



浙江东方基因生物制品股份有限公司
Zhejiang Orient Gene Biotech Co., LTD

**SARS-COV-2 variants, implications for and monitoring of impact
on Orient Gene Coronavirus Ag Rapid Test Cassette (Swab)
performance**

Updated on 13 Dec., 2021

As a manufacturer for infectious disease detection reagents, during the outbreak of COVID-19 pandemic, Zhejiang Orient Gene Biotech Co., Ltd formed a group of COVID-19 Task Force team with seven scientists to monitor and study the development of novel coronavirus around the world. This team includes four doctoral supervisors and three biomedical engineers with specialties in virology, genetics and immunology. Under the guidance of this scientist team, we have commercialized the clinical application of COVID-19 detection reagents and test cassettes. Orient Gene brand and Healgen brand are of the same quality.

We will continue staying high alert about and monitor as closely as possible for any new Coronavirus variants that may emerge around the world, and take our role and responsibilities:

1. Orient Gene's Responsibility:

We monitor the possible impact of SARS-Covid-2 variants on Lateral Flow Test (LFT) performance and advise the appropriate regulatory authority if there is wet lab or in silico evidence of any known or potential risk of test failure.

2. Share information for end users assurance as details in below:

- the target antigen(s) detected by the assays, such as Nucleocapsid protein, Spike protein or recombinant fragments/ subunits or domains of such antigens used to generate/select for the antibodies and test target molecules used in the LFT assay
- the nature of antibodies and test molecules used for detection/labelling of the antigens, monoclonal/polyclonal antibodies or combinations thereof
- the nature of the antibodies/testing molecules used to capture the antigen at the test line, including monoclonal/polyclonal antibodies or combinations thereof
- the characterized (sequenced) strains of SARS-Covid-2 that are detected by the LFT and LOD for these strains



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The articles, studies, researches, and reports in the inside pages are to be updated monthly.

The Coronavirus Ag Rapid Test Cassette (Swab) (colloidal gold method) manufactured by Zhejiang Orient Gene Biotech Co., Ltd is used for the qualitative detection of nucleocapsid protein of SARS-CoV-2 in samples. This test cassette is based on colloidal gold immunochromatography for the specific detection of novel coronavirus Nucleocapsid (N) protein using a double antibody sandwich method. Two antibodies highly specific to COVID-19 N protein are used in our SARS-CoV-2 antigen assay kit, both of the two monoclonal antibodies are made from CHO cells. One of them (product No. Mab117) is known to bind to 44-175aa region of the N protein, because deleting this region prevents its binding to the recombinant N protein. However, it does not bind with 4 synthesized peptides within the 44-175aa of N protein. Therefore, its epitope is located at 44-175aa, but is likely conformational. 3D modeling has not been done for this antibody, it was used in test line, and is a singular epitope. Another antibody (product No. Mab30) can bind with 74-105aa peptides, thus, it is a linear singular epitope, and was used on the conjugate pad. Together, the pair of the monoclonal antibodies used in this test kit mainly recognize the N protein epitope of 74-105aa (see below highlighted part and Fig.2) .

N protein amino acid sequence(accession No. NC_045512):

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MSDNQPQNQRNAPRITFGGSPDSTGNSQNGERSGARSKQRRPQGLPNNTASWFTALTQHGKED  
LKFRPGQGVPIINTNSSPDDQIGYYRRATRRIRGGDGKMKDLSPRWYFYLLGTGPEAGLPYGANKD  
GIIWVATEGALNTPKDHIGTRNPANNAIIVLQLPQGTTLPKGFYAEGSRGGSQASSRSSSRSRNSS  
RNSTPGSSRGTSPTARMAGNGGDAALALLLDRLNQLESKMSGKGGQQGQTVTKKSAEASKKP  
RQKRTATKAYNVTQAFGRGPEQTQGNFGDQELIRQGTDYKHWPQIAQFAPSASAFFGMSRIGM  
EVTPSGTWLTYTGAIKLDDKDPNFKDQVILLNKHIDAYKTFPPTEPKKDKKKKADETQALPQRQKKQ  
QVTLLPAADLDDFSKQLQQSMSSADSTQA
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Silico Analysis

