Clinical evaluation of the TaqPath COVID-19 CE-IVD RT-PCR Kit for the detection of SARS-CoV-2

Summary

The objective of this study was to determine the clinical performance of the Applied Biosystems™ TaqPath™ COVID-19 CE-IVD RT-PCR Kit. In this study, 446 retrospective upper respiratory samples from patients of all ages were tested for SARS-CoV-2 using the cobas™ SARS-CoV-2 Test (Roche) and the TaqPath COVID-19 CE-IVD RT-PCR Kit, and their results were compared. Discordant results were resolved using the EasySeq™ SARS-CoV-2 Whole Genome NGS Sequencing Kit (NimaGen).

Of the 446 samples analyzed, the cobas SARS-CoV-2 Test reported 172 samples positive and 274 samples negative for SARS-CoV-2. The TaqPath COVID-19 CE-IVD RT-PCR Kit confirmed 171 of the 172 samples positive for SARS-CoV-2 and 261 of 274 negative for SARS-CoV-2, resulting in a positive percent agreement of 99.4% and a negative percent agreement of 95.3%. Of the 14 discordant results, six were resolved by sequencing and confirmed the presence of SARS-CoV-2 in concordance with the TaqPath COVID-19 CE-IVD RT-PCR Kit results.

This clinical study confirms that the TaqPath COVID-19 CE-IVD RT-PCR Kit is a highly sensitive and specific RT-qPCR test for the identification of SARS-CoV-2 infection.

Introduction

The outbreak of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), causing coronavirus disease 2019 (COVID-19), was first reported by the end of 2019. The subsequent rapid worldwide spread of SARS-CoV-2 resulted in a global need for accessible and reliable diagnostic testing solutions.

Thermo Fisher Scientific immediately developed the TaqPath COVID-19 CE-IVD RT-PCR Kit to enable clinical and public health laboratories to quickly diagnose COVID-19 caused by SARS-CoV-2 infection. The TaqPath COVID-19 CE-IVD RT-PCR Kit is intended for the qualitative detection of nucleic acid from SARS-CoV-2 in upper respiratory specimens (including bronchoalveolar lavage, mid-turbinate swabs, nasal swabs, nasopharyngeal swabs, nasopharyngeal aspirate, oropharyngeal swabs, and saliva* collected with the SpectrumDNA™ SDN (Spectrum Solutions)) from individuals suspected of having a SARS-CoV-2 infection. Extraction of samples is conducted manually or using the automated Thermo Scientific™ KingFisher™ Flex Purification System. The TaqPath COVID-19 CE-IVD RT-PCR Kit is compatible with seven Applied Biosystems™ real-time PCR instruments, including the 7500 systems (7500, 7500 Fast, 7500 Fast Dx), the QuantStudio™ 5 system (in 96-well, 0.1 mL and 0.2 mL blocks, and 384-well block format), and the QuantStudio™ 7 Flex system (384-well block format).

In this study, the objective was to evaluate the clinical performance of the TaqPath COVID-19 CE-IVD RT-PCR Kit using upper respiratory samples from patients of all ages. The cobas SARS-CoV-2 Test was used as a comparator, and discordant results were resolved using the EasySeq™ SARS-CoV-2 Whole Genome NGS Sequencing Kit on an Illumina™ NGS sequencing platform.

*** Regulatory requirements vary by country; saliva testing and workflow may not be applicable in your geographic area or may require additional approval by local authorities.**

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Results

A total of 450 retrospective upper respiratory tract samples, from routine leftover clinical specimens from patients of all ages, were tested to evaluate the clinical performance of the TaqPath COVID-19 CE-IVD RT-PCR Kit. The samples were collected in Germany in February 2021, and the study was carried out by MVZ Labor Dr. Limbach (Heidelberg, Germany). All samples were deidentified and randomized before being tested concurrently using the TaqPath COVID-19 CE-IVD RT-PCR Kit and the cobas SARS-CoV-2 Test as a comparator. NGS was used to resolve discordant results between the two CE-IVD RT-PCR tests. A schematic overview of the study design is shown in Figure 1.

During the concurrent testing, for 4 samples, the cobas SARS-CoV-2 Test only detected target 2, one of two targeted amplicons. In accordance with the study design, these samples were deemed "inconclusive" and were removed from the comparison, resulting in a total of 446 samples that were compared. Of the 446 specimens, the cobas test deemed 172 samples to be positive and 274 to be negative for SARS-CoV-2.

