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Clinical performance of the TaqPath™ COVID-19 CE-IVD RT-PCR Kit as compared to Cobas® SARS-CoV-2 Assay

Jasmin Köffer[1], Ulrich Eigner[1], Xuemei Wang[2], Thu Pham[2], Pius Brzoska[2], Oscar Aceves[2],Erene Mina[2], Géraldine Mercier[2], Manoj Gandhi[2]

[1]MVZ Labor Dr. Limbach & Kollegen GbR, Heidelberg, Germany; [2]Thermo Fisher Scientific, South San Francisco, CA, USA

MVZ Labor Dr. Limbach HEIDELBERG

Background

The TaqPath™ COVID-19 CE-IVD RT-PCR Kit and Cobas® SARS-CoV-2 Assay are among the most widely used RT-PCR tests for the detection of SARS-CoV-2. The TaqPath™ COVID-19 CE-IVD RT-PCR Kit targets three viral genes (ORF1ab, N and S), while the Cobas® SARS-CoV-2 Assay targets two viral genes (ORF1ab, E-gene). In this study, we compared the clinical performance of the two above-mentioned RT-PCR diagnostic tests using Next Generation Sequencing (NGS) to resolve discordant results.

Methods

The retrospective study was performed on 450 (175 positive and 275 negative) upper respiratory tract samples from routine leftover clinical specimens collected in Germany in February 2021 with an attempt to include any SARS-CoV-2 variants. All samples were previously characterized for SARS-CoV-2 status using the Cobas® SARS-CoV-2 Assay. Positive cohort spanned the dynamic range of the Cobas® SARS-CoV-2 Assay with Ct values in even distribution (Ct<20, 20≤Ct<25, 25≤Ct<30, and Ct≥30). The samples were thawed following storage at -80°C and tested using both tests in a blinded, randomized fashion. Inconclusive or invalid results from either test method were excluded from analysis. Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA) were assessed and discordant samples were analyzed using the EasySeq™ SARS-CoV-2 Whole Genome Sequencing (WGS) kit on the Illumina® NGS sequencing instrument. Sequencing results were analyzed using ncbi-blast-2.11.0+ by alignment to the SARS-CoV-2 reference sequence, NC-045512.2. Clinical Sensitivity and Specificity for both assays were calculated using a combination of concordant results, and discordant results in which the resolver method was used to define true positive and true negative. Discordant results not resolved by WGS were excluded from the analysis for Clinical Sensitivity and Specificity.

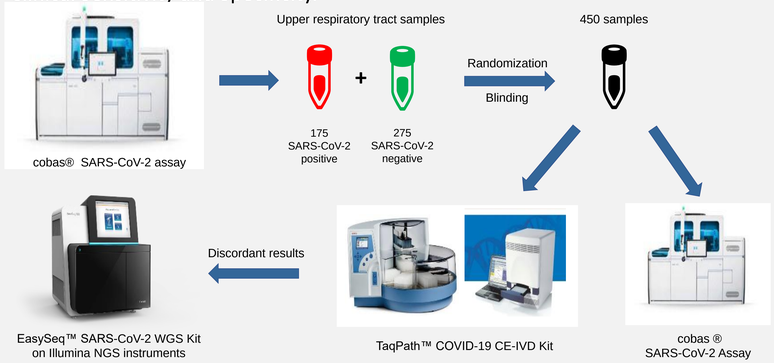

Figure 1: Design for the performance comparison study with discordant resolution using WGS

Results

		Cobas® SARS-CoV-2 Assay		
		Positive	Negative	Total
TaqPath™ COVID-19 CE-IVD RT-PCR Kit	Positive	171	13	184
	Negative	1	261	262
	Total	172	273	446
Positive Percent Agreement (95% CI)		**99.4%**	(96.8% to 100.0%)	
Negative Percent Agreement (95% CI)		**95.3%**	(92.0% to 97.4%)	

Table 1. Concordance between TaqPath™ COVID-19 CE-IVD RT-PCR Kit and Cobas® SARS-CoV-2 Assay

