OnSite COVID-19 Ag Rapid Tests Detects Newly Identified SARS-CoV-2 Variants

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Summary

The *OnSite* COVID-19 Ag Rapid Test is a lateral flow immunoassay for the qualitative detection of SARS-CoV-2 virus in nasopharyngeal (NP) or nasal swab specimens from symptomatic individuals suspected of COVID-19. The test detects the presence of antigens from the SARS-CoV-2 virus, namely the viral Nucleocapsid Protein (NP). The performance of the test has been extensively validated both internally and by external evaluations by MOH (Ministry of Health), end-users, and international agencies and institutions.

Multiple SARS-CoV-2 variants are now circulating globally. In a previous study, we evaluated the *OnSite* COVID-19 Ag Rapid Test on detecting variants B.1.1.7, B.1.351, and P.1[1]. In addition, we mapped our antibodies' epitopes. All three variants do not harbor mutations within the antibodies' epitopes, and the *OnSite* COVID-19 Ag Rapid Test can detect these variants.

In this study, we analyzed the NP sequences from new emerging variants, B.1.617+, B.1.525, B.1.526, B.1.617.1, C.37, B.1.427/B.1.429, P2, B.1.620 and B.1.1.529. None of these variants have mutations with the *OnSite* COVID-19 Ag Rapid Test antibodies' epitopes. Therefore, the performance of the *OnSite* COVID-19 Ag Rapid Test to detect these new variants should not be affected.

1. Background

SARS-CoV-2, the virus that causes COVID-19, changes over time. Most changes have little to no impact on the virus' properties. However, some changes may affect the virus's properties, such as how easily it spreads, the associated disease severity, or the performance of vaccines, therapeutic medicines, diagnostic tools, or other public health and social measures.

WHO has been monitoring and assessing the evolution of SARS-CoV-2 since January 2020. In late 2020, the emergence of variants that posed an increased risk to global public health prompted the characterization of specific Variants of Interest (VOIs) and Variants of Concern (VOCs) to prioritize global monitoring and research ultimately to inform the ongoing response to the COVID-19 pandemic[2]. VOCs have one or more of the following changes: 1) Increase in transmissibility or detrimental change in COVID-19 epidemiology; 2) Increase in virulence or change in clinical disease presentation; 3) Decrease in the effectiveness of public health and social measures or available diagnostics, vaccines, and therapeutics. The current VOCs include B.1.1.7, B.1.351, P.1, B.1.617.2, and B.1.1.529. VOIs have one or more of the following changes: 1) has been identified to cause community transmission/multiple COVID-19 cases/clusters, or has been detected in multiple countries; 2) is otherwise assessed to be a VOI by WHO in consultation with the WHO SARS-CoV-2 Virus Evolution Working Group. The current VOIs include B.1.525, B.1.526, B.1.617.1, and C.37.

The *OnSite* COVID-19 Ag Rapid Test detects the presence of antigens from the SARS-CoV-2 virus within the first seven days of the onset of symptoms. Test results can be interpreted at 15-20 minutes, by minimally skilled personnel, without the use of cumbersome laboratory equipment. With the emergence of multiple SARS-CoV-2 variants in the last few months, in a

previous study, we evaluated the possible impact of the mutations identified in variants B.1.1.7, B.1.351, and P.1 on the performance of the test[1]. Our results showed that the *OnSite* COVID-19 Ag Rapid Test detects these variants. We also mapped the epitopes of both capture and conjugate antibodies used in the *OnSite* COVID-19 Ag Rapid Test. All B.1.1.7, B.1.351, and P.1 do not have mutations within these antibodies' epitopes.

In this study, we analyzed the NP sequences from new variants, B.1.617+, B.1.525, B.1.526, B.1.617.1, C.37, B.1.427/B.1.429, P2, B.1.620, and the newly identified B.1.1.529 variant (Omicron) to see if they have any mutations within the epitopes of the antibodies used in the *OnSite* COVID-19 Ag Rapid Test.

2. Assay description

The *OnSite* COVID-19 Ag Rapid Test is a lateral flow chromatographic immunoassay. The test cassette consists of: 1) a colored conjugate pad containing anti-SARS-CoV-2 antibodies conjugated with colloidal gold (antibody conjugates) and 2) a nitrocellulose membrane strip containing a test line (Ag line) and a control line (C line). The test line is pre-coated with anti-SARS-CoV-2 antibodies, and the C line is pre-coated with control antibodies.

