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Performance Evaluation of a Highly Mutation-Resilient RT-PCR Assay to Address Diagnostic Challenges with SARS-CoV-2 Variants of Concern

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Introduction

One of the considerations regarding SARS-CoV-2 variants of concern (VOC) is the impact of mutations on the performance of diagnostic tests. The TaqPath™ COVID-19 RNase P Combo Kit 2.0* was designed with a total of 8 targets in 3 genes: *orf1a*, *orf1b* and *N* to compensate for emerging SARS-CoV-2 variants. In the current study we evaluated the clinical performance of the TaqPath™ COVID-19 RNase P Combo Kit 2.0* as compared to the cobas® SARS-CoV-2 Assay (2 targets, 1 each in *orf1ab* and *E*-gene).

Methods

The retrospective study was performed on 675 nasopharyngeal swab samples collected in Germany from February to August 2021. SARS-CoV-2 status was characterized using the cobas® SARS-CoV-2 Assay. The samples were tested using both tests in a blinded, randomized fashion and Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA) were assessed. Discordant results were resolved using the TaqPath™ COVID-19 RT-PCR CE-IVD kit* and sensitivity and specificity were calculated. Variant status for 116 samples was determined using the TIB MOLBIOL SARS-CoV-2 combi assay (Figure 1).

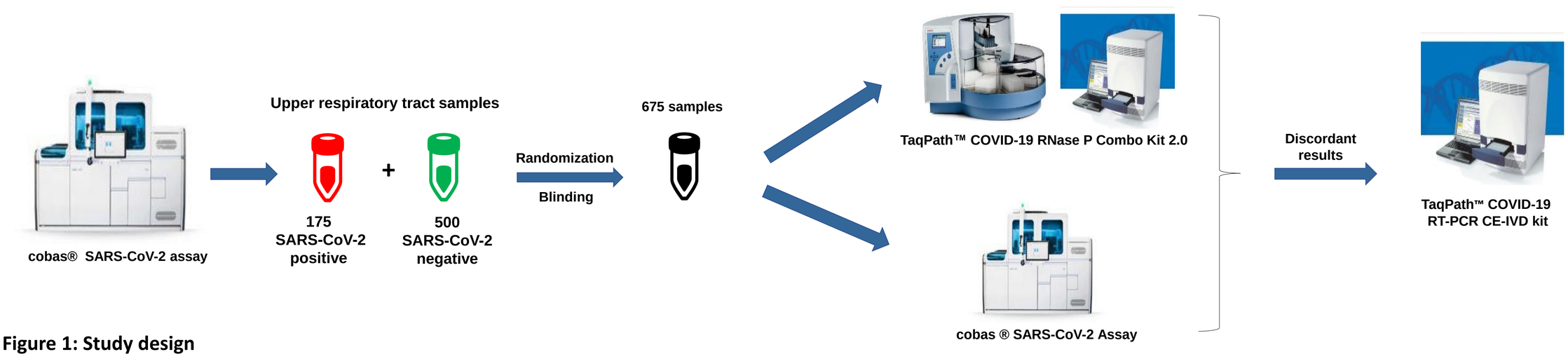

Figure 1: Study design

Results

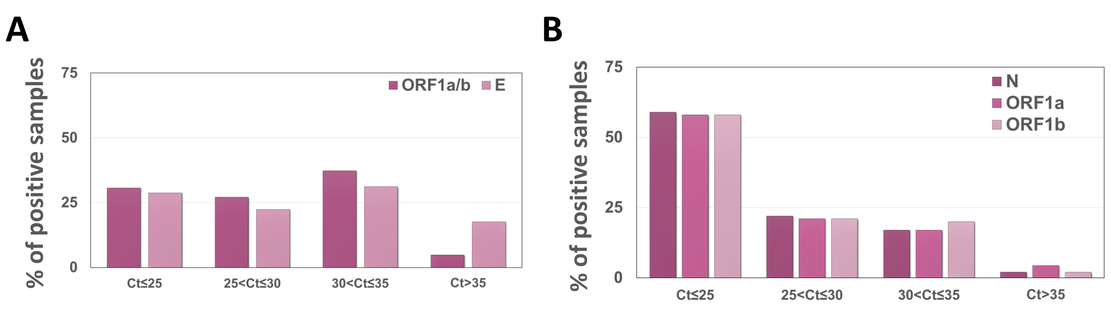

Figure 2. Distribution of Ct values for positive cohort based on A) cobas® SARS-CoV-2 assay and B) TaqPath™ COVID-19 RNase P Combo Kit 2.0* testing