Of the 450 samples, 4 samples yielded inconclusive or invalid results and were excluded from the analysis. Of the 172 samples positive by the Cobas® SARS-CoV-2 Assay, 171 samples yielded a positive result using TaqPath™ COVID-19 CE-IVD RT-PCR Kit with only 1 sample showing a discordant result, resulting in a PPA of 99.4%. Of the 274 samples negative by the Cobas® SARS-CoV-2 Assay, 261 samples yielded a negative result using TaqPath™ COVID-19 CE-IVD RT-PCR Kit with 13 samples showing discordant results, resulting in a NPA of 95.3%. (Table 1)

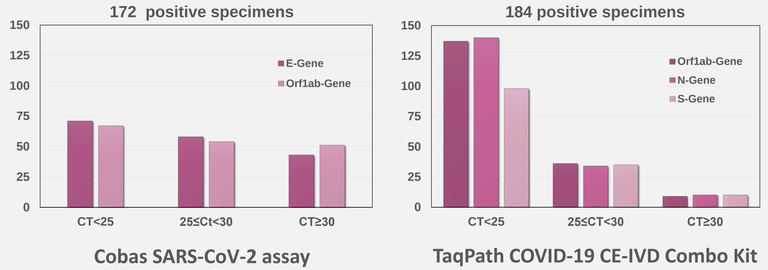

Figure 2: Range of viral titers for the parallel RT-PCR testing using cobas and TaqPath.

Of the 14 discordant samples, WGS did not yield a valid result in 8 samples due to low RNA concentration and need to be resolved by an alternate method. In the remaining 6 discordant samples, WGS results yielded valid results and revealed the presence of SARS-CoV-2 RNA in all 6 samples. All the valid NGS results agreed with the results from the TaqPath™ COVID-19 CE-IVD RT-PCR Kit. (Table 2) For the TaqPath™ COVID-19 CE-IVD RT-PCR Kit, the Clinical Sensitivity and Specificity were, both, 100%. For the Cobas® SARS-CoV-2 Assay, the Clinical Sensitivity was 96.61% and the Clinical Specificity was 100%. (Table 3)

Results (contd.)

Sr No	Orf1ab	N	S	MS2	TaqPath	Roche	WGS
1	31.49	32.31	33.36	23.63	Positive	Negative	Invalid
2	32.48	31.43	32.17	25.19	Positive	Negative	Invalid
3	28.73	29.12	29.32	23.86	Positive	Negative	Invalid
4	29.06	28.73	28.97	23.20	Positive	Negative	Invalid
5		32.16	33.09	24.70	Positive	Negative	Invalid
6		31.06	32.44	24.84	Positive	Negative	Invalid
7	27.42	26.67	27.57	24.50	Positive ✓	Negative	Present
8	20.75	21.23		24.29	Positive ✓	Negative	Present
9	23.10	23.31	24.78	24.88	Positive ✓	Negative	Present
10	23.75	24.35	23.82	24.38	Positive ✓	Negative	Present
11	28.73	27.89		25.42	Positive	Negative	Invalid
12					Negative	Positive	Invalid
13	36.13	34.76	33.17	24.90	Positive ✓	Negative	Present
14	35.05	32.99		23.29	Positive ✓	Negative	Present

Table 2. Discordant sample resolution using WGS. Grey rows represent samples that could not be resolved by WGS (Average Ct values >30)

	TaqPath™ COVID-19 CE-IVD RT-PCR Kit	cobas® SARS-CoV-2 Assay
Clinical Sensitivity	**100%**	**96.61%**
Clinical Specificity	**100%**	**100%**

Table 3. Clinical Sensitivity and Specificity after discordant sample resolution using WGS.

Conclusion

The TaqPath™ COVID-19 CE-IVD RT-PCR Kit showed good concordance with the Cobas® SARS-CoV-2 Assay (>95% PPA and NPA). The TaqPath test had excellent agreement after arbitration testing by Whole Genome Sequencing (100% Sensitivity, 100% Specificity). Samples with low viral loads are not ideal for SARS-CoV-2 detection using WGS and alternate methods with higher sensitivity may be needed for such samples.