Comparison of TaqMan™ and TIB Molbiol SARS-CoV-2 Genotyping Assays for the Identification of B.1.1.7 in S-gene Target Failure

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Introduction

SARS-CoV-2 variant identification is critical as COVID-19 transitions from a pandemic to an endemic phase. While sequencing is the conventional method to perform variant identification, it is resource intensive and the increased turnaround time (TAT) precludes swift public health measures to be implemented. PCR-based genotyping assays (GA), specific for functional mutations found in different SARS-CoV-2 variants, enable rapid and scalable alternative for COVID-19 surveillance. The B.1.1.7 (*Alpha*) variant contains a 6-nt deletion (del69_70) that results in the S-Gene Target Failure (SGTF) with the TaqPath™ COVID-19 CE-IVD RT-PCR Kit and this, in turn, serves as a useful proxy for detection of B.1.1.7. The purpose of this study was to evaluate the performance of two SARS-CoV-2 genotyping assays for the detection of del69_70 in SGTF positive samples

Methods

The study was performed on 38 upper respiratory tract samples collected in Germany in February 2021. SARS-CoV-2 positivity was determined using the Cobas® SARS-CoV-2 Assay. All samples were also tested using the TaqPath™ COVID-19 kit and displayed SGTF. The range of Ct values is shown in Fig. 1. The samples were then tested using TaqMan SARS-CoV-2 GA targeting 5 mutations: del69_70, N501Y, P681H, E484K, and K417N as well as the TIB Molbiol del69,70+484K+501Y multiplex kit (Fig. 2).

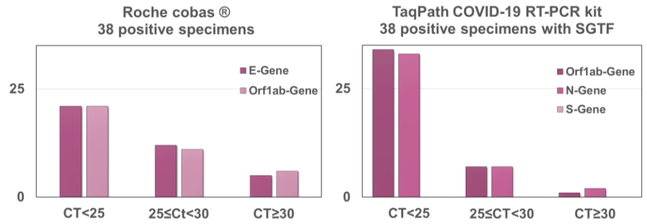

Figure 1. Range of viral titers as detected by parallel testing using cobas® and TaqPath™ COVID-19 assays

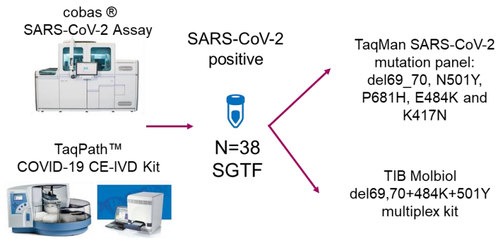

Figure 2. Study design

Results

Of the 38 SGTF samples, one sample (434) failed to show amplification using both, the TaqMan and TIB Molbiol GA due to low viral load and was excluded from the analysis. For B.1.1.7 defining mutations, (N501Y, del69_70 and P681H), TaqMan GA identified the mutant allele for all three mutations in 28/37 (75.7%) of samples. del69_70 mutation was detected by TaqMan GA in 37/37 (100%) and by TIB Molbiol kit in 34/37 (91.9%) of samples; while 3 samples (8.1%) failed to show amplification by TIB Molbiol kit. (Table 1, Fig. 3A)