The specimen is collected with a nasopharyngeal or nasal swab, and the SARS-CoV-2 antigen is extracted from the swab with an extraction buffer. Alternatively, samples stored in a viral transport medium (VTM) can be directly tested. When applied to the sample well, the extracted specimen migrates across the test strip by capillary action. SARS-CoV-2 antigen, if present in the extract, binds to the antibody conjugates, and the immunocomplex is then captured on the membrane by the pre-coated anti-SARS-CoV-2 antibody, forming a colored Ag line that indicates a COVID-19 positive test result.

The test contains an internal control (C line), which should exhibit a colored line regardless of color development on the Ag line. If the C line does not develop, the test result is invalid, and the specimen must be retested with a new device.

3. Methods

3.1 GISAID Initiative EpiCoV database

The GISAID Initiative (www.epicov.org) helps monitor emerging hCoV-19 variants that could become relevant due to signs of increased spread (estimated by change in the number of locations) combined with potential effects on receptor or antibody binding as annotated in CoVsurver. Currently, 147 amino acid changes and deletions in the Spike protein occur in at least 10 different geographical locations and were identified in studies to cause antibody escape. As well as an increase in ACE2 binding or increase in Spike protein expression and stability are considered part of combinations or constellations forming potential variants to be monitored.

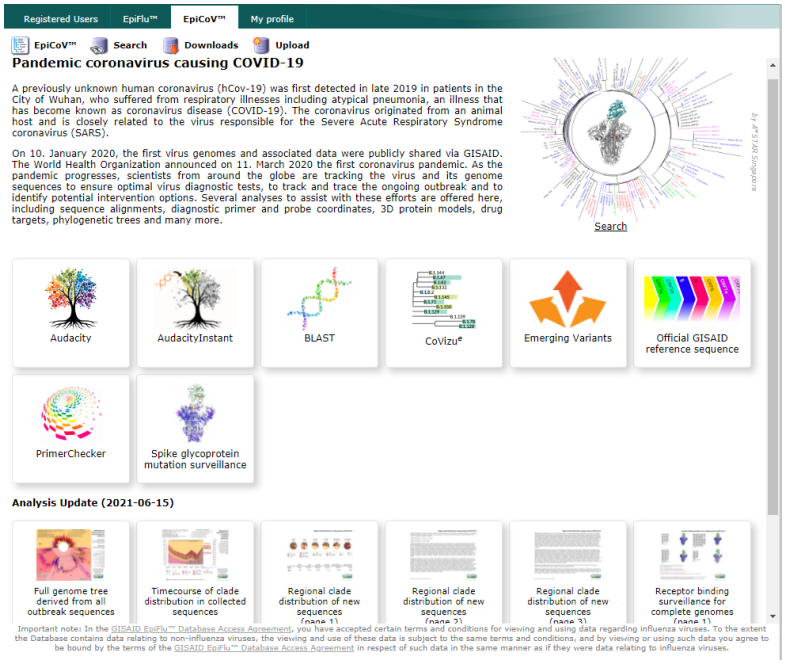


Figure 1. GISAID Initiative EpiCoV database.

The variants for each month (by collection date) are ranked by SxC (S: **Δ#Loc, C: #aachanges**) (Figure 2). This is the product of the change in a number of locations (compared to previous months; akin towards the spread S) and the number of relevant amino acid changes with potential effects contributing to a constellation (C). The variants also are ranked by #Locations.

