Detection of SARS-CoV-2 Omicron variants using RT-PCR based genotyping assays

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Background

The newly emerged SARS-CoV-2 Omicron variant was designated a variant of concern (VOC) in November 2021, and shortly thereafter, has spread worldwide, rapidly replacing the Delta VOC. Whole genome sequencing (WGS) is considered the method of choice for identification of new variants, but it has major limitations such as time to result (>several days) and resources required. To track variant spread in real-time and enable timely public health action, a RT-PCR-based genotyping approach can be used. This study evaluated the performance of a custom TaqMan SARS-CoV-2 mutation panel for detection of Omicron BA.1 and BA.2 variants in comparison to WGS.

Methods

We tested 6 assays of the TaqMan mutation panel on 69 SARS-CoV-2 positive samples collected between December 2021 and January 2022 in South Africa (Figure 1). The assays targeted mutations in the S-gene of SARS-CoV-2 and lineage determination was based on the mutation profile (Table 1). Wild type signal on all 6 genotyping assays was presumed to be Delta variant. All samples in parallel underwent WGS (Figure 1). The SARS-CoV-2 positive status was determined using the TaqPath™ COVID-19 CE-IVD RT-PCR Kit and S-gene target failure (SGTF) was observed in 42% of samples.

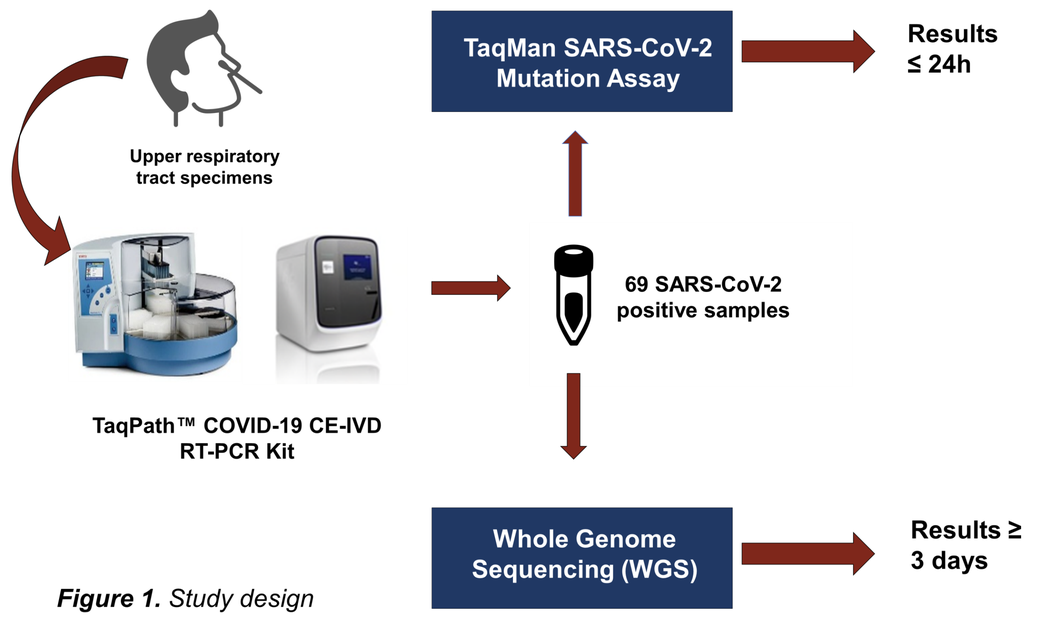

Figure 1. *Study design*

Table 1. *TaqMan SARS-CoV-2 mutation panel for variant detection*

TaqMan SARS-CoV-2 Mutation Assay	Delta / Non-omicron	Omicron – BA.1	Omicron – BA.2
Del69_70	Wild-type	Mutant	Wild-type
G339D	Wild-type	Mutant	Mutant
Q493R	Wild-type	Mutant	Mutant
T547K	Wild-type	Mutant	Wild-type
N856K	Wild-type	Mutant	Wild-type
Q954H	Wild-type	Mutant	Mutant

Results

WGS data was available for 50 samples. The genotyping assays correctly assigned the lineage in 96% of the cases when compared to WGS. In total, BA.1 variant was detected in 29 samples using the mutation panel (Figure 2), all of which displayed SGTF (Figure 3). Of these 29 samples, WGS could determine the lineage in 24 samples, while 5 samples did not yield a result by WGS. BA.2 variant was detected in 11 cases using both the mutation panel and WGS (Figure 2) and all of them did not show SGTF when tested with the TaqPath™ COVID-19 CE-IVD RT-PCR Kit (Figure 3).

Several genotyping assays (4/6) showed presence of both the wild-type and mutant sequence in 2 samples, and the sequencing data confirmed that these 2 samples did indeed contain sequences of both Delta and Omicron variants (Figure 4).

The 6 tested assays showed excellent performance across different SARS-CoV-2 viral loads (Figure 5). In 3 samples with Ct>30 1/6 assays did not amplify, and in one sample with Ct>36 2/6 assays did not amplify, while the others could detect the mutations present.

