Diagnostic & Clinical Impacts of SARS-CoV-2 Variants

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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
## Variants and mutations of interest

<table>
<thead>
<tr>
<th>Variants</th>
<th>Mutations</th>
</tr>
</thead>
<tbody>
<tr>
<td>B.1.427/429</td>
<td>E484K</td>
</tr>
<tr>
<td>B.1.526/526.1/526.2</td>
<td>L452R</td>
</tr>
<tr>
<td>B.1.617/617.1/617.3</td>
<td>N501Y</td>
</tr>
<tr>
<td>P.2</td>
<td>N501Y + E484K</td>
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<tr>
<td></td>
<td>S131I + L452R</td>
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<tr>
<td>&quot;California&quot;</td>
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<tr>
<td>&quot;New York&quot;</td>
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<tr>
<td>&quot;India&quot;</td>
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<tr>
<td>&quot;Brazil&quot;</td>
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</table>
Lineage B.1.1.7 (aka “the UK variant”)

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

- 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”)

- Explosive outbreaks in Manaus (reinfection?)
- Has spread fairly quickly
- Troika of mutations also in B.1351
B.1.617.2 (aka “the Indian variant”)

- 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 → rt then multiplex PCR → library prep → Sequencing

Viral RNA → 109 overlapping 400 bp amplicons → Sequencing

Analysis – 1 additional day

Sequencing

4 days sample to data

7 days sample to data

Pangolin COVID-19 Lineage Assigner
Phylogenetic Assignment of Named Global Outbreak Lineages

Mutation calls
Sample QC

Demultiplex to sample sequences

AGT\textsuperscript{CGCAGAGTGG...}
AGTTG\textsuperscript{GCAGAGTGG...}
AGTTGC\textsuperscript{AGAGTGG...}
AGTTGC\textsuperscript{AGAATGG...}

Lineage call
B.1.1.7, B.1351...

Analysis – 1 additional day
Quality control by sequencing defined templates

1. Mix
2. RT-PCR
3. Sequence

Wuhan-Hu-1

EPI_ISL_418227

Viral Load

Frequency
0%, 0.25%, 0.5%, 1%, 2%, 5%, 10%, 100%

Wuhan-Hu-1

EPI_ISL_418227

C241U  C2416U
C335U  C3037U
C14408U
A23403G  G25563U

C25
C50
C75
C100
Quality control by sequencing defined templates

Many false positives with low input viral load
Non-sequence diagnostics

ThermoFisher TaqPath
N, ORF1ab, S

“S gene target failure”
Other variants have SGTF (del 69-70)

Brito et al. and Larsen and Worobey, Virological.org
SARS-CoV-2 Viral Mutations: Impact on COVID-19 Tests

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:

- Genetic Variations: Background and Considerations
- General Information for Clinical Laboratory Staff and Healthcare Providers
- Molecular Tests Impacted by SARS-CoV-2 Mutations
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

<table>
<thead>
<tr>
<th>Lineage with Spike Protein Substitution</th>
<th>Key Substitutions Tested</th>
<th>Fold Reduction in Susceptibility</th>
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<tbody>
<tr>
<td>B.1.1.7 (UK origin)</td>
<td>N501Y^a</td>
<td>no change^c</td>
</tr>
<tr>
<td>B.1.351 (South Africa origin)</td>
<td>K417N, E484K, N501Y^b</td>
<td>no change^c</td>
</tr>
<tr>
<td>P.1 (Brazil origin)</td>
<td>K417T + E484K</td>
<td>no change^c</td>
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<td>L452R</td>
<td>no change^c</td>
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<tr>
<td>B.1.526 (New York origin)^d</td>
<td>E484K</td>
<td>no change^c</td>
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*| Lineage with Spike Protein Substitution | Key Substitutions Tested^a | Fold Reduction in Susceptibility |
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<td>&gt;2,360^c</td>
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<td>K417N + E484K + N501Y</td>
<td>&gt;45^c</td>
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<td>P.1 (Brazil origin)</td>
<td>K417T + E484K + N501Y</td>
<td>&gt;511^c</td>
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<td>B.1.427/B.1.429 (California origin)</td>
<td>L452R</td>
<td>7.4</td>
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*Information provided through the FDA EUA Fact Sheets, with thanks to Jason Pogue and Dan Kaul.*
Surveillance informing empiric therapy

In fact, despite increasing local rates of L452 and E484K (~15% of viruses between the two) we currently preferentially use BAM-ETE to reserve CAS-IMD in case B.1.351 or P.1 become more prevalent. We also think it important that this product be available for areas with lower rates.

The local frequency of B.1.351/P.1 that will cause us to switch to preferential use of CAS-IMD is 10%, & local data suggest we remain < 5%. Our rationale is based on the impact of different degrees of "failure" due to mutations on the NNT. After 10% the impact starts to sharply increase.

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

#### A. ID₅₀ Titers

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<tr>
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<tr>
<td>D614G</td>
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<td>B.1.351</td>
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<td>P &lt; 0.001</td>
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#### B. ID₅₀ Titers

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<td><img src="image11" alt="Graph" /></td>
<td><img src="image12" alt="Graph" /></td>
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<tr>
<td>B.1.351</td>
<td><img src="image13" alt="Graph" /></td>
<td><img src="image14" alt="Graph" /></td>
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<tr>
<td>P &lt; 0.001</td>
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<td><img src="image17" alt="Graph" /></td>
<td><img src="image18" alt="Graph" /></td>
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*Shen et al. NEJM 2021*
What serum titer is “protective”?

MacMahon et al. Nature 2020
Serology is only part of the story

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)...

7:34 AM · Jan 19, 2021 · Twitter Web App
Vaccines – efficacy and effectiveness

Janssen, FDA filing
- 72%
- 68%
- 64%

Pfizer, SIREN study, Lancet
- 85%
  (Dec-Feb)

Novavax, Press release
- 89.3%
  (post-hoc similar)
- 60.1% HIV neg
  (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 Hatzioannou, 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