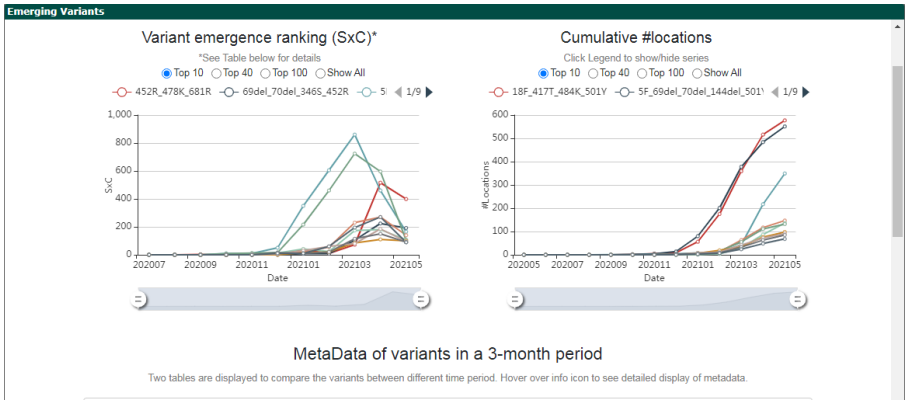
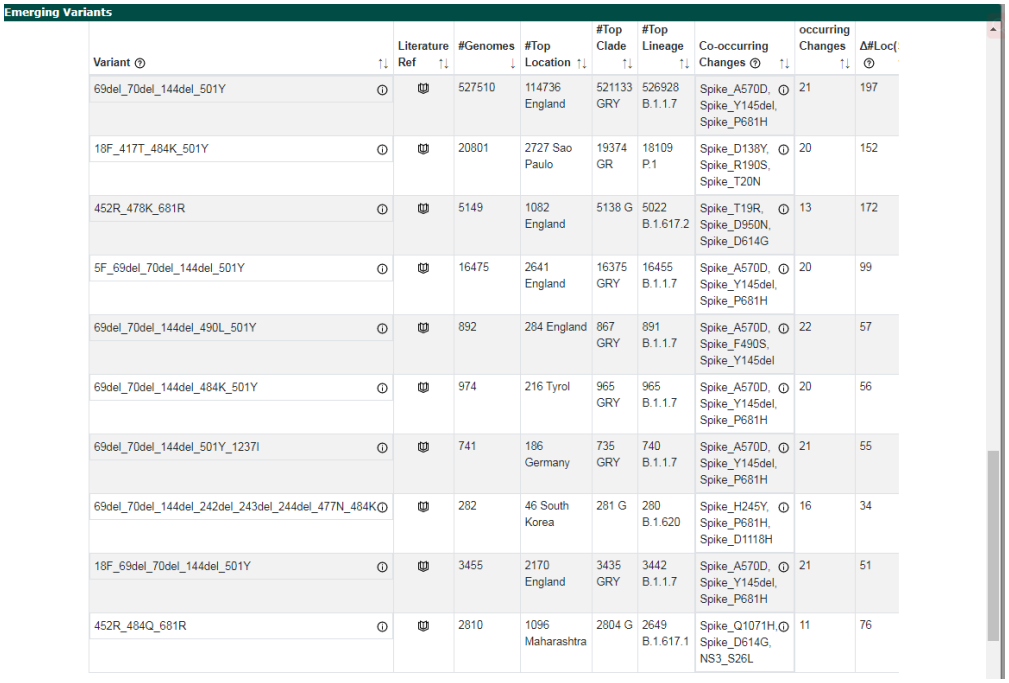


Figure 2. SARS-CoV-2 emerging variants. The variant emergence ranking (SxC) and variant cumulative #locations are shown on left and right, respectively.

The detais of variants in April 2021 are shown in Figure 3. The top 3 variants were **B.1.1.7** 69del_70del_144del_501Y, **P.1** 18F_417T_484K_501Y and **B.1.617.2** 452R_478K_681R. The variants were ranked by SxC value.



