

Variant Testing Update: SNPsig[®] SARS-CoV-2 EscapePLEX[™]

The SNPsig SARS-CoV-2 EscapePLEX[™] kit is a qPCR test for the detection and differentiation of SARS-CoV-2 variants: Delta, Delta with K417N and Omicron. It will also detect Beta and Gamma, although circulation of these variants is currently (May 2022) extremely low.

The kit uses two SARS-CoV-2 gene targets (ORF1ab and M gene) to confirm infection, whilst screening for the following clinically significant mutations associated with variants of concern (VOCs):

- E484K
- K417N
- K417T
- P681R

In the presence of the Omicron variant, EscapePLEX[™] will produce a K417N-positive, E484K-negative, K417T-negative, and P681R-negative mutation profile. This mutation profile can be used to distinguish Omicron from other variants of concern (VOCs). Please refer to the technical bulletin of MM176¹ for details of the mutation profiles of other variants.

As previously described, the kit will detect both the BA.1 and BA.2 sublineages of Omicron without relying on the S-Gene Target Failure (SGTF) screening strategy used by some labs at the beginning of the Omicron surge. This SGTF strategy distinguishes BA.1, which has the S gene 69/70 deletion (SGTF), from Delta which does not have the deletion and will therefore still be S-Gene Target Positive (SGTP). Since BA.2 is also SGTP, this screening strategy risked missing Omicron BA.2 identification soon after the emergence of this subvariant.

Variant	Δ69/70 Status	S-Gene Target Status
Omicron BA.1	Deletion	SGTF
Omicron BA.2	No deletion	SGTP
Delta	No deletion	SGTP

Table 1. Table showing the S-Gene Target status of Delta, Omicron BA.1 and Omicron BA.2. SGTF, S-Gene Target Failure; SGTP, S-Gene Target Positive.

Spread of the BA.2 Sublineage of Omicron and the Emergence of Further Sublineages

By the 1st January 2022, BA.2 accounted for 5% of S-gene target positive (SGTP) cases identified in England, UK, therefore SGTF was acknowledged as no longer adequate in tracking the spread of Omicron². Since then, BA.2 has continued to spread, prompting it to be classified as a Variant Under Investigation by the UK Health Security Agency on the 19th January (VUI-22JAN-01). The World Health Organisation, however, decided that BA.2 should still be considered with the Omicron VOC. By 31st January BA.2 accounted for 97% of SGTP and was then considered a reasonable proxy for the presence of BA.2³. Further analysis in March identified a small number of BA.2 sequences which showed SGTF, revealing that this variant identification strategy was still problematic.

On 6th April 2022, two further Omicron sublineages were identified, BA.4 and BA.5, which share mutations with BA.2 plus additional ones but in contrast, do have the 69/70 deletion. Case numbers of these newly identified sublineages are still very low in the UK⁴, but are on the rise in South Africa⁵. This mirrors the previously rapidly changing situation with the Delta variant which also diverged into a number of sublineages. Please note that BA.3 was identified in November 2021 and continues to circulate but in low numbers.

SNPsig® VariPLEX™ (COVID-19)

The SNPsig VariPLEX (COVID-19) kit, which was launched in April 2021, was designed to detect the Alpha, Beta and Gamma Variants of Concern (VOC) and the Epsilon Variant under Investigation (VUI) as well as the mutations of biological interest N501Y and E484K. It is a reflex test which works by allelic discrimination, each assay having probes for both the wild type (as defined by the SARS-CoV-2 reference sequence) and mutant (MUT) in the same reaction, labelled with different fluorophores and sharing a set of primers. Therefore for each assay, if the mutant is not present, a signal should be obtained from the wild type (WT) probe in the qPCR reaction. Conversely, if the mutant sequence was present in a sample, the mutant probe would give a signal.

