UK Health Security Agency

# Fast-track SARS-CoV-2 Variant and Mutation Profiling for Public Health Action in the United Kingdom Using RT-PCR Based Genotyping Assays

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## Introduction

- Whole genome sequencing (WGS) is the reference standard for identifying and monitoring SARS-CoV-2 variants and mutations.
- The TaqMan RT-PCR genotyping assay (GA) detects a panel of four mutations indicative of high-priority variants for positive SARS-CoV-2 samples with a Ct≤30. GAs offer shorter turn-around-time (12-24h after initial PCR result) compared to WGS and increase variant diagnostic test coverage.
- A rule-based decision algorithm (RBDA) was developed to optimize assignment of “Variant and mutation Profiles” (VAMPs) using the GA mutation panel, plus where available, “S-gene target failure” (SGTF) status for cases initially detected by the TaqPath PCR assay.
- The aim of this study was to evaluate the performance of VAMPs assigned by the GA RBDA compared to VAMPS assigned by WGS.

## Methods

- A dataset of GA mutation profiles was extracted from the PHE Second Generation Surveillance System and paired with WGS-derived VAM Profiles for specimens from 1 March to 11 May 2021.
- The GA mutation profile included E484K, K417N, K417T, N501Y GA mutations, plus SGTF status (where available).
- The RBDA (Figure 1) was applied to assign GA-based VAMPs for Alpha (B.1.1.7), Beta (B.1.351), and Gamma (P.1) variants, plus “Undetermined+E484K” (for samples containing the E484K Mutation of Concern).
- The GA- and WGS-based VAMPs for each sample were then compared and sensitivity, specificity and Positive Predictive Value (PPV) calculated for each variant.
- For the Alpha variant, a sensitivity analysis was conducted regarding the inclusion of SGTF status using a later extract of data extracted on 27/05/2021.

| Variant (Lineage) | Mutation profile N501Y | E484K | K417N | K417T | SGTF | Assignment order | Additional rules |
|---|---|---|---|---|---|---|---|
| Beta (B.1.351) | | | | | | 1st | |
| Gamma (P.1) | | | | | | 2nd | |
| Undetermined+E484K | | | | | | 3rd | |
| Alpha (B.1.1.7) | | | | | | 4th | Mutation present in N501Y or SGTF |
| Undetermined | All other results | | | | | 5th | |

| Requirement for mutation: | Confirmed present | Not confirmed absent | Present or absent | Not confirmed present |
|---|---|---|---|---|

***Figure 1. Rules-based decision algorithm (RBDA) first used for distinguishing SARS-CoV-2 Variant and Mutation Profiles of public health importance circulating in the United Kingdom. 'Undetermined' refers to samples with an assay mutation profile not used to call a current GA variant VAMP. 'Undetermined+E484K' refers to Undetermined samples containing the E484K mutation of concern, for which public health action is also required.***

## Results

- Paired results were available for 14,766 specimens. SGTF status was available for 71.6%.
- Using the RBDA, GA were able to accurately assign a variant for 86.4% of all specimens (n=12,761), of which 86.1% were Alpha (n=12,761), 0.5% were Beta (n=72) and 0.1% were Gamma (n=18).
- The sensitivity of the RA was 99.1% for Alpha, 98.6% for Beta and 85.7% for Gamma.
- The specificity of the RA was 97.6% for Alpha and 100% for both Beta and Gamma variants.
- The PPV of the RA was 99.6% for Alpha, 100% for Beta and 90% for Gamma variants.
- 17,141 paired samples were used for the SGTF sensitivity analysis for Alpha, 70.7% of which had an SGTF status (n=12,127). The results for samples without SGTF status had reduced sensitivity (93.9%) and specificity (98.3%) compared to those where it was available. PPV remained unchanged (99.6%).

| GA for B.1.1.7 (Alpha) profiling | Without SGTF | With SGTF |
|---|---|---|
| Sensitivity | 93.9% | 99.5% |
| Specificity | 98.3% | 98.5% |
| Positive Predictive Value (PPV) | 99.6% | 99.6% |

***Figure 3. Comparison of GA RBDA sensitivity, specificity and PPV for Alpha variant with and without SGTF status***

***Figure 2. Performance of GA for variant detection compared to Whole Genome Sequencing (for variants detectable by GA during March-May 2021)***

| | | Whole Genome Sequencing | | |
|---|---|---|---|---|
| | | B.1.1.7 (Alpha) | Other | Total |
| Genotyping Assays | B.1.1.7 (Alpha) | 12,671 | 48 | 12,719 |
| | Other | 116 | 1,931 | 2047 |
| | Total | 12,787 | 1,979 | 14,766 |
| Sensitivity | | | 99.1% | |
| Specificity | | | 97.6% | |
| Positive Predictive Value (PPV) | | | 99.6% | |

| | | Whole Genome Sequencing | | |
|---|---|---|---|---|
| | | B.1.351 (Beta) | Other | Total |
| Genotyping Assays | B.1.351 (Beta) | 72 | 0 | 72 |
| | Other | 1 | 14,693 | 14,694 |
| | Total | 73 | 14,693 | 14,766 |
| Sensitivity | | | 98.6% | |
| Specificity | | | 100% | |
| Positive Predictive Value (PPV) | | | 100% | |

| | | Whole Genome Sequencing | | |
|---|---|---|---|---|
| | | P.1 (Gamma) | Other | Total |
| Genotyping Assays | P.1 (Gamma) | 18 | 2 | 20 |
| | Other | 3 | 14,743 | 14,746 |
| | Total | 21 | 14,745 | 14,766 |
| Sensitivity | | | 85.7% | |
| Specificity | | | 100% | |
| Positive Predictive Value (PPV) | | | 90% | |

## Conclusions

- **Genotyping assays can be highly specific and sensitive tests for detection of SARS-CoV-2 variants.**
- **Including SGTF status from primary RT-PCR diagnosis improves the specificity and sensitivity of genotyping variant detection.**
- **While genotyping assays cannot replace WGS for identification of new variants, the rapid diagnosis and increased variant diagnostic coverage they offer can aid national efforts to reduce transmission of SARS-CoV-2 variants.**