Emerging Variants

Variant	Literature Ref	#Genomes	#Top Location	#Top Clade	#Top Lineage	Co-occurring Changes	occurring Changes	Δ#Loc(
69del_70del_144del_501Y		527510	114736 England	521133 GRY	526928 B.1.1.7	Spike_A570D, Spike_Y145del, Spike_P681H	21	197
18F_417T_484K_501Y		20801	2727 Sao Paulo	19374 GR	18109 P.1	Spike_D138Y, Spike_R190S, Spike_T20N	20	152
452R_478K_681R		5149	1082 England	5138 G	5022 B.1.617.2	Spike_T19R, Spike_D950N, Spike_D614G	13	172
5F_69del_70del_144del_501Y		16475	2641 England	16375 GRY	16455 B.1.1.7	Spike_A570D, Spike_Y145del, Spike_P681H	20	99
69del_70del_144del_490L_501Y		892	284 England	867 GRY	891 B.1.1.7	Spike_A570D, Spike_F490S, Spike_Y145del	22	57
69del_70del_144del_484K_501Y		974	216 Tyrol	965 GRY	965 B.1.1.7	Spike_A570D, Spike_Y145del, Spike_P681H	20	56
69del_70del_144del_501Y_1237I		741	186 Germany	735 GRY	740 B.1.1.7	Spike_A570D, Spike_Y145del, Spike_P681H	21	55
69del_70del_144del_242del_243del_244del_477N_484K		282	46 South Korea	281 G	280 B.1.620	Spike_H245Y, Spike_P681H, Spike_D1118H	16	34
18F_69del_70del_144del_501Y		3455	2170 England	3435 GRY	3442 B.1.1.7	Spike_A570D, Spike_Y145del, Spike_P681H	21	51
452R_484Q_681R		2810	1096 Maharashtra	2804 G	2649 B.1.617.1	Spike_Q1071H, Spike_D614G, NS3_S26L	11	76

Figure 3. Top ten emerging variants in April 2021.

3.2 SARS-CoV-2 variants NP sequence analysis

The criterion for selection of SARS-CoV-2 variants sequences included the following information: select variant, with host information, with complete and high coverage, with patient status, and with collection date (Figure 4).

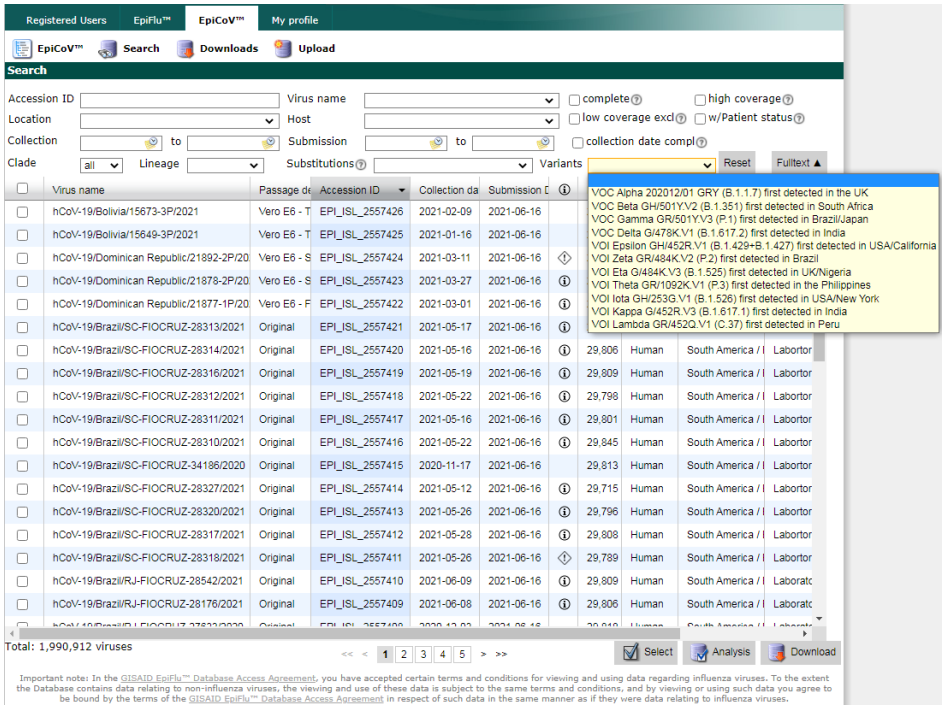


Figure 4. SARS-CoV-2 variants sequences in EpiCoV database.

Table 1 showed variants analyzed in this study. The sequence details are shown in the supplementary table.

Table 1. SARS-CoV-2 variants sequence number.

