The Importance of Monitoring Emerging SARS-CoV-2 Variants to Ensure Antigen Assay Effectiveness

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SARS-CoV-2 is the RNA coronavirus responsible for the COVID-19 global pandemic. Just when newly available vaccines (and many more in development) offered the prospect of a return to normalcy, the emergence of highly circulating variants has raised significant concerns about vaccine efficacy.[1-3] To better understand the potential impact of SARS-CoV-2 variants on testing and vaccines, it is important to know the differences between mutants, variants, and strains, and understand how variants might compromise SARS-CoV-2 antigen testing.

Antigen tests contribute to overall COVID-19 testing capacity, offering advantages in workflow, rapid time to first result, and reduced costs, especially in situations in which RT-PCR testing capacity is limited. They detect the presence of SARS-CoV-2 using antibodies directed against the nucleocapsid (or N-protein) of the virus. Antigen tests cannot differentiate the viral variant, but they help to reduce further transmission through early detection of highly infectious patients, enabling contact tracing to begin immediately and isolation of contagious people.

Antigen tests have the potential to be affected by mutations if the mutation causes an alteration to the protein or physical structure of the virus targeted by the test.[1-6] It is expected that many of the antigen tests detecting the nucleocapsid and currently used in primary detection of SARS-CoV-2 will be unaffected by mutations, but laboratories should be aware of potential effects on certain diagnostic tests.[7] Siemens Healthineers has designed its Atellica® IM and ADVIA Centaur® SARS-CoV-2 Antigen (CoV2Ag) assays* to use five monoclonal antibodies to detect the nucleocapsid to the N-terminal and C-terminal domains of the nucleocapsid, while many other assays use fewer monoclonal or polyclonal antibodies. Studies have been conducted to demonstrate that the Siemens Healthineers SARS-CoV-2 Antigen assays can detect the presence of the nucleocapsid even with the variants of concern (VOCs) recently defined by the World Health Organization (WHO) and the Center for Disease Control and Prevention (CDC).[4,5]

Difference between mutants, variants, and strains

The term "viral variant" can be confusing and is often (and incorrectly) used interchangeably with other terms, such as mutations, strains, and lineages. Figure 1 includes formal definitions and distinctions. Mutations are normal, abundant, and expected, especially with an RNA virus. When a mutation or group of mutations confers an advantage, a new variant can emerge. If the altered phenotype allows it to outcompete existing virus (for example, if it is more infectious or more capable of evading immune pressure), it may become the dominant strain.

Variants of concern definition
A Variant of Concern (VOC) is a variant associated with an experimentally verified functional change in the virus: Increased transmissibility, increased pathogenicity, drug resistance, immune escape, vaccine escape. Because of their increased risk to public health, VOCs have been identified as a priority for surveillance and response.[4-5]

Defining terms

Mutant: A change in the viral (RNA) genome

Synonymous mutation: No change in amino acid sequence of the resulting protein

Missense or nonsense mutation: Change in amino acid sequence of the resulting protein

Variant and lineage: No universally accepted definition across viruses, but generally a new "variant" or "lineage" is named based on accumulation of a significant number of mutations

Strain: A virus variant that shows distinct phenotype (differences in antigenicity, transmissibility, or virulence)

Figure 1. Definition of mutant, variant, lineage, and strain

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Variants have been with us since the beginning

As confirmed by the recent joint investigation by the WHO and scientific teams from China, variants have been with us since the dawn of the pandemic. The identification of VOCs in several parts of the world as of August 2021, (including those first identified in the United Kingdom [Alpha B.1.1.7], South Africa [Beta B.1.351], Brazil [Gamma P.1] and India [Delta B.1.617.2]), now detected in multiple countries, has elevated recognition and prompted investigation.[1-3,5,6] In 2021, the WHO released a new nomenclature to denote variants of the SARS-CoV-2 to simplify their names and discourage the practice of associating these to regions or countries. The new nomenclature uses Greek letters (Figure 2).[6]