The TaqPath COVID-19 CE-IVD RT-PCR Kit reported a total of 184 positive and 262 negative for SARS-CoV-2 of the 446 total samples. The TaqPath COVID-19 CE-IVD RT-PCR Kit confirmed 171 of the 172 samples that were reported positive by the reference method. A total of 14 samples showed discordant results between the TaqPath COVID-19 CE-IVD RT-PCR Kit and the cobas test. The TaqPath COVID-19 CE-IVD RT-PCR Kit detected SARS-CoV-2 in 13 samples that were reported negative for the virus by the cobas test. The results are summarized in Table 1.

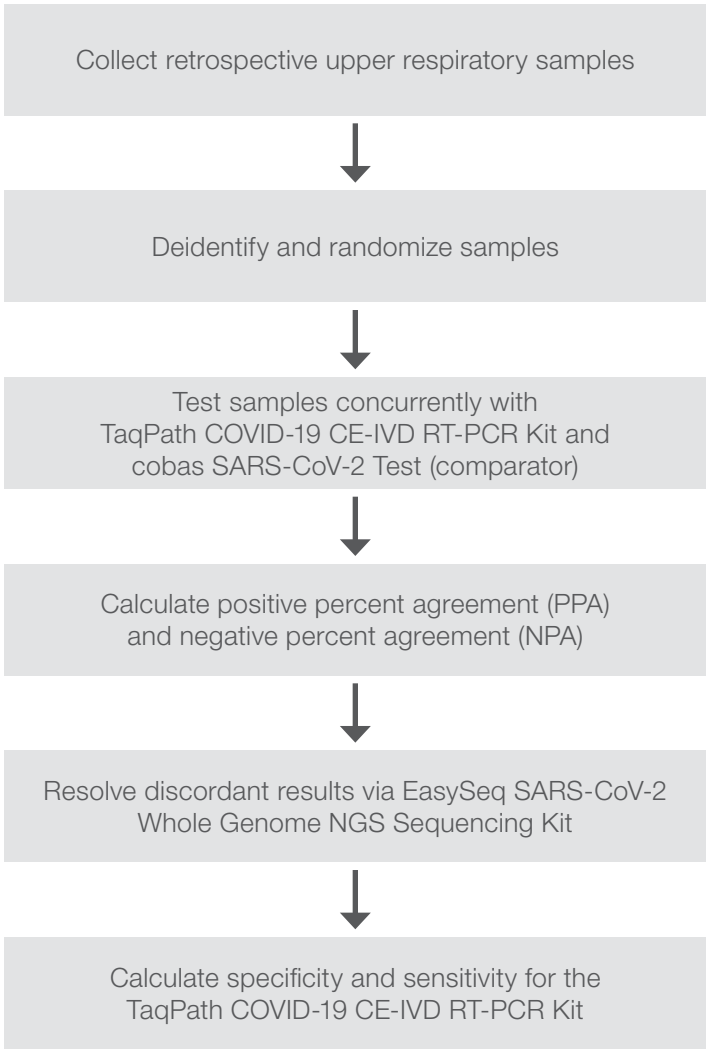

Figure 1. Schematic overview of study design.

Table 1. Detection of SARS-CoV-2 in 446 samples using the TaqPath COVID-19 kit and the cobas SARS-CoV-2 Test.

Detection of SARS-CoV-2	Comparator: cobas SARS-CoV-2 test		
TaqPath COVID-19 kit (CE-IVD)	Positive	Negative	**Total**
Positive	171	13	**184**
Negative	1	261	**262**
Total	**172**	**274**	**446**

The positive percent agreement (PPA) and negative percent agreement (NPA) were calculated based on the formulas below (Equations 1, 2) to determine the concordance between the TaqPath COVID-19 CE-IVD RT-PCR Kit and the cobas SARS-CoV-2 Test. The TaqPath COVID-19 CE-IVD RT-PCR Kit showed a PPA of 99.4% and an NPA of 95.3% (Table 2).

$$PPA = \frac{\text{Number of positive results shared by both evaluation method and its comparator}}{\text{Number of positive results by comparator method}} \times 100\% \quad (1)$$

$$NPA = \frac{\text{Number of negative results shared by both evaluation method and its comparator}}{\text{Number of negative results by comparator method}} \times 100\% \quad (2)$$

Table 2. PPA and NPA of the TaqPath COVID-19 CE-IVD RT-PCR Kit with the cobas SARS-CoV-2 Test.

