SARS-CoV-2 Variant Determination Using PCR-based Genotyping Assays and Whole Genome Sequencing

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Introduction

SARS-CoV-2 variants of concern (VOC) pose an increased risk to public health due to higher transmissibility and/or immune escape. Whole genome sequencing (WGS) is the conventional method for variant determination, however its implementation in surveillance is limited as it is resource-intensive, and the relatively long time-to-results can impact timely public health action. PCR-based genotyping assays present an alternative surveillance tool for known VOC with faster results. In this study we assessed the performance of a custom TaqMan SARS-CoV-2 mutation panel* compared to WGS for identification of 4 VOC (Alpha, Beta, Gamma, and Delta) circulating in Europe in 2021.

Methods

331 randomly selected SARS-CoV-2-positive samples collected during routine PCR screening (Ct<32) between May and July 2021 in two regions in the Netherlands were analyzed using 10 selected assays of the TaqMan SARS-CoV-2 mutation panel* covering the 4 VOC: N501Y, E484K, K417N, K417T, del69_70, P681H, delY144, A701V, P681R and L452R. VOC lineage was determined based on the detected mutation profile (Figure1). In parallel, all samples underwent WGS with the Ion AmpliSeq SARS-CoV-2 research panel* on the Ion S5 instrument.

Results

Of 331 samples the WGS identified 62.5% Alpha (N=207); 36.6% Delta (N=121); 0.6% Beta (N=2) and N=1 of the C.36.3.1 variant (Table 1). Matching results were obtained using the TaqMan SARS-CoV-2 mutation panel assays* in 330 out of 331 samples, demonstrating accuracy of genotyping assays in VOC detection. Sample with the C.36.3.1 could not be assigned to a specific lineage based on the genotyping approach.

Variant of Concern	Whole Genome Sequencing	TaqMan Mutation Panel
Alpha	207	207
Delta	121	121
Beta	2	2
C.36.3.1	1	0
Total	331	330

Table 1: SARS-CoV-2 Variants of Concern identified using whole genome sequencing and TaqMan SARS-CoV-2 mutation panel

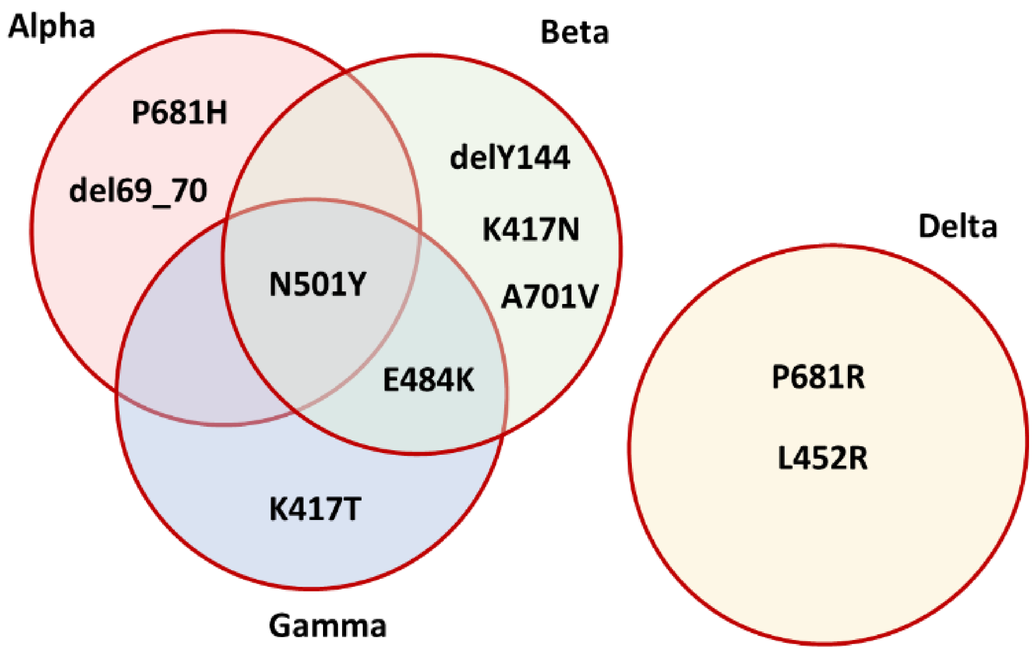

Figure 1: TaqMan SARS-CoV-2 mutation panel for VOC detection

Temporal analysis of our dataset illustrates the rapid increase in Delta variant prevalence from 0% in June 2021 to 100% of SARS-CoV-2 positive cases by July 2021 (Figure 2).

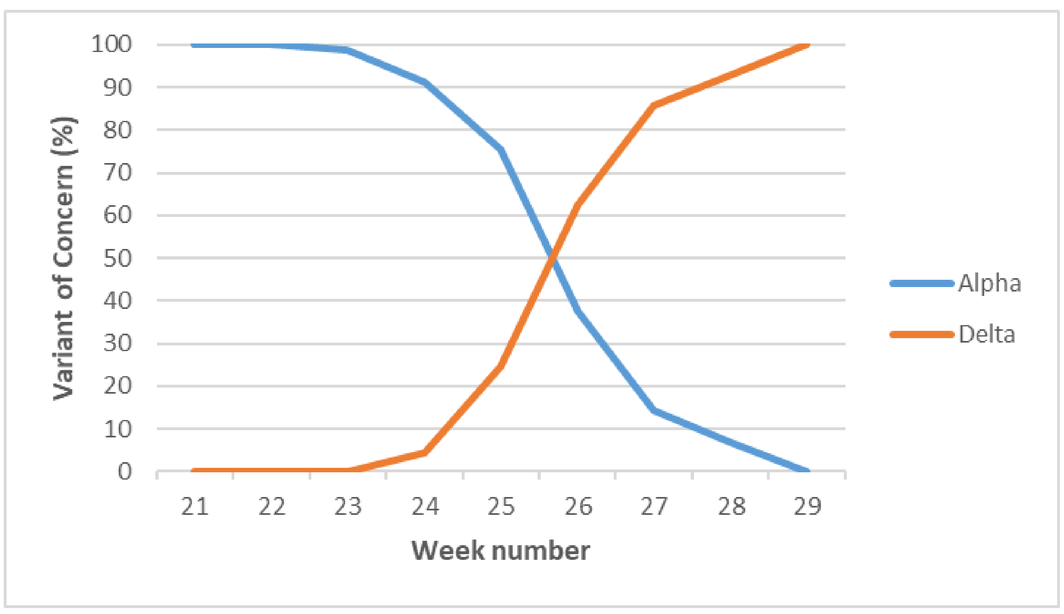

Figure 2: SARS-CoV-2 Variants of Concern detected in the Netherlands, May-July 2021

Conclusion

Genotyping assays enable fast detection of SARS-CoV-2 VOC in a highly accurate manner. They are an easily implementable tool complementary to WGS which can enable rapid time-to-result and include higher proportion of SARS-CoV-2 positive cases in the VOC surveillance testing. The genotyping approach enables labs to reserve valuable WGS resources for identification of new variants and can be used as an alternative when WGS is not available. In addition, a new genotyping assay can be set up within 2 weeks when a new mutation is discovered by WGS.

*Research use only, not for use in diagnostic procedures