# Laboratory detection of mutations/variants of concern (VOC)

**Version 1.1 6.4.2021**

| Version | Date | Changes from previous version |
|---|---|---|
| 1.1 | 6.4.2021 | Information on the Eurofins ViroBOAR screening assay included<br>Turnaround times updated |

Following initial confirmation that SARS-CoV-2 RNA is detected using PCR assay, specimens may be further characterised using a number of **variant screening assays**

**The ThermoFisher TaqPath assay** is a PCR assay that detects three distinct SARS-CoV-2 targets: orf1-ab, N gene, and S gene. Due to a deletion (at position 69-70) in the Spike protein of the UK variant (lineage B.1.1.7) the TaqPath assay S gene component yields a Not Detected result when testing the UK variant (but the two other targets are Detected). This is referred to as S gene target failure (SGTF) or 'S dropout'.

➢ If S drop out/S gene target failure is seen, then in the Irish setting, this is most probably the UK variant. Of note, SGTF has been reported in other (non-B.1.1.7) lineages, including B.1.525

➢ If all three targets are detected in the TaqPath assay, then the specimen does not contain B.1.1.7.

**The 501 allele-specific PCR** identifies the N501Y amino acid (AA) change in the spike protein: this AA change (or mutation) is present in B.1.1.7, B.1.351, and P.1

➢ If the N501Y AA change is not detected, then no further testing is required. The virus can be reported as wild-type SARS-CoV-2.

➢ If the N501Y is present, then further testing is required to distinguish between the three variants.

**The Eurofins ViroBOAR assay** combines SNP detection assays to identify the N501Y and A570D AA changes, both of which are present in B.1.1.7, but only N501Y is present in B.1.351 and P.1

➢ If neither AA change is detected, then no further testing is required. The virus can be reported as wild-type SARS-CoV-2.

➢ If both AA changes are detected, then the virus can be reported as B.1.1.7.

➢ If any other combination of 501Y and A570D is detected, the virus can be provisionally considered as a variant of concern, with further testing required to distinguish between the variants.

**The 484 allele-specific PCR** identifies the E484K amino acid (AA) change in the spike protein: this AA change (or mutation) is present in B.1.351 and P.1, but NOT in B.1.1.7.

➢ If the E484K AA change is not detected, the virus can be reported as wild-type SARS-CoV-2, or B.1.1.7, depending on the other results available.

➢ If the E484K AA change is detected, the virus can be provisionally considered as a variant of concern, with further testing required to distinguish between the variants.

Samples provisionally characterised as VOC samples require further testing. They may go for Sanger sequencing of the S gene alone (or a portion thereof) or for whole genome sequencing (WGS); either of these sequencing methods can identify the B.1.351 or P.1 variants.

**Testing Timescales**

The allele-specific PCRs, TaqPath, and ViroBOAR assays can be completed within 48-72 hours, depending on when samples arrive at the laboratory; these tests are undertaken when the NVRL receives samples of interest and are currently scheduled to be performed twice weekly. Sanger sequencing of the relevant portion of the S gene takes approximately 36-48 hours and is done on an "as required" basis. Whole Genome Sequencing takes 4-5 days and is currently being done weekly.

Specimens of particular concern or interest should be brought to the attention of the laboratory in advance, and will be prioritised insofar as is possible.