More variants continue to be identified globally as countries initiate enhanced sequence surveillance programs, with the greatest focus on mutations in the spike protein. As of August 2021, the Epsilon variant [B1.427, B1.429; first identified in California, U.S.] is now classified as a Variant of Interest by WHO and recently by the U.S. CDC.[5,6] Concerns about the impact of variants include impairment of some diagnostic tests, including a small subset of molecular tests in which the mutation affects primer annealing, and the possibility of changes that enhance pathogenesis or transmission. For the antigen tests using antibodies to the Nucleocapsid (N-protein), the mutations of the spike protein will not impact the detection of the virus, only the mutations on the N-terminal or C-terminal domains of the nucleocapsid may cause issues for the detection. The N protein is the most abundant protein in virus-infected cells. Its primary function is to package the 30 kb single stranded, 5′-capped positive strand viral genome RNA molecule into a ribonucleoprotein complex called the capsid.

Name (previous WHO nomenclature)	Alpha (B.1.1.7[8-16])	Beta (B.1.351)[9,14-16]	Gamma (P.1)[10,14-16]	Delta B.1.617.2 [11,13-16]
First detected	September 2020	October 2020	January 2021	March 2021
Country of first detection	United Kingdom	South-Africa	Japan Brazil	India
Detected in other countries	Yes	Yes	Yes	Yes
Concern	Increased transmissibility >40% and potentially increased disease severity	Increased transmissibility 50% and reduced neutralization by antibodies including reduction of vaccine effectiveness	Possible reduced neutralization by antibodies including reduction of vaccine effectiveness	Increased transmissibility and possible reduced neutralization by antibodies including reduction of vaccine effectiveness
Nucleocapsid (N) mutation	**D3L, S235F R203K, G204R**	**T2051**	**P80R, R203K G204R**	**R203M, D377Y, G215C D63G**
Spike (S) protein mutation	69-70HVdel. Y144 del, **N501Y**, A57OD, P681H	K417N **E484K N501Y 242/24 3/244 del**	**K417T E484K N501Y**	D614G P681R T19R T478K L452R D950N E156G Del157/158

Figure 2. Variants of concern description.

Variant Testing
Siemens Healthineers is committed to the continuous monitoring of emerging variants and conducting evaluations to ensure that our assays remain effective at detecting them. Siemens Healthineers Global Assay Development, Tarrytown, NY, USA, tested patient respiratory specimens infected with the Alpha, Beta, Gamma and Epsilon variants, and more recently, the Delta variant, using the Atellica® IM SARS-CoV-2 Antigen Assay (CoV2Ag) and/or ADVIA Centaur CoV2Ag assay.*

Alpha (B.1.17), Beta (B.1.351) and Epsilon (B.1.429 Variants Study
Seventeen nasopharyngeal specimens with different viral load collected in VTM or 0.45% saline media, positive by RT-PCR and sequenced using next generation sequencing were evaluated with the Atellica IM CoV2Ag Assay.

Interpretation and result
The CoV2Ag assay cutoff is 1.0 index. Results ≥1.0 are reactive, and results <1.0 are non-reactive. The results in Table 1 indicate that the three variants Alpha, Beta, and Epsilon can be detected with the CoV2Ag assay.

