PDL UPDATE:

Impact of SARS-CoV-2 Mutations on COVID assays

January 14, 2021

Overview

With the publication of the FDA's communique[1] on the potential for false negative results when interrogating SARS-CoV-2 by molecular methods, health care workers are scurrying to learn about how genetic variants occur and what impact (if any) these variants have on vaccination, targeted therapies, and laboratory testing.

Mutational variation is a normal process of viral replication, best evidenced by the annual changes associated with the flu virus. The rate of mutation is higher in mRNA than DNA viruses, but lower in coronaviruses than other mRNA viruses due to an enzyme that corrects some of the errors made during replication.

Nomenclature[2]

- A m*utation* refers to the actual change in nucleic acid sequence (for instance, D614G is an aspartic acid-to-glycine substitution at position 614 of the spike glycoprotein);
- Genomes that differ in sequence are often called *variants*, although variants can differ by more than one mutation;
- A variant is a *strain* when it demonstrates a phenotypic difference in antigenicity, transmissibility, or virulence.

While clinicians and genomic epidemiologists are interested in the effect of mutations on transmissibility, virulence and antigenicity, laboratorians are more focused on the impact of mutations on molecular and antigen detection.

SARS-CoV-2 Mutations

Mutations in N501Y were first identified in South Africa and reported at a WHO conference in December 2020. Upon hearing of this mutation, British investigators realized that a phylogenetic cluster in the county of Kent, UK had similar properties, and upon sequencing positive specimens previously collected from patients from this cluster, determined that this variant was similar to and predated the South African variant, having first appeared in September 2020.[3] The UK variant became known as 501Y.V1, and the South African variant designated 501Y.V2. This mutation became known as lineage B.1.1.7, with 17 lineage-defining mutations identified[4]:

Gene	Nucleotide	amino acid
ORF1ab	C3267T	T1001I
	C5388A	A1708D
	T6954C	I2230T
	11288-11296 deletion	SGF 3675-3677 deletion
Spike	21765-21770 deletion	HV 69-70 deletion
	21991-21993 deletion	Y144 deletion

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Gene	Nucleotide	amino acid
	A23063T	N501Y
	C23271A	A570D
	C23604A	P681H
	C23709T	T716I
	T24506G	S982A
	G24914C	D1118H
ORF8	C27972T	Q27stop
	G28048T	R52I
	A28111G	Y73C
N	28280 GAT->CTA	D3L
	C28977T	S235F

Eight of the lineage B.1.1.7 mutations are in the spike glycoprotein (S gene); three of these mutations have potential biological effects that have been described previously[4]:

- Mutation N501Y is one of six key contact residues within the receptor-binding domain (RBD) and has been identified as increasing binding affinity to human and murine ACE2.
- The spike deletion 69-70del has been described in the context of evasion to the human immune response but has also occurred a number of times in association with other RBD changes.
- Mutation P681H is immediately adjacent to the furin cleavage site, a known location of biological significance.

Preliminary data suggests that the resulting amino acid changes in N501Y in the spike glycoproteins are associated with an increased ability of the virus to bind to human cells making it easier for a person to become infected; the mutated virus accounts for >60% of new infections in London and surrounding areas.[5]

Another variant recently emerged in Nigeria, but little is known about this variant at this time. And just Sunday January 10th, Japan's National Institute of Infectious Disease (NID) announced it has detected a new variant in four travelers arriving from Brazil. The NID further indicated that the new variant has similar characteristics of the UK and South African variants, belongs to the B.1.1.248 strain, and has 12 mutations in the spike protein.[6] If this new variant behaves

like its predecessor variants, the hope is that there is minimal impact on vaccine efficacy, and the effect on performance of molecular assays is predictable and similar to how other variants impact testing.

Genomic Surveillance

The new SARS-CoV-2 variant present in Britain has also been identified in 47 countries, with 30 countries reporting genomic sequences confirming the variant.[7]

Although public health officials were calling for routine genetic surveillance of viral outbreaks, most countries are either ill-equipped to sequence, or haven't committed the necessary resources. While Britain sequences ~10% of samples that test positive for the virus, the U.S. only sequences ~1% of its positive samples![3]

Genomic surveillance has largely focused on mutations in the S gene for spike glycoprotein, which mediates attachment to cells, and is the major viral antigen associated with vaccines. Furthermore, if mutations in the S gene (spike) increase transmissibility, that variant could outcompete, replace, and become the dominant variant. However, since current vaccines trigger an immune response to the entire spike protein, protection may still occur despite a few changes at antigenic sites in SARS-CoV-2 variants. This has been supported by preliminary studies underwritten by Pfizer that demonstrated that the N501Y variants did not impact vaccine efficacy as evidenced by no reduction in neutralization activity against the variant.[8]