Of 675 samples, 8 samples were excluded from the analysis due to inconclusive or invalid results. The positive cohort spanned the dynamic range of the assay with a minimum of 20% of samples in each category: Ct≤25, 25<Ct≤30, 30<Ct≤35 and Ct>35 (Figure 2). The results showed an excellent concordance between the TaqPath™ COVID-19 RNase P Combo Kit 2.0* and the cobas® SARS-CoV-2 Assay, with PPA of 97.7% and NPA of 98.2% (Table 1). All 116 VOC samples (Alpha (N=104) and Delta (N=12)) showed a positive result using the TaqPath™ COVID-19 RNase P Combo Kit 2.0 without any inconclusive calls.

Table 1. Concordance between TaqPath™ COVID-19 RNase P Combo Kit 2.0* and cobas® SARS-CoV-2 Assay

		cobas® SARS-CoV-2 assay		
		Positive	Negative	Total
TaqPath™ COVID-19 RNase P Combo Kit 2.0*	Positive	173	9	182
	Negative	4	481	485
	Total	177	490	667
Positive Percent Agreement (95% CI)		**97.7%**	(94.3% to 99.4%)	
Negative Percent Agreement (95% CI)		**98.2%**	(96.5% to 99.2%)	

Table 2. Discordant sample resolution using TaqPath™ COVID-19 RT-PCR CE-IVD kit*

Sample number	TaqPath™ COVID-19 RNase P Combo Kit 2.0*					cobas® SARS-CoV-2 Assay	TaqPath™ COVID-19 RT-PCR CE-IVD kit*
	N	ORF1a	ORF1b	RNAse P	Result		
1	31.21	31.72	32.62	24.40	Positive	Negative	Positive
2	34.94	33.74	33.84	24.81	Positive	Negative	Negative
3	29.34	29.89	30.40	23.41	Positive	Negative	Negative
4	31.98	37.09	34.73	25.53	Positive	Negative	Positive
5	31.24	36.41		20.90	Positive	Negative	Positive
6	28.62	30.82	30.93	23.63	Positive	Negative	Positive
7	35.35	36.65		22.20	Positive	Negative	Negative
8	27.50	28.46	29.02	21.17	Positive	Negative	Positive
9	32.64	36.17	38.22	27.97	Positive	Negative	Negative
10				25.48	Negative	Positive	Positive
11				28.71	Negative	Positive	Negative
12				24.49	Negative	Positive	Negative
13				23.73	Negative	Positive	Negative

Upon arbitration testing of the 13 discordant samples (Table 2), the sensitivity and specificity of the TaqPath™ COVID-19 RNase P Combo Kit 2.0* was 99.4% and 99.2% respectively (Table 3).

Table 3. Clinical Sensitivity and Specificity of the TaqPath™ COVID-19 RNase P Combo Kit 2.0* after discordant sample resolution

	TaqPath™ COVID-19 RNase P Combo Kit 2.0	
Clinical Sensitivity (95% CI)	**99.4%**	(96.9 %to 99.9%)
Clinical Specificity (95% CI)	**99.2%**	(97.9% to 99.7%)

Conclusion

The highly mutation-resilient TaqPath™ COVID-19 RNase P Combo Kit 2.0* has excellent concordance with the cobas® SARS-CoV-2 Assay and is a highly accurate method for detection of SARS-CoV-2 including VOC.

*CE-IVD In Vitro Diagnostic Use