Index results

Samples	Swab	Variant Type	RLU	Index
1	Nasopharyngeal	Alpha (B.1.1.7)	5123543	**>1000**
2	Nasopharyngeal	Alpha (B.1.1.7)	15113	**13.82**
3	Nasopharyngeal	Alpha (B.1.1.7)	4706	**2.43**
4	Nasopharyngeal	Alpha (B.1.1.7)	7202984	**>1000**
5	Nasopharyngeal	Alpha (B.1.1.7)	1220594	**>1000**
6	Nasopharyngeal	Alpha (B.1.1.7)	2221	**0.16**
7	Nasopharyngeal	Alpha (B.1.1.7)	6055	**4.31**
8	Nasopharyngeal	Beta (B.1.351)	800419	**895.72**
9	Nasopharyngeal	Beta (B.1.351)	106310	**115.48**
10	Nasopharyngeal	Beta (B.1.351)	155232	**169.06**
11	Nasopharyngeal	Beta (B.1.351)	170389	**185.66**
12	Nasopharyngeal	Beta (B.1.351)	250757	**273.81**
13	Nasopharyngeal	Epsilon (B.1.429)	4212707	**>1000**
14	Nasopharyngeal	Epsilon (B.1.429)	1567537	**>1000**
15	Nasopharyngeal	Epsilon (B.1.429)	6063204	**>1000**
16	Nasopharyngeal	Epsilon (B.1.429)	4086	**1.86**
17	Nasopharyngeal	Epsilon (B.1.429)	700843	**779.90**

Table 1. Atellica IM SARS-CoV-2 Antigen Assay demonstrated reactivity in samples with known Alpha, Beta, and Epsilon variants confirmed by genomic sequencing.

Gamma (P.1) Variant Study
Fifteen combined nasal and oropharyngeal specimens collected in Brazil in 0.45% saline media or viral transport media (VTM), known to be positive with RT-PCR and sequenced using next generation sequencing, and carrying the Gamma (P.1) variant were evaluated with the Atellica IM CoV2Ag Assay.

Interpretation and result
The CoV2Ag assay cutoff is 1.0 index. Results ≥1.0 are reactive, and results <1.0 are non-reactive. The results in Table 2 indicate that 12 of 15 specimens were detectable with the CoV2Ag assay.