Variants	Other names	Sequence No.	Note
B.1.1.7	Alpha/UK variant	5492	Reference 1
B.1.351	Beta/South Africa variant	273	Reference 1
P.1	Gamma/Brazil variant	101	Reference 1
B.1.617+	Delta/India variant	541	This study
B.1.525	Eta/Nigeria variant	105	This study
B.1.526	Iota/USA	250	This study
B.1.617.1	Kappa/India	246	This study
C.37	Lambda/Peru	500	This study
B.1.429 + B.1.427	California variant	586	This study
P.2	Brazil	500	This study
B.1.620	None	500	This study
B.1.1.529	Omicron	76	This study

The sequences were aligned with Clustal Omega online tools (Figure 5) at EMBL-EBI (https://www.ebi.ac.uk/Tools/msa/clustalo/). The full-length NP genes (NP Position 28274 to 29533) from aligned sequences were translated to amino acid sequence, and a consensus sequence was generated using BioEdit software. All the NP consensus sequences from variants were aligned with the official reference NP sequence from hCoV-19/Wuhan/WIV04/2019 (WIV04, EPI_ISL_402124).

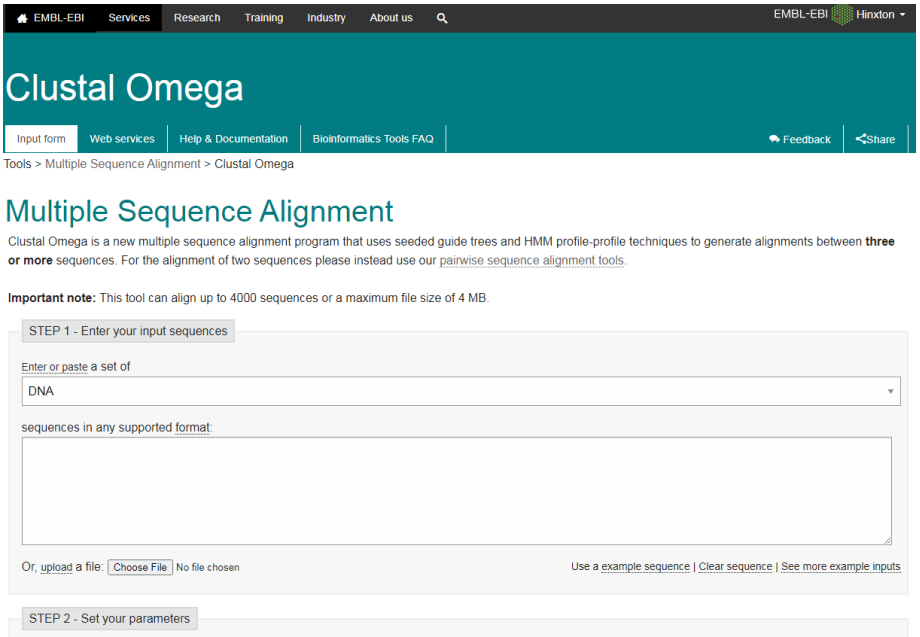


Figure 5. Multiple Sequence Alignment at EMBL-EBI.

4. Results

4.1 Epitope information of antibodies used in *OnSite* COVID-19 Ag Rapid Test

In a previous study, we evaluated our *OnSite* COVID-19 Ag Rapid Test on detecting U.K., S.A., and Brazil SARS-CoV-2 variants[2]. In that study, we mapped antibodies' epitopes by using peptide scanning (Figure 6). The conjugate antibody's epitope is within peptide 10, and the capture antibody's epitope is within peptide 9. Sequence analysis showed that all the mutations from U.K., S.A., and Brazil variants were not in the conjugate or the capture antibodies' epitopes. We also introduced all mutations from three variants to WT NP gene and expressed mutants in mammalian cells. The *OnSite* COVID-19 Ag Rapid Test can still detect these NP mutants derived from three variants. So, as long as the SARS-CoV-2 variant does not have a mutation within conjugate and capture antibodies' epitopes, the *OnSite* COVID-19 Ag Rapid Test should still be able to detect it.

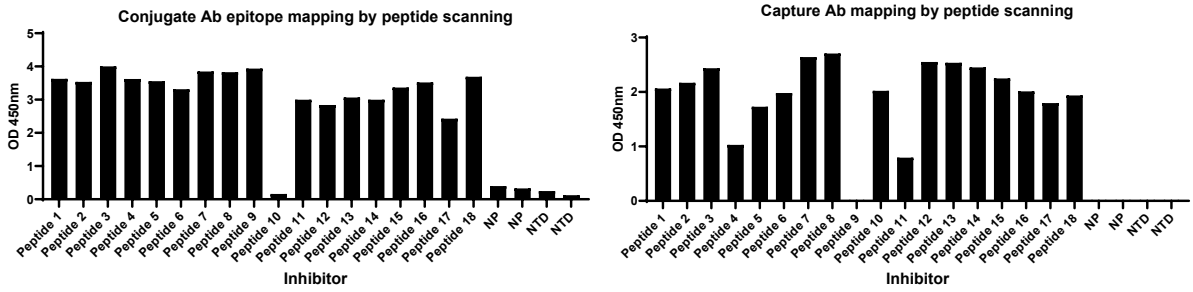


Figure 6. Competition ELISA of conjugate antibody with peptides.