Sample ID	Roche cobas® Ct Values		TaqPath™ COVID-19 kit Ct Values			TaqMan Mutation Panel	TIB Molbiol multiplex kit
	E-gene	ORF1ab	N gene	ORF1ab	S gene		
008	27.15	26.23	14.1	13.66	SGTF	B1.1.7 (Alpha)	B1.1.7 (Alpha)
011	21.07	21.85	16.56	16.95	SGTF	B1.1.7 (Alpha)	B1.1.7 (Alpha)
021	18.91	18.45	9.51	7.86	SGTF	B1.1.7 (Alpha)	B1.1.7 (Alpha)
022	23.5	23.72	11.83	9.57	SGTF	B1.1.7 (Alpha)	B1.1.7 (Alpha)
058	25.48	25.76	21.67	21.66	SGTF	B1.1.7 (Alpha)	B1.1.7 (Alpha)
080	29.08	30.26	21.92	21.54	SGTF	B1.1.7 (Alpha)	B1.1.7 (Alpha)
092	31.1	32.97	26.64	27.23	SGTF	B1.1.7 (Alpha)	B1.1.7 (Alpha)
107	21.2	21.26	14.43	12.41	SGTF	B1.1.7 (Alpha)	B1.1.7 (Alpha)
125	20.4	20.57	16.71	15.68	SGTF	B1.1.7 (Alpha)	B1.1.7 (Alpha)
149	28.44	27.81	17.27	14.73	SGTF	B1.1.7 (Alpha)	B1.1.7 (Alpha)
162	20.98	21.91	14.56	13.68	SGTF	B1.1.7 (Alpha)	B1.1.7 (Alpha)
181	25.7	26.57	18.79	18.76	SGTF	B1.1.7 (Alpha)	B1.1.7 (Alpha)
194	19.51	19.57	12.56	12.82	SGTF	B1.1.7 (Alpha)	B1.1.7 (Alpha)
197	32.11	34.85	29.33	29.34	SGTF	B1.1.7 (Alpha)	B1.1.7 (Alpha)
219	29.51	29.06	20.37	17.18	SGTF	B1.1.7 (Alpha)	B1.1.7 (Alpha)
226	26.4	27.48	18.8	18.6	SGTF	B1.1.7 (Alpha)	B1.1.7 (Alpha)
232	23.15	22.52	13.71	11.26	SGTF	B1.1.7 (Alpha)	B1.1.7 (Alpha)
242	21.35	21.94	17.61	16.35	SGTF	B1.1.7 (Alpha)	B1.1.7 (Alpha)
248	26.05	27.64	17.07	17.19	SGTF	B1.1.7 (Alpha)	B1.1.7 (Alpha)
256	20.73	21.49	14.19	14.21	SGTF	B1.1.7 (Alpha)	B1.1.7 (Alpha)
270	20.88	20.97	18.63	18.54	SGTF	B1.1.7 (Alpha)	B1.1.7 (Alpha)
321	21.41	21.78	11.48	8.91	SGTF	B1.1.7 (Alpha)	B1.1.7 (Alpha)
335	22.89	24.6	15.45	14.78	SGTF	B1.1.7 (Alpha)	no amplification
364	22.72	23.47	18.14	17.42	SGTF	B1.1.7 (Alpha)	B1.1.7 (Alpha)
384	24.09	24.45	19.59	18.73	SGTF	B1.1.7 (Alpha)	B1.1.7 (Alpha)
400	22.1	21.86	13.24	11.38	SGTF	B1.1.7 (Alpha)	B1.1.7 (Alpha)
417	19.32	19.52	14.55	13.51	SGTF	B1.1.7 (Alpha)	B1.1.7 (Alpha)
445	25.9	26.99	18.46	18.38	SGTF	B1.1.7 (Alpha)	B1.1.7 (Alpha)
051	33.81	35.77	34.69	29.94	SGTF	del69_70	del69_70
176	20.33	20.68	11.75	8.41	SGTF	del69_70	del69_70
217	26.86	26.76	19.75	18.91	SGTF	del69_70	del69_70
234	20.53	20.56	11.8	8.64	SGTF	del69_70	del69_70
298	20.26	21.16	11.11	9.29	SGTF	del69_70	del69_70
353	28.52	29.44	22.04	21.23	SGTF	del69_70	del69_70
377	23.93	24.7	19.34	19.23	SGTF	del69_70	del69_70
177	26.65	28.09	18.42	18.12	SGTF	del69_70	no amplification
320	31.7	33.54	25.8	24.07	SGTF	del69_70	no amplification
434	33.48	35.61	36.8	34.23	SGTF	no amplification	no amplification

Table 1. TaqMan SARS-CoV-2 mutation panel confirms the presence of Alpha or other variant containing del69_70 in samples displaying S-gene target failure

Results (contd.)

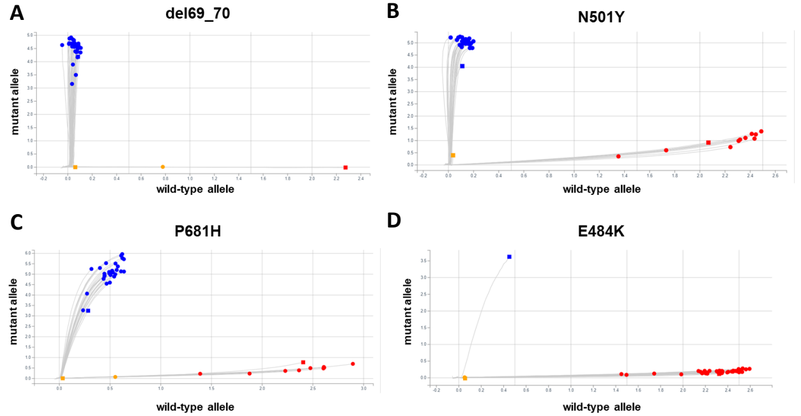

Figure 3. Allelic discrimination plots for del69_70, N501Y, P681H and E484K mutations in the 38 samples analyzed. *The mutant (blue dots) or wild-type (red dots) alleles are detected with each assay. The yellow circle represents one sample in which amplification failed due to low viral load. The squares represent controls.*

9 samples showed *wt* pattern for N501Y and P681H mutations (Fig. 3B,3C red dots). All samples showed *wt* result for E484K (Fig. 3D) and K417N assays (not shown). In all 34 samples for which TIB Molbiol results were obtained, genotyping results for the 3 mutations were 100% concordant with the TaqMan SARS-CoV-2 GA.

Conclusions

- **SGTF is a good proxy for the presence of del69_70 mutation in SARS-CoV-2 positive samples.**
- **The TaqMan SARS CoV-2 genotyping assay shows excellent concordance with the TIB Molbiol multiplex kit for identification of B.1.1.7 and SARS-CoV-2 variants with del69_70.**
- **The TaqMan SARS-CoV-2 mutation panel is able to detect mutations in samples with low viral loads and combined with a short TAT allows for variant surveillance in a greater proportion of COVID-19 positive cases.**