Index results

Sample	Swab	Variant Type	Mutations found	RLU	Index
17025796	Nasopharyngeal + Oropharyngeal	Gamma (P.1)	L18F, T20N, P26S, D138Y, R190S, K417T, E484K, N501Y, D614G, H655Y, T1027I + V1176F (29908pb, 37,77GC, 16x)	**348510**	**375.32**
17025931	Nasopharyngeal + Oropharyngeal	Gamma (P.1)	p.R190S, p.L18F, p.T20N, p.P26S, p.D138Y, p.K417T, p.E484K, p.N501Y, p.D614G, p.H655Y, p.T1027I, p.V1176F	**9171864**	**>1000**
17025753	Nasopharyngeal + Oropharyngeal	Gamma (P.1)	p.D614G, p.H655Y, p.T1027I, p.V1176F, p.L18F, p.T20N, p.P26S, p.D138Y, p.R190S, p.K417T, p.E484K, p.N501Y	**10639395**	**>1000**
17026210	Nasopharyngeal + Oropharyngeal	Gamma (P.1)	p.K417T, p.L18F, p.T20N, p.P26S, p.D138Y, p. R190S, p.V1176F, p.T1027I, p.N960S, p.H655Y, p.D614G, p.N501Y, p.E484K, p.S477Ip.N501Y, p. E484K, p.S477I	**250004**	**268.72**
17025869	Nasopharyngeal + Oropharyngeal	Gamma (P.1)	L18F, T20N, P26S, D138Y, R190S, K417T, E484K, N501Y, D614G, H655Y, T1027I + V1176F (2 contigs: 23856+5988pb, 38GC, 65x)	**37191**	**39.50**
17025699	Nasopharyngeal + Oropharyngeal	Gamma (P.1)	p.T1027I, p.V1176F, p.N501Y, p.D614G, p.T20N, p.L18F, p.P26S, p.E484K, p.K417T, p.R190S, p.H655Y, p.D138Y, p.I1018I	**10332882**	**>1000**
17025834	Nasopharyngeal + Oropharyngeal	Gamma (P.1)	p.T20N, p.L18F, p.P26S, p.D138Y, p.R190S, p.K417T, p.E484K, p.N501Y, p.D614G, p.H655Y, p.T1027I, p.V1176F	4819320	**>1000**
17026148	Nasopharyngeal + Oropharyngeal	Gamma (P.1)	p.N501Y, p.H655Y, p.S659L, p.E661D, p.V1176F, p.L18F, p.T20N, p.P26S, p.D138Y, p.R190S, p.K417T, p.E484K	1891	**0.20**
17025842	Nasopharyngeal + Oropharyngeal	Gamma (P.1)	p.V1176F, p.H655Y, p.N501Y, p.E484K, p.K417T, p.R190S, p.D138Y, p.P26S, p.T20N, p.L18F	7822	**7.05**
17026040	Nasopharyngeal + Oropharyngeal	Gamma (P.1)	p.H655Y, p.V1176F, p.K417T, p.E484K, p.N501Y, p.L18F, p.T20N, p.P26S, p.D138Y, p.R190S	710160	**776.14**
17025966	Nasopharyngeal + Oropharyngeal	Gamma (P.1)	p.E484K, p.L18F, p.G1167A, p.H655Y, p.N501Y, p.T20N, p.P26S, p.D138Y, p.R190S, p.K417T	2526	**0.96**
17026016	Nasopharyngeal + Oropharyngeal	Gamma (P.1)	p.T1027I, p.H655Y, p.D614G, p.E484K, p.N501Y, p.T20N, p.L18F, p.E1182G, p.V1176F	611187	**664.73**
17025877	Nasopharyngeal + Oropharyngeal	Gamma (P.1)	p.K417T, p.L18F, p.T20N, p.P26S, p.D138Y, p.R190S, p.V1176F, p.H655Y, p.D614G, p.N606N, p.N501Y, p.E484K	9678109	**>1000**
17026024	Nasopharyngeal + Oropharyngeal	Gamma (P.1)	p.D138Y, p.P26S, p.T20N, p.L18F, p.H655Y, p.N501Y, p.D614G, p.E484K, p.K417T, p.R190S	218108	**234.37**
17026091	Nasopharyngeal + Oropharyngeal	Gamma (P.1)	p.D614G, p.R190S, p.K417T, p.E484K, p.N501Y, p.N606N, p.L18F, p.A845S, p.H655Y, p.V1176F, p.T20N, p.P26S, p.D138Y	1576	**0.00**

Table 2. Atellica IM SARS-CoV-2 Antigen Assay demonstrated reactivity in 12 out of 15 samples with known Gamma variant confirmed by genomic sequencing.

Delta (B.1.617.2) Variant Study
Five nasopharyngeal specimens in 0.45% saline media known to be positive by RT-PCR (Taqpath Covid19 combo) and carrying the Delta variant were evaluated with the Atellica IM CoV2Ag Assay and the ADVIA Centaur CoV2Ag assay.

Interpretation and result
The CoV2Ag assay cutoff is 1.0 Index. Results ≥1.0 Index are reactive, and results <1.0 index are non-reactive. The results in Table 3 indicate that the 5 samples were detectable with the CoV2Ag assay on both ADVIA Centaur XP (Table 3a) and Atellica IM (Table 3b) with an Index value above 1.0 Index.

Index results

Samples	Swab	Variant Type	RLU	Index
1	Nasopharyngeal	Delta (B.1.617.2)	746058	**>1000**
2	Nasopharyngeal	Delta (B.1.617.2)	1966897	**>1000**
3	Nasopharyngeal	Delta (B.1.617.2)	1851151	**>1000**
4	Nasopharyngeal	Delta (B.1.617.2)	3662828	**>1000**
5	Nasopharyngeal	Delta (B.1.617.2)	1283265	**>1000**

Table 3a. ADVIA Centaur XP SARS-CoV-2 Antigen Assay demonstrated reactivity in samples with known Delta variant confirmed by genomic sequencing.