Since there are more and more emerging variants, we developed a strategy to monitor if SARS-CoV-2 variants have mutations within conjugate and capture antibodies' epitopes and if the *OnSite* COVID-19 Ag Rapid Test would still be able to detect these new variants.

Our previous study analyzed the NP sequences from B.1.351, B.1.1.7, and P.1 variants. Here we added sequences from B.1.617+, B.1.525, B.1.526, B.1.617.1, C.37, B.1.427/B.1.429, P2, B.1.620 and B.1.1.529 variants. The NP consensus sequences from each variant were aligned to reference sequence WIV04 and the mutations shown in Figure 7 and Table 2.

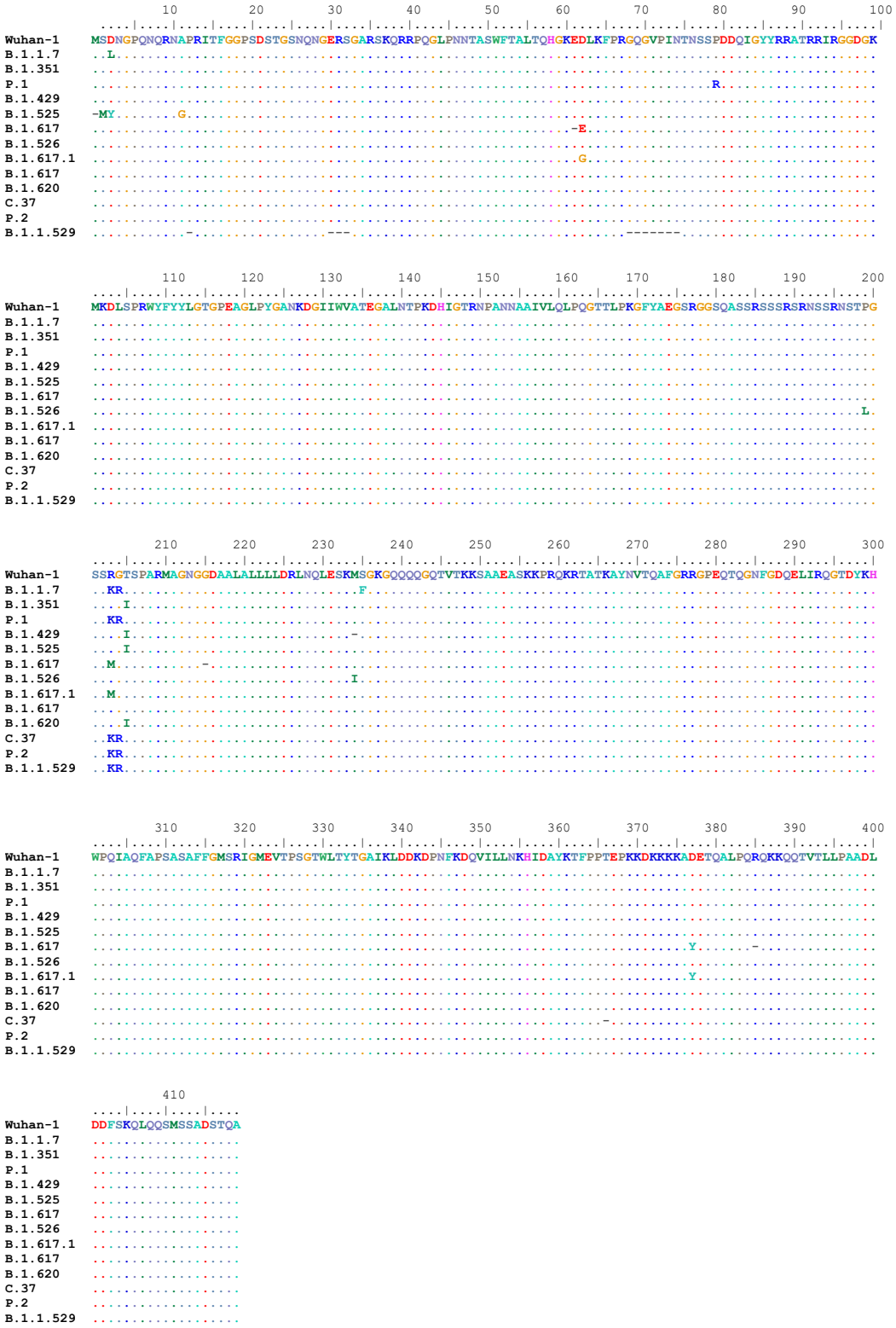


Figure 7. SARS-CoV-2 variants NP sequence alignment.

The mutations from B.1.351, B.1.1.7, and P.1 variants have been described and tested in the previous study[2]. B.1.617.2 variant has 3 mutations (D63E, R203M, and D377Y) and 3 deletions (Δ E62, Δ G214, and Δ R385). B.1.525 variant has S2Y, A12G and T205I mutations and D3 deletion. B.1.526 variant has P199L and M234I mutations. B.1.617.1 has D63G, R203M, and D377Y mutations. C.37 has R203K, G204R, and Δ T366 deletion. B.1.427 and B.1.429 variant has T205I mutation and M249 deletion. P.2 has R203K and G204R mutations. B.1.620 has T205I mutation. B.1.1.529 has R203K, G204R mutations, and multiple deletions (Δ P13, Δ 31ERS33, and Δ69-GQGVPIN-75. **These mutations and deletions do not fall within the *OnSite* COVID-19 Ag Rapid Test conjugate and capture antibodies' epitopes.**

Table 2. SARS-CoV-2 variants NP mutations.

Variants	B.1.1.7	B.1.351	P.1	B.1.617.2	B.1.525	B.1.526	B.1.617.1	C.37	B.1.429 + B.1.427	P.2	B.1.620	B.1.1.529
Other names	Alpha/ UK variant	Beta/ South Africa variant	Gamma/ Brazil variant	Delta/ India variant	Eta/ Nigeria variant	Iota/ USA	Kappa/ India	Lambda / Peru	Californi a variant	Zeta/Br azil	Lithuan ian strain	Omicron