Most molecular methods interrogate multiple viral nucleic acid sequences, often targeting a combination of viral genes sequences present in the N or S genes and/or the open reading frame ORF1ab. Some methods also target the virus's RNA-dependent RNA polymerase (RdRp), which is required for SARS-CoV-2 to replicate in human and animal hosts. Patient specimens can have any individual gene or a combination of viral genes detected; however, response to the S-gene alone is not considered a reliable indicator of presence of the virus.

Since these variants of SARS-CoV-2 have genetic changes in the S-gene, the S-gene may not be detected in current molecular tests, whether purchased commercially (IVD) or lab developed (LDTs). It's possible that cases that would have previously been positive on all genes are now positive only for the ORF1ab, the N-gene, or the RdRp gene (not the S-gene).

Consequently, absence of the S-gene has become one of the flags indicating the potential presence of a new variation in COVID-19; the percentages of this type of positive has increased since November, providing much of the evidence supporting the growth of the new variant.

Recently, the US FDA has alerted clinical laboratorians that false negative results may occur with any molecular test if a mutation occurs in the part of the viral S gene interrogated by that particular test.[1] Assays that detect multiple genetic targets are less susceptible to the effects of genetic variation than tests designed to detect a single viral target.

To date, three commercially available EUA approved molecular assays are known to be affected:

- **Mesa Biotech Inc. Accula SARS-Cov-2 Test** may be impacted when a SARS-CoV-2 virus patient sample has a genetic variant at position 28881 (GGG to AAC).

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- If the B.1.1.7 variant carries a double deletion at positions 69 and 70 on the spike protein gene (S-gene), the following two kits may be affected, although both assays detect multiple genetic targets, so overall sensitivity may not be compromised, and the pattern of detection may help identify new variants:
  - **TaqPath COVID-19 Combo Kit/Combo Kit Advanced**
  - **Linea COVID-19 Assay Kit**

The FDA recommends that clinical laboratory staff and health care providers:

- Be aware that genetic variants of SARS-CoV-2 arise regularly in S, N, and E genes and false negative test results can occur.
- Be aware that tests that use multiple genetic targets to determine a final result are less likely to be impacted by increased prevalence of genetic variants.
- Consider negative results in combination with clinical observations, patient history, and epidemiological information.
- Consider repeat testing with a different test (with different genetic target such as the RdRp gene) if COVID-19 is still suspected after receiving a negative test result.

Impact of Variants on Antigen Testing

SARS-CoV-2 antigen (Ag) tests uses antibodies (NOT nucleic acid based-primers like PCR) to capture SARS CoV-2 antigen, most often the viral nucleocapsid antigen (not the spike protein). Antibodies typically recognize 8-15 amino acid target sequences (equivalent to 24-45 nucleotide sequences); consequently, single nucleic acid point mutations are not likely to affect the performance of the Ag assays on the market. Furthermore, mutations outside of the nucleocapsid viral coding region (e.g. Spike protein) should have no effect on assay performance.

Impact of Variants on Antibody Testing

SARS-CoV-2 Antibody tests are designed to detect immunoglobulins (IgG and IgM or IgG, IgM and IgA isotypes) to either the spike protein or nucleocapsid protein of SARS-CoV-2 in whole blood, serum and/or plasma from individuals who have either had or were vaccinated against SARS CoV-2.

Since vaccinations to date target the spike protein, it is thought that an assay targeting antibodies to the spike protein would best assess vaccine efficacy, although that has not yet been proven nor approved by the FDA. Furthermore, it remains to be seen if a quantitative assay that correlates to the presence of neutralizing antibodies is necessary to assess immunity, or whether a qualitative or semi-quantitative assay is sufficient.

If this is the case, it may prove beneficial to use a supplemental assay targeting antibodies to nucleocapsid protein to differentiate between an antibody response due to vaccination vs. one due to infection. That said, if the vaccine stimulates the immune response in someone who is already infected, the antibodies could be responding to all viral proteins.

Nucleic acid changes in the viral variants may in theory change the immunodominant epitopes. Since SARS CoV-2 Ab tests use S and N viral proteins as target antigens, the changes in the variants could modulate the Ab

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response detected. But since Ab responses are most often to many epitopes on an antigenic target, false negative Ab results would be unlikely.

Citations and General References

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General References:

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