Samples	Swab	Variant Type	RLU	Index
1	Nasopharyngeal	Delta (B.1.617.2)	1143260	**>1000**
2	Nasopharyngeal	Delta (B.1.617.2)	2859772	**>1000**
3	Nasopharyngeal	Delta (B.1.617.2)	2895231	**>1000**
4	Nasopharyngeal	Delta (B.1.617.2)	5321621	**>1000**
5	Nasopharyngeal	Delta (B.1.617.2)	1819081	**>1000**

Table 3b. Atellica IM SARS-CoV-2 Antigen Assay demonstrated reactivity in samples with known Delta variant confirmed by genomic sequencing.

Conclusion

SARS-CoV-2 variants of concern will continue to emerge, and enhanced surveillance will support earlier identification and assessment of potential effects on testing.

Assay design contributes to potential vulnerability of assays to detection altered by variants. For example, a design that incorporates features such as multiple monoclonal antibodies may help reduce the likelihood of missed detection of variants.

Based on testing, the SARS-CoV-2 Antigen assays available on ADVIA Centaur systems and Atellica IM Analyzer can detect samples that contain the SARS-CoV-2 variants Alpha, Beta, Gamma, Delta and Epsilon.

References:

1. Lauring AS, Hodcroft EB. Genetic variants of SARS-CoV-2—what do they mean? JAMA. 2021; 325(6):529-531. doi: 10.1001/jama.2020.27124
2. Fontanet A, Autran B, Lina B, et al. SARS-CoV-2 variants and ending the COVID-19 pandemic. Lancet. 2021. doi: 10.1016/s0140-6736(21)00370-6
3. Mascola JR, Graham BS, Fauci AS. SARS-CoV-2 viral variants—tackling a moving target. JAMA. 2021. doi: 10.1001/jama.2021.2088
4. https://www.euro.who.int/__data/assets/pdf_file/0003/492177/SARS-CoV-2-VOC-factsheet-eng.pdf
5. SARS-CoV-2 Variant classifications and definitions. 2021 Apr 27. https://www.cdc.gov/coronavirus/2019-ncov/cases-updates/variant-surveillance/variant-info.html
6. Tracking SARS-CoV-2 variants https://www.who.int/en/activities/tracking-SARS-CoV-2-variants/
7. https://www.medrxiv.org/content/10.1101/2021.05.05.21256527v1.full.pdf
8. https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/961037/NERVTAG_note_on_B.1.1.7_severity_for_SAGE_77__1_.pdf
9. Pearson CAB, Russell TW, Davies NG, Kucharski AJ, COVID-19 working group, Edmunds WJ, et al. Estimates of severity and transmissibility of novel South Africa SARS-CoV-2 variant 501Y.V2 [cited 14 January 2021]. https://cmmid.github.io/topics/covid19/reports/sa-novel-variant/2021_01_11_Transmissibility_and_severity_of_501Y_V2_in_SA.pdf
10. Brief report: new variant strain of SARS-CoV-2 identified in travelers from Brazil [updated 12 January 2021]. Available from: https://www.niid.go.jp/niid/en/2019-ncov-e/10108-covid19-33-en.html
11. Deng X, Garcia-Knight MA, Khalid MM, et al. Transmission, infectivity, and antibody neutralization of an emerging SARS-CoV-2 variant in California carrying a L452R spike protein mutation. MedRxiv 2021. doi: https://doiorg/10.1101/2021.03.07.21252647
12. https://www.ecdc.europa.eu/sites/default/files/documents/Emergence-of-SARS-CoV-2-B.1.617-variants-in-India-and-situation-in-the-EUEEA.pdf
13. Heliyon 7 (2021) e06167; The molecular assessment of SARS-CoV-2 nucleocapsid phosphoprotein variants among Indian isolates
14. SARS-CoV-2 Spike Mutant Protein (sinobiological.com)
15. A standardized, open-source database of COVID-19 resources and epidemiology data: htpps://outbreak.info
16. https://covariants.org/

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