Mutations	D3L R203K G204R S235F	T205I	P80R R203K G204R	ΔE62 D63E R203M ΔG214 D377Y ΔR385	S2Y ΔD3 A12G T205I	P199L M234I	D63G R203M D377Y	R203K G204R ΔT366	T205I ΔM249	R203K G204R	T205I	ΔP13 Δ31ERS33 Δ69GQGVPIN75 R203K G204R

5. Discussion and Conclusions

The B.1.617.2, also known as the Delta variant, was first discovered in India. Descendant of lineage B.1.617, which also includes B.1.617.1, was first discovered in October 2020 and has since spread internationally[3-5]. On 6 May 2021, British scientists declared B.1.617.2 as a "variant of concern," labeling it VOC-21APR-02, after they flagged evidence that it spreads more quickly than the original version of the virus and could spread as quickly as Alpha (B.1.1.7)[6-7]. On 3 June 2021, Public Health England reported that twelve of the 42 deaths from the B.1.617.2 variant in England were among the fully vaccinated and that it was spreading almost twice as fast as the B.1.1.7 variant[8]. Also, on 11 June 2021, Foothills Medical Centre in Calgary, Canada, reported that half of their 22 cases of the B.1.617.2 variant occurred among the fully vaccinated[9]. On 14 June 2021, India detected a mutated variant of B.1.617.2 or Delta variant known as A.Y or 'Delta Plus' variant[10].

The lineage B.1.525 or Eta variant, does not carry the same N501Y mutation found in B.1.1.7, B.1.351, and P.1, but carries the same E484K-mutation as found in the P.1, P.2, and B.1.351 variants, and also carries the same Δ H69/ Δ V70 deletion (a deletion of the amino acids histidine and valine in positions 69 and 70) as found in B.1.1.7[15]. B.1.525 differs from all other variants by having both the E484K-mutation and a new F888L mutation. As of March 5, 2021, it had been detected in 23 countries, including the UK, Denmark, Finland, Norway, Netherlands, Belgium, France, Spain, Nigeria, Ghana, Jordan, Japan, Singapore, Australia, Canada, Germany, Italy, Slovenia, Austria, Malaysia, Switzerland, the Republic of Ireland and the US[15-17].