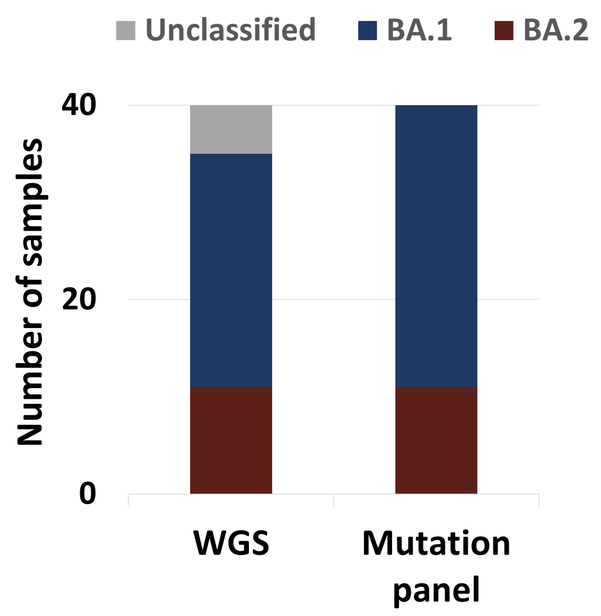

Figure 2. *Samples determined to be BA.1 or BA.2 Omicron variant using whole genome sequencing (WGS) or TaqMan SARS-CoV-2 mutation panel*

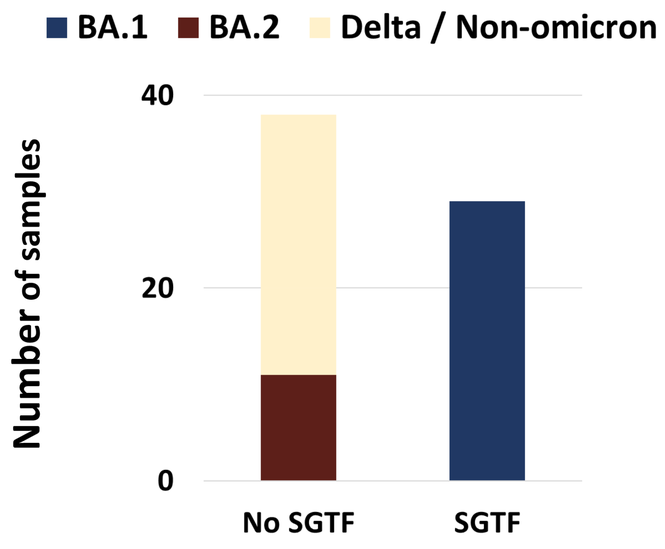

Figure 3. *S-gene target failure (SGTF) status of SARS-CoV-2 positive samples tested using the TaqPath™ COVID-19 CE-IVD RT-PCR Kit and characterized for variants with TaqMan SARS-CoV-2 mutation panel*

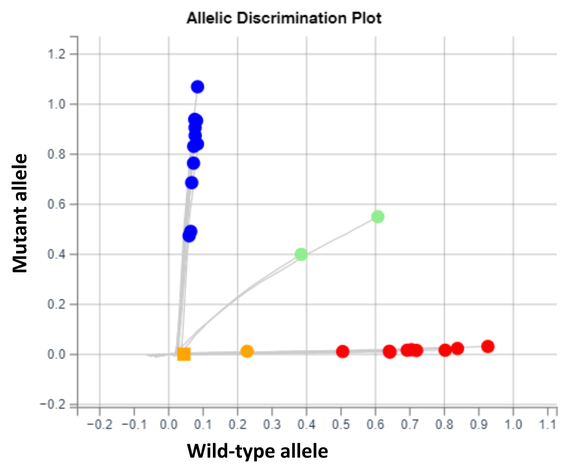

Figure 4. *Allelic discrimination plot for Q493R mutation. The mutant (blue dots), wild-type (red dots) or samples with both wild-type and mutant (green dots) alleles are detected.*

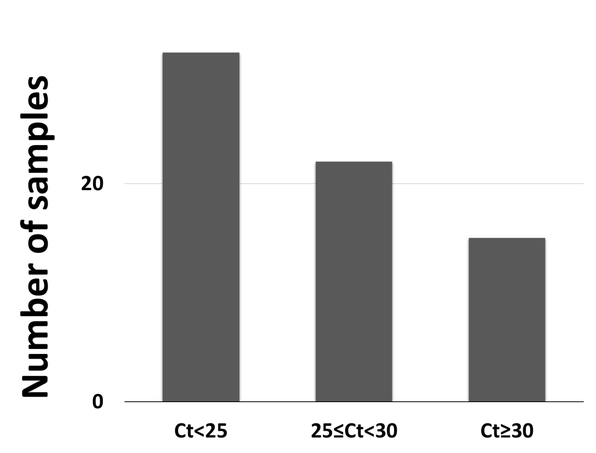

Figure 5. *Distribution of Ct values for the ORF1ab target in the positive cohort based on TaqPath™ COVID-19 CE-IVD RT-PCR Kit*

Conclusion

TaqMan SARS-CoV-2 mutation assays enable quick and accurate detection of Omicron VOC using RT-PCR and can even detect presence of two variants in a sample. This approach can enable surveillance testing of a higher proportion of SARS-CoV-2 positive cases, while reserving WGS for identification of new variants, or can be an excellent alternative if WGS is not available.