For in silico monitoring of possible antigen variations in novel coronavirus variants, linear epitopes are analyzed in silico (sequence alignments and structure prediction method(alpha regions-Chou Fasman, antigen index-Jameson Wolf, surface probability-Emini). Alpha, Beta, Gamma, Delta, Lambda, Kappa, Mu, etc. SARS-CoV-2 variants were analyzed, these N protein mutations have no noticeable impact on our antibodies affinity and binding(Fig.1 & Fig.2).3D modeling has not been performed. Wet lab tests have being performed constantly as part of our continuous VOC monitoring activities.



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VOC: Current Variants of Concern and amino acid changes in nucleocapsid, update Dec.10, 2021 by ECDC, WHO and GISAID (Table1) :

WHO label	Lineage+additional mutations	Nucleocapsid protein mutation	Accession No./GISAID ID
Alpha	B.1.1.7	D3L, G204R, R203K, S235F,	EPI_ISL_6141708
Beta	B.1.351	T205I	EPI_ISL_5479908
Gamma	P.1	P80R	EPI_ISL_6121603
Delta	B.1.617.2,AY.1, AY.2, AY.3, AY.33, AY.34	D63G, D377Y, R203M, G215C	EPI_ISL_5794703
Omicron	B.1.1.529 BA.1 B.1.1.529 BA.2	E31del, G204R, P13L, R32del, R203K, S33del	EPI_ISL_6590782 EPI_ISL_7190366

VOI: Variants of Interest (VOI) and amino acid changes in nucleocapsid, update Dec.3, 2021 by ECDC and WHO (Table2) :

WHO label	Lineage+additional mutations	Nucleocapsid protein mutation	Accession No./GISAID ID
Lambda	C.37+C37.1	G204R, G214C, P13L, R203K,T366I	EPI_ISL_6121600
MU	B.1.621/B.1.621.1	T205I,D377Y	EPI_ISL_6175289
Delta+	AY.4.2	D63G, D377Y, G215C, M322I, R203M,	EPI_ISL_5794703

VUI: Variants under monitoring (VUM) and amino acid changes in nucleocapsid, update Dec.3, 2021 by ECDC (Table3)

WHO label	Lineage+additional mutations	Nucleocapsid protein mutation	Accession No./GISAID ID
Eta	B.1.525	A12G,T205I	MZ362451
Kappa	B.1.617.1	D63G, D377Y, R203M,	MZ571142
	B.1.1.318	A208G, G204R, R203K, R209del,	EPI_ISL_1913078
	C.36.3	G204R, G212V, R203K,	EPI_ISL_1509923
	B.1.640	D22Y, E378Q, Q272L, T205I,	EPI_ISL_6781731

The VOC and VOI strains (table1 & table2), including B.1.1.7, B. 1.351(P.1) Japan ex Brazil, B.1.617.2+AY.x, C.37 and MU have main mutations in the region of the S protein. A few mutations are reported in the region of the N protein, which are not in the epitope region of the N protein detected by the pair of antibodies used in this test kit. N protein epitope analysis demonstrated that the amino acid changes of P80R in the



(P.1) Japan ex Brazil variants do not affect the antibody binding affinity. (Fig.1). Wet tests have been performed on some mutated recombinant antigens with slightly higher mutation frequencies (ex. P80R, T205I, R203K, details see the list of tested N antigen with amino acid change). Under the same concentrations of the mutated recombinant antigens and antigens of the wild type N protein, none of the mutations present in **Omicron** variant N protein (P13L, E31del, R32del, S33del, R203K, G204R), including the novel deletion of aa31-33 is in the same region as our antibody binding epitope which is in the following region. (aa44-175, aa74-105) (Fig.2). There is no change in sensitivity of detection with this SARS-CoV-2 Ag Rapid Test Cassette (swab). Accordingly, the detection by this test cassette is not affected by these mutant strains analyzed (Fig.2).

