

AzureSeq Detection of the Omicron (B.1.1.529) Variant

Date:

Jan. 25, 2022

Scope:

Evaluation of the AzureSeq components to detect and amplify RNA template derived from the SARS-CoV-2 Omicron variant (B.1.1.529).

Conclusion:

An initial *in silico* analysis of the variant shows a mutation P13L that overlaps the 3rd 5' base of the N1 probe. The predicted T_m change in the probe is a reduction from 72.8°C to 67.0°C with the mismatch (using updated chemical conditions). Since the annealing/extension stage of the PCR thermal cycling protocol is performed at 60°C, the mismatch was not expected to result in any reduction in performance of the N1 probe (FAM). Additionally, inspection of the three sub-lineages of Omicron; specifically, BA.1, BA.2, and BA.3 showed that they share the same mutation (P13L) and are predicted to be detected with the AzureSeq assay.

To confirm the results of the *in silico* analysis, RT-qPCR was performed using the AzureSeq kit following the standard IFU to amplify synthetic RNA (Twist Bioscience, Inc.) from the original alpha strain (WT), as well as UK/SA (B.1.1.7), Delta (B.1.627.2), and Omicron variant (B.1.1.529) RNA. As predicted by the *in silico* analysis, AzureSeq reagents successfully amplify N1 and N2 targets against all tested RNA variants. N1 performance against the Omicron template, which contains a single mismatch in the N1 probe, was not affected and performed equally to the other variants. To note, these tests were performed with variant synthetic RNA diluted with sterile water and could not be tested across all possible Viral Transport Media.

In summary the AzureSeq reagents perform equally against all major strains of SARS-CoV-2 RNA seen in global circulation to date. Therefore, it is expected that AzureSeq reagents will continue to amplify and detect SARS-CoV-2 Omicron variant RNA from individuals who have detectable levels of SARS-CoV-2 present in appropriately collected sample types.

Materials and Methods:

1. AzureSeq kit
2. Twist Bioscience, Inc Synthetic RNA Templates:
 - a. WT (Control 2)
 - b. B.1.1.7 UK/SA (Control 15)
 - c. B.1.627.2 Delta (Control 23)
 - d. B.1.1.529 Omicron (Control 48)

RNA was diluted in nuclease free water and added to the RT-qPCR reactions containing 1X AzureSeq reagents.

Table 1: Omicron Sub-lineage Mutational Landscape:

Characteristic mutations of BA.1		Characteristic mutations of BA.2		Characteristic mutations of BA.3	
gene	amino acid	gene	amino acid	gene	amino acid
ORF1a	K856R	ORF1a	S135R	ORF1a	S135R
ORF1a	S2083I	ORF1a	T842I	ORF1a	G1307S
ORF1a	del2084/2084	ORF1a	G1307S	ORF1a	T3090I
ORF1a	A2710T	ORF1a	L3027F	ORF1a	T3255I
ORF1a	T3255I	ORF1a	T3090I	ORF1a	P3395H
ORF1a	P3395H	ORF1a	L3201F	ORF1a	A3657V
ORF1a	del3674/3676	ORF1a	T3255I	ORF1a	del3675/3677
ORF1a	I3758V	ORF1a	P3395H	ORF1b	P314L
ORF1b	P314L	ORF1a	del3675/3677	ORF1b	I1566V
ORF1b	I1566V	ORF1b	P314L	S	A67V
S	A67V	ORF1b	R1315C	S	del69/70
S	del69/70	ORF1b	I1566V	S	T95I
S	T95I	ORF1b	T2163I	S	G142D
S	G142D	S	T19I	S	del143/145
S	del143/145	S	L24S	S	N211I
S	N211I	S	del25/27	S	del212/212
S	del212/212	S	G142D	S	G339D
S	G339D	S	V213G	S	S477N
S	S371L	S	G339D	S	T478K
S	S373P	S	S371F	S	E484A
S	S375F	S	S373P	S	Q493R

S	S477N	S	S375F	S	Q498R
S	T478K	S	T376A	S	N501Y
S	E484A	S	D405N	S	Y505H
S	Q493R	S	R408S	S	D614G
S	G496S	S	K417N	S	H655Y
S	Q498R	S	N440K	S	N679K
S	N501Y	S	S477N	S	P681H
S	Y505H	S	T478K	S	N764K
S	T547K	S	E484A	S	D796Y
S	D614G	S	Q493R	S	Q954H
S	H655Y	S	Q498R	S	N969K
S	N679K	S	N501Y	E	T9I
S	P681H	S	Y505H	M	Q19E
S	N764K	S	D614G	M	A63T
S	D796Y	S	H655Y	ORF8	S84L
S	N856K	S	N679K	N	P13L
S	Q954H	S	P681H	N	del31/33
S	N969K	S	N764K	N	R203K
S	L981F	S	D796Y	N	G204R
E	T9I	S	Q954H	N	S413R
M	D3G	S	N969K		
M	Q19E	ORF3a	T223I		
M	A63T	E	T9I		
ORF8	S84L	M	A63T		
N	P13L	ORF6	D61L		
N	del31/33	ORF8	S84L		
N	R203K	N	P13L		
N	G204R	N	del31/33		
		N	R203K		
		N	G204R		
		N	S413R		

Table 1 summarizes all mutations found in each sub-lineage of the Omicron variant B.1.1.529. The mutations within each block correspond to the region targeted by N1 in the AzureSeq assay. The bold test highlights the mutation that is found in the 3rd 5' base of the N1 probe.

Table 2: Sensitivity of the AzureSeq assay against all major SARS-CoV-2 RNA variants, synthetic RNA

Template	Copies/Reaction	240 copies		120 copies		60 copies		30 copies	
	Assay	N1	N2	N1	N2	N1	N2	N1	N2
WT RNA	Positive/Total	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
	Mean Ct	30.67	31.01	31.73	31.97	32.60	32.95	34.28	34.87
B.1.1.7 RNA (UK/SA)	Positive/Total	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
	Mean Ct	30.80	31.27	31.73	32.18	32.72	33.28	34.07	34.75
B.1.617.2 (Delta)	Positive/Total	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
	Mean Ct	30.26	30.87	31.61	32.10	33.28	33.70	33.93	34.34
B.1.1.529 (Omicron)	Positive/Total	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
	Mean Ct	29.19	29.04	30.03	29.97	26.94	31.26	32.68	32.55
	Copies/Reaction	15 copies		7.5 copies		3.75 copies		1.9 copies	
	Assay	N1	N2	N1	N2	N1	N2	N1	N2
WT RNA	Positive/Total	5/5	5/5	4/5	5/5	2/5	3/5	4/5	3/5
	Mean Ct	34.99	35.34	35.24	35.55	37.17	37.30	36.64	37.54
B.1.1.7 RNA (UK/SA)	Positive/Total	5/5	5/5	4/5	4/5	4/5	4/5	1/5	1/5
	Mean Ct	35.20	35.27	36.00	36.25	36.29	36.58	36.17	37.40
B.1.617.2 (Delta)	Positive/Total	5/5	5/5	4/5	3/5	3/5	3/5	1/5	1/5
	Mean Ct	35.11	35.59	36.37	38.09	36.59	37.25	29.64	37.50
B.1.1.529 (Omicron)	Positive/Total	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
	Mean Ct	33.82	33.75	34.15	34.15	35.41	35.25	36.39	36.33

Figure 1. Example of RT-qPCR Amplification Plots