The variant B.1.526 was first discovered in November 2020 in New York City[18]. As of 11 April 2021, the variant has been detected in at least 48 U.S. states and 18 countries. In a pattern mirroring Epsilon, Iota initially reached relatively high levels in some states, but by May 2021, it was outcompeted by the more transmissible Delta and Alpha[19].

The Kappa variant B.1.617.1[2] is one of the three sub-lineages of lineage B.1.617. It is also known as lineage B.1.617.1, 21B[20], or 21A/S:154K[21] and was first detected in India in December 2020[22]. By the end of March 2021, Kappa accounted for more than half of the submitted sequences from India[23]. On the 1st of April 2021, it was designated a variant under investigation (VUI-21APR-01) by Public Health England[8]. It has the notable mutations L452R, E484Q, P681R[24].

The Lambda variant, also known as lineage C.37, was first detected in Peru in August 2020 and was designated by the WHO as a variant of interest on 14 June 2021[2]. It spread to at least 30 countries[25] around the world, and, as of July 2021, it is unknown whether it is more infectious and resistant to vaccines than other strains[26-27].

The lineage B.1.429, or Epsilon variant, was of particular concern[11-12]. B.1.429 is possibly more transmissible, but further study is necessary to confirm this[12]. CDC has listed B.1.429 and the related B.1.427 as "variants of concern," and cites a preprint for saying that they exhibit a ~20% increase in viral transmissibility, have a "Significant impact on neutralization by some, but not all," therapeutics that have been given Emergency Use Authorization (EUA) by FDA for treatment or prevention of COVID-19, and moderately reduce neutralization by plasma collected by people who have previously been infected by the virus or who have received a vaccine against the virus[13-14]. According to WHO, it has been labeled as Epsilon variant.

Zeta variant or lineage P.2, a sub-lineage of B.1.1.28 like Gamma (P.1), was first detected in circulation in Rio de Janeiro; it harbors the E484K mutation but not the N501Y and K417T mutations[28]. It evolved independently in Rio de Janeiro without being directly related to the Gamma variant from Manaus[29]. Though previously Zeta was labeled a variant of interest, as of July 2021, it is no longer considered as such by the WHO[2].

In March 2021, Lineage B.1.620 was discovered in Lithuania. It was named lineage B.1.620[30], also known as the 'Lithuanian strain.' It is found in Central Africa as well as North America[31]. Apart from Lithuania, other European countries, including France and Belgium, have also found presence of this variant[30]. This lineage has 23 mutations and deletions compared to the reference strain, some of which are unique mutations. The lineage contains an E484K mutation[31-32]. D614G, a mutation present in most circulating strains, is also found in this variant[33]. Other notable mutations include P681H and S477N[31].

The SARS-CoV-2 Omicron variant (B.1.1.529) is a variant of SARS-CoV-2, the virus that causes COVID-19. The variant was first reported to the World Health Organization (WHO) from South Africa on 24 November 2021[34]. On 26 November 2021, the WHO designated it as a variant of concern and named it Omicron. The variant contains an unusually large number of mutations, several of which are novel (also known as autapomorphy), and several of which affect the spike protein used for most available vaccines. This level of variation has led to concerns regarding transmissibility, immune system evasion, and vaccine resistance. As a result, the variant was rapidly designated as "of concern," and several countries introduced travel restrictions to limit or slow its international spread.

Here we have analyzed NP sequences from three new variants, B.1.617.2, B.1.525, B.1.526, B.1.617.1, C.37, B.1.427/B.1.429, P2, B.1.620 and B.1.1.529. All these variants have mutations and deletions within the NP protein. However, none of the variants have mutations within the epitopes of conjugate and capture antibodies used in the *OnSite* COVID-19 Ag Rapid

Test. Therefore, the *OnSite* COVID-19 Ag Rapid Test should still be able to detect these variants without any impact on its performance.

6. References

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