The GISAID database updated 6 cases SARS-CoV-2 sequences collected in Vietnam on JUNE 1st, of which 5 cases belonged to Delta variants with more other mutations, and their N protein mutation sites were D63G+D377Y+R203M. At present, this new recombinant mutated N protein antigen has been prepared and were used to evaluate its influence on SARS-CoV-2 antigen detection. The results demonstrated that the response to the mutant antigen and the wild-type antigen was equivalent at the same concentration, and there was no significant difference in reactivity. The SARS-CoV-2 antigen test kits (Zhejiang Orient Gene Biotech co.,ltd) will not miss the detection of the SARS-CoV-2 variants in Vietnam currently (B.1.617.2).

Wet Lab Testing

Full length recombinant mutated N proteins are used in our wet lab testing for VOC monitoring, including VOC /VOI / VUI SARS-CoV-2 strains, as well as other strains with higher frequency mutation. We use the company's own antigen ncov-ps-Ag6 in wet lab testing. The test unit is pg/ml (Table 4), and the results are correlated with the purity and concentration definition of recombinant proteins.

Reference Sequence ncov-ps-Ag6

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MSDNGPONORNAPRITFGGSPDSTGSONGERSGARSKORRPOGLPNNTAS  
WFTALTOHGKEDLKFPRGOGVPINTNSSPDDOIGYYRRATRRIRGGDGKMKDL  
SPRWYFYLLGTGPEAGLPYGANKDGIWVATGALNTPKDHIGTRNPANNAIIV  
LOLPOGTTLPKGFYAEGSRGGSQASSRSSSRSRNSSRNSTPGSSRGTSARMAG  
NGGDAALALLLLDRLNOLESKMSGKGOOOOGOTVTKKSAAEASKKPROKRTA  
TKAYNVTOAFGRRGPEOTQGNFNGDOELIROGTDYKHWPOIAOAFAPSAFAFFG  
MSRIGMEVTPSGTWTLYTGAIKLDDKDPNFKDOVILLNKHIDAYKTFPPTPEPKK  
KKKKADETQALPQRQKKQQTVTLLPAADLDDFSKQLQQSMSSADSTQA
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CONCLUSIONS:

Based on the above monitoring of SARS-CoV-2 variants and the evaluation of our company's products, the SARS-CoV-2 Ag Rapid Test Cassette (colloidal gold method) manufactured by Zhejiang Orient Gene Biotech co., ltd can be used for reliable detection of existing new coronavirus variants.

