


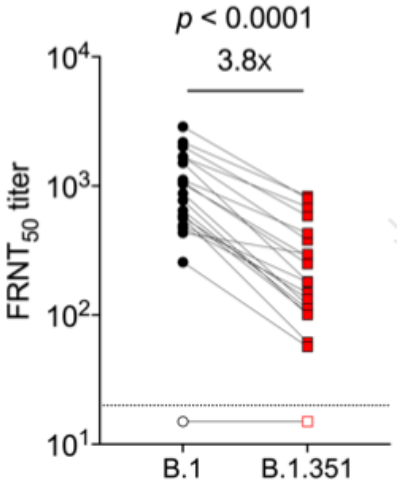




Wrapp et al. Science 2020

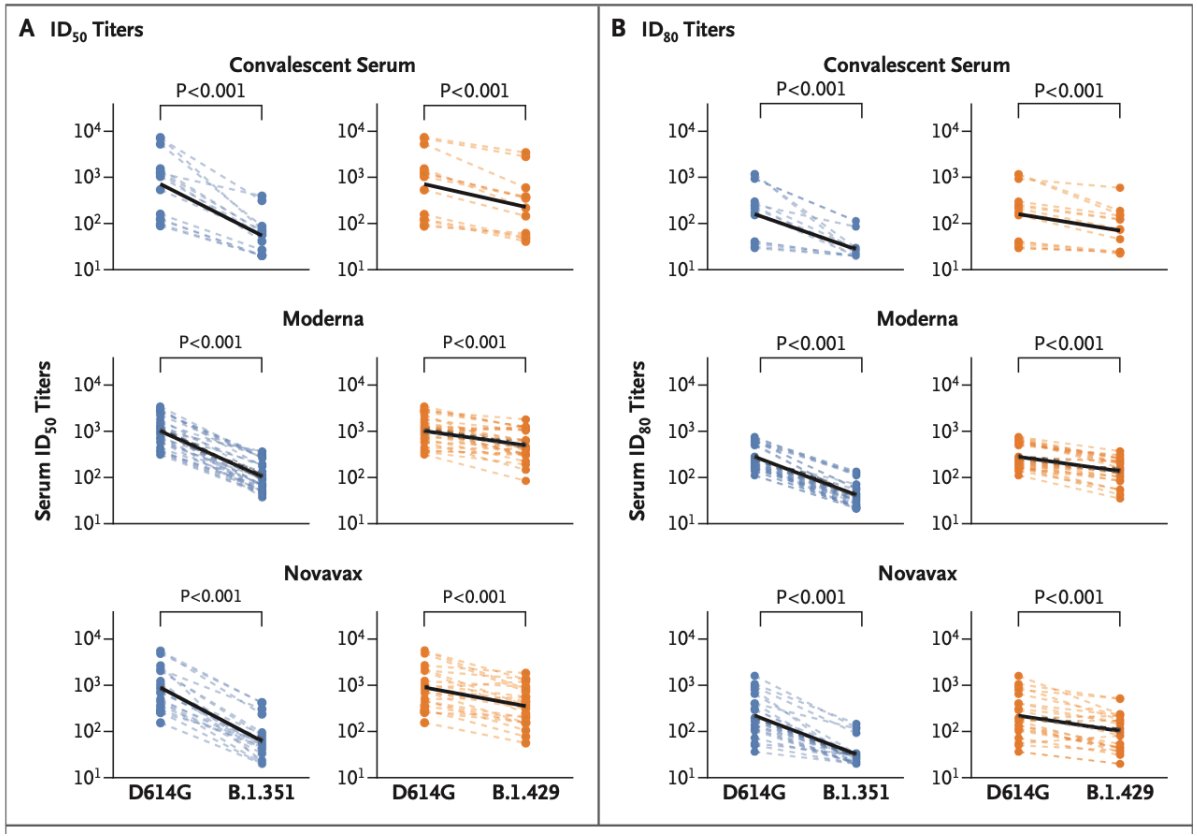
Vaccines – serological responses





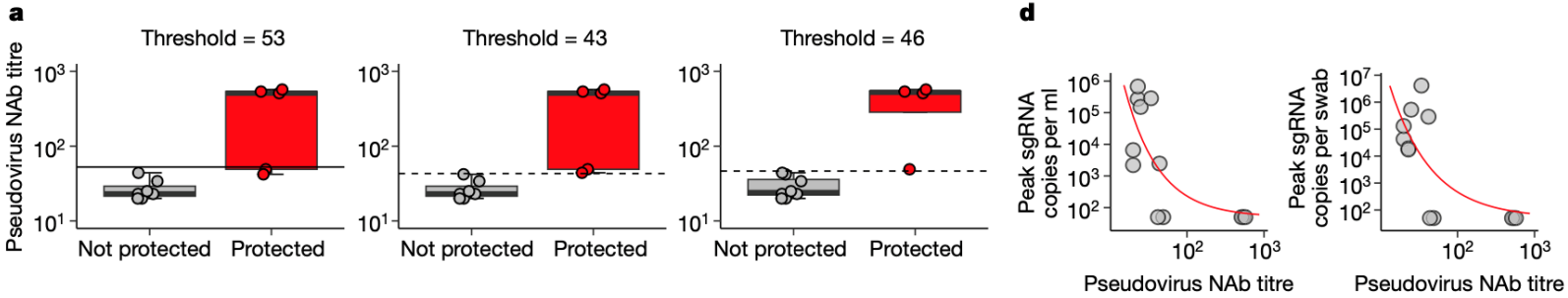
Liu et al. NEJM 2021 (Pfizer)
Edara et al. Cell Host Microbe (Moderna)
See also Werner NEJM 2021 (Moderna)
Want Nature 2021 (Moderna/Pfizer)

Vaccines – serological responses



Shen et al. NEJM 2021

What serum titer is “protective”?



MacMahon et al. Nature 2020

Serology is only part of the story

Adam Kucharski 
@AdamJKucharski

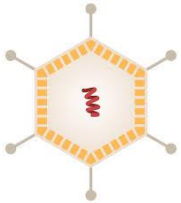
A few people have asked "do new variants mean vaccines won't work"? Important to avoid simple categories of 'works' and 'doesn't work'. Some variants may alter the extent of protection (and some probably won't) and question is whether this change matters (and at what scale)... 1/

7:34 AM · Jan 19, 2021 · Twitter Web App

A/HongKong/2014	”cell”	1:160
A/HongKong/2014	“egg”	1:2560
A/Singapore/2016	“cell”	1:160
A/Singapore/2016	“egg”	1:5120

Vaccines – efficacy and effectiveness

Janssen, FDA filing



72%

68% P.1*

64% B.1.351
(94.5% cases, variant)

Pfizer, SIREN study, Lancet

85% B.1.1.7
(Dec-Feb)

Novavax, Press release

89.3% B.1.1.7
(post-hoc similar)

60.1% HIV neg B.1.351
(93% cases, variant)

* 69% cases P2, has E484K

Estimating variant-specific vaccine effectiveness

Vaccine Breakthrough Infections with SARS-CoV-2 Variants

