



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

Requirement for mutation: Confirmed present (Present), Not confirmed absent (Absent), Present or absent (Present), Not confirmed present (Absent)

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	2,047
	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.