During the emergence and subsequent dominance of the Delta variant, the kit appeared to be of less utility in the identification of variants. However, with the spread of Omicron BA.1 and BA.2 sublineages, the VariPLEX kit can once again be used as an alternative to expensive and time-consuming Next Generation Sequencing of SARS-CoV-2 positive samples and avoids reliance on a sample's SGTF or SGTP status. Specifically, the BA.1 sublineage has mutations in the E484K and Alpha identifier probe targets which cause complete target failure. Rather than being an issue, this gives BA.1 the following unique mutation profile with the VariPLEX kit: no detection in Tube 1, Beta identifier MUT in tube 2, N501Y MUT and Epsilon identifier WT in Tube 3. See *Table 2 and Appendix for details*.

Similarly, BA.2 with mutations in both versions of the E484K and Epsilon identifier probes also suffer target failure. This means that VariPLEX yields unique profiles for BA.1 and BA.2 and these can therefore be identified and distinguished from Delta and any other variants which may still be circulating. The patterns of assay failure of these two sublineages obtained with the VariPLEX kit is highlighted in Table 2 with full details in the Appendix. Since the numbers of BA.4 and BA.5 are still very low, it is a reasonable assumption that the second profile in Table 2 will be from BA.2. This could change with the course of time if these sublineages spread further.

Lineage	VariPLEX Assay	
	Alpha Identifier Tube 1	Epsilon Identifier Tube 3
BA.1*	No amplification	ROX amplification (WT)
BA.2*/BA.4/BA.5	ROX amplification (WT)	No amplification

Table 2. The differences in mutation profiles of BA.1 and BA.2/BA.4/BA.5 using the VariPLEX kit. No amplification will be obtained due to mutations in both wild type (WT) and mutant probe targets. *In silico prediction confirmed by laboratory testing.

Recombinants

Recently there have been reports of recombinants between Omicron and Delta, dubbed Deltacron by the media, namely XD and XF, along with a BA.1 and BA.2 recombinant named XE. The numbers of these are relatively small but are being monitored by agencies such as the UKHSA⁶. For details of the mutation profiles of these recombinants please contact Tech Support.

References

1. Novacyt Group. Variant Testing Update: SNPsig® SARS-CoV-2 EscapePLEX™ Technical Bulletin MM191.
2. UK Health Security Agency. SARS-CoV-2 Variants of Concern and Variants Under Investigation in England: Technical Briefing 34. UKHSA publications gateway number GOV-10924; 14 January 2022.
3. UK Health Security Agency. SARS-CoV-2 Variants of Concern and Variants Under Investigation in England: Technical Briefing 37. UKHSA publications gateway number GOV-11462; 1 February 2022.
4. UK Health Security Agency. SARS-CoV-2 Variants of Concern and Variants Under Investigation in England: Technical Briefing 40. UKHSA publications gateway number GOV-11916; 8 April 2022.
5. World Health Organisation COVID-19 Weekly Epidemiological Update Edition 90, published 4 May 2022.
6. UK Health Security Agency. SARS-CoV-2 Variants of Concern and Variants Under Investigation in England: Technical Briefing 39. UKHSA publications gateway number GOV-11753; 25 March 2022.

Appendix

Mutation Profiles for the Dominant Variants from January to May 2022. with the SNPsig® VariPLEX™ (COVID-19) Kit

Putative Delta	FAM	HEX	ROX	Cy5
Tube 1	+	-	+	-
Tube 2	+	-	-	+/-
Tube 3	-	-	+	-

Putative Delta with K417N	FAM	HEX	ROX	Cy5
Tube 1	+	-	+	-
Tube 2	-	+	-	+/-
Tube 3	+	-	+	-

Putative Omicron BA.1	FAM	HEX	ROX	Cy5
Tube 1	-	-	-	-
Tube 2	-	+	-	+/-
Tube 3	-	+	+	-

Putative Omicron BA.2*	FAM	HEX	ROX	Cy5
Tube 1	-	-	+	-
Tube 2	-	+	-	+/-
Tube 3	-	+	-	-

* <1% of sequences were designated BA.3 BA.4 or BA.5 out of all sequences uploaded to GISAID which were collected between 1 April and 6 May 2022 and identified as a particular variant. If the mutation profile obtained does not match those presented here, it is advisable to sequence the sample for definitive variant identification.

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