Ezgi Hacisuleyman, Ph.D., Caryn Hale, Ph.D., Yuhki Saito, Ph.D., Nathalie E. Blachere, Ph.D., Marissa Bergh, B.S.N., Erin G. Conlon, Ph.D., Dennis J. Schaefer-Babajew, Ph.D., Justin DaSilva, M.S., Frauke Muecksch, Ph.D., Christian Gaebler, M.D., Richard Lifton, M.D., Ph.D., Michel C. Nussenzweig, M.D., Ph.D., Theodora Hatziioannou, Ph.D., Paul D. Bieniasz, Ph.D., and Robert B. Darnell, M.D., Ph.D.

SUMMARY

Emerging variants of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) are of clinical concern. In a cohort of 417 persons who had received the second dose of BNT162b2 (Pfizer–BioNTech) or mRNA-1273 (Moderna) vaccine at least 2 weeks previously, we identified 2 women with vaccine breakthrough infection. Despite evidence of vaccine efficacy in both women, symptoms of coronavirus disease 2019 developed, and they tested positive for SARS-CoV-2 by polymerase-chain-reaction testing. Viral sequencing revealed variants of likely clinical importance, including E484K in 1 woman and three mutations (T95I, del142–144, and D614G) in both. These observations indicate a potential risk of illness after successful vaccination and subsequent infection with variant virus, and they provide support for continued efforts to prevent and diagnose infection and to characterize variants in vaccinated persons. (Funded by the National Institutes of Health and others.)

Thank You!

REUTERS/Kyle Grillot