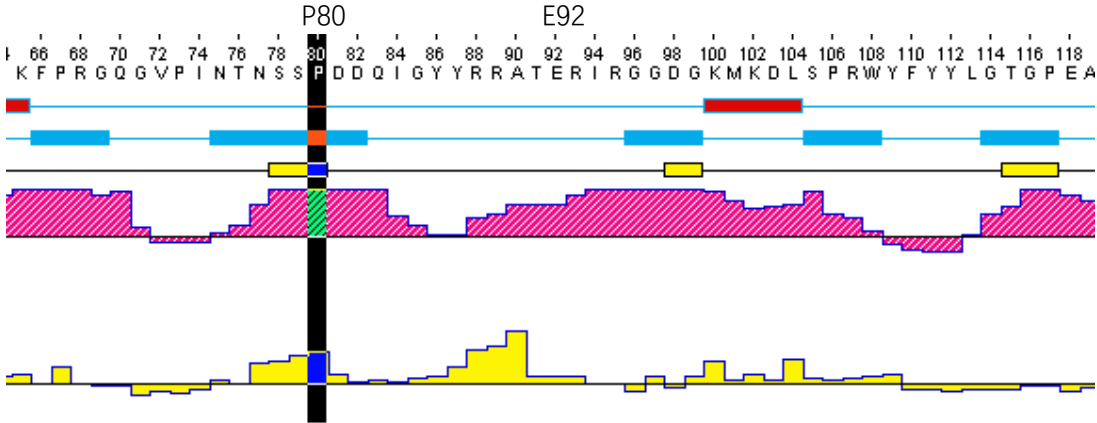


Fig.1 Epitope and binding analysis of N antigen region

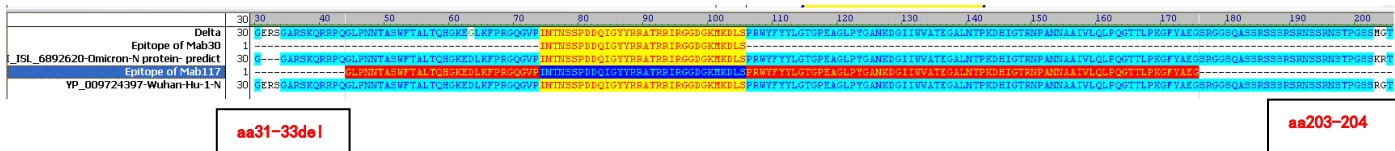


Fig.2 Binding epitope of Mab117&Mab30

List of tested N antigen with amino acid change

- 1、S197L
- 2、RG203KR+I292T
- 3、P13L
- 4、S202N
- 5、S197L+P13L
- 6、A220V
- 7、S194L
- 8、A208G
- 9、RG203KR+K375N
- 10、D3L+S235F+RG203KR
- 11、T205I
- 12、D3L+H145Y+S235F+ RG203KR



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- 13、P80R+ RG203KR
- 14、A119S+M234I+RG203KR
- 15、A12G+T205I
- 16、P67S+P199L
- 17、P67S+P199L+Q389L
- 18、D377Y+R203M
- 19、D3Y+D377Y+R203M
- 20、D3L+G204P+R203K+S235F
- 21、A376T+M234I
- 22、G18S +A119S+A217S++M234I+E367Q
- 23、M234I+T205I
- 24、P365S+ A220V
- 25、S194L+T391I
- 26、Q418H+ RG203KR
- 27、L139F
- 28、S187L+Q418H+RG203KR
- 29、Q9H+ T135I+ P207L+T391I

Table 4 LOD of mutant SARS-CoV-2 antigens

Related strains	mutant antigen	LoD (pg/ml)
Wild	ncov-PS-Ag6	10
	ncov-PS-Ag84	25
	ncov-PS-Ag55	50
	ncov-PS-Ag90	25
	ncov-PS-Ag56	25
	ncov-PS-Ag57	25
	ncov-PS-Ag58	10
	ncov-PS-Ag59	10
	ncov-PS-Ag60	25
	ncov-PS-Ag89	25
Alpha, B. 1. 1. 318, C. 36. 3, Omicron*	ncov-PS-Ag37	25
Beta, Omicron*, Mu	ncov-PS-Ag40	25
	ncov-PS-Ag61	50
Gamma	ncov-PS-Ag42	10
	ncov-PS-Ag86	50
Eta	ncov-PS-Ag54	10
	ncov-PS-Ag64	25
	ncov-PS-Ag65	10



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	ncov-PS-Ag66	25
Kappa	ncov-PS-Ag44	25
	ncov-PS-Ag67	25
	ncov-PS-Ag68	25
	ncov-PS-Ag46	25
	ncov-PS-Ag69	50
	ncov-PS-Ag70	25
	ncov-PS-Ag71	25
	ncov-PS-Ag72	50
	ncov-PS-Ag78	50
	ncov-PS-Ag82	50
	ncov-PS-Ag73	10
	ncov-PS-Ag74	10
	ncov-PS-Ag83	10
	ncov-PS-Ag75	50
	ncov-PS-Ag52	25
Delta, AY. 4. 2	ncov-PS-Ag76	50
	ncov-PS-Ag53	25
	ncov-PS-Ag77	50
	ncov-PS-Ag79	100
	ncov-PS-Ag51	25
	ncov-PS-Ag88	25
	ncov-PS-Ag80	25
	ncov-PS-Ag81	25
	ncov-PS-Ag87	25
	ncov-PS-Ag91	25
	ncov-PS-Ag92	25
	ncov-PS-Ag93	25
	ncov-PS-Ag94	25
Omicron	ncov-PS-Ag150	25
Lambda	ncov-PS-Ag105	25
B. 1. 640	ncov-PS-Ag151	50
	ncov-PS-Ag98	10
	ncov-PS-Ag99	25

*R203K, G204R