	Concordance	Percentage	95% confidence interval*
Positive percent agreement (PPA)	171/172	99.4%	96.8–100.0%
Negative percent agreement (NPA)	261/274	95.3%	92.0–97.4%

* The two-sided 95% confidence intervals (CI) were calculated using the Clopper–Pearson method.

To calculate the clinical sensitivity and specificity of the TaqPath COVID-19 CE-IVD RT-PCR Kit, we aimed to determine the SARS-CoV-2 status on the discordant samples using a set of orthogonal assays. First, we tested the 14 discordant samples along with 20 concordant samples (10 positives, 10 negatives) using whole-genome sequencing (WGS) on an Illumina NGS instrument. WGS was able to conclusively determine SARS-CoV-2 status in all the 20 concordant samples, justifying the use of this method for discordant resolution. Of the 14 discordant samples, WGS was able to determine the SARS-CoV-2 status in 6 samples, all of which were positive for SARS-CoV-2, confirming the results obtained by the TaqPath COVID-19 CE-IVD RT-PCR Kit. In the remaining 8 samples, WGS was not able to provide a definitive result on the SARS-CoV-2 status. These unresolved 8 cases were additionally analyzed using 2 separate genotyping panels: the Applied Biosystems™ TaqMan® SARS-CoV-2 Mutation Panel comprising 5 assays (S.delH69V70, S.K417N, S.E484K, S.N501Y, S.P681H), as well as the TIB MOLBIOL panel (del69, 70+484K+501Y multiplex kit). Neither of the genotyping panels resulted in conclusive results in these 8 unresolved cases, possibly due to low viral loads or poor sample quality. Both sequencing and genotyping assays have limited sensitivity, thus it is likely the samples had low viral loads and thus no result could be obtained. Methods with higher sensitivity would be needed to resolve the status of the remaining 8 discordant samples. As a result, these 8 cases remain unresolved and were removed from the sensitivity and specificity calculation. The complete results of the discordant sample testing are shown in Table 3.

Table 3. Results for the discordant samples.

Discordant result	cobas SARS-CoV-2 Test (comparator) results	TaqPath COVID-19 test results	WGS results	Genotyping assays results	Resolved
1	Negative	Positive	No result	No result	No
2	Negative	Positive	No result	No result	No
3	Negative	Positive	No result	No result	No
4	Negative	Positive	No result	No result	No
5	Negative	Positive	No result	No result	No
6	Negative	Positive	No result	No result	No
7	Negative	Positive	Positive	Not tested	Yes
8	Negative	Positive	Positive	Not tested	Yes
9	Negative	Positive	Positive	Not tested	Yes
10	Negative	Positive	Positive	Not tested	Yes
11	Negative	Positive	No result	No result	No
12	Negative	Positive	Positive	Not tested	Yes
13	Negative	Positive	Positive	Not tested	Yes
14	Positive	Negative	No result	No result	No

The clinical sensitivity and specificity were calculated based on the formula below (Equations 3, 4). The clinical sensitivity of the TaqPath COVID-19 CE-IVD RT-PCR Kit is 100% and the clinical specificity is 100% (Table 4).

$$\text{Sensitivity} = \frac{\text{Number of true positives}}{\text{Number of true positives + false positives}} \times 100\% \quad (3)$$

$$\text{Specificity} = \frac{\text{Number of true negatives}}{\text{Number of true negatives + false negatives}} \times 100\% \quad (4)$$

We analyzed the reported C_t values of all positive samples, to ensure that the study covered a wide range of viral titers. Based on C_t values from the cobas SARS-CoV-2 Test, we clustered SARS-CoV-2 viral loads into high ($C_t < 25$), medium ($25 \leq C_t < 30$) and low ($C_t \geq 30$).

From the results from the cobas SARS-CoV-2 Test, about 75% of the samples tested had viral loads in the high ($C_t < 25$) and medium ($25 \leq C_t < 30$) clusters for both viral gene targets supported by the assay. The remaining 25% of specimens showed C_t values in the low viral titer range ($C_t \geq 30$). An overview of the C_t values detected for target 1 and 2 can be seen in Figure 2A.

The TaqPath COVID-19 CE-IVD RT-PCR Kit showed the majority of C_t values in the high and medium clusters, and only 5–7% of the samples showed $C_t \geq 30$. An overview of the C_t values detected by the TaqPath kit can be seen in Figure 2B.

Table 4. Summary of results and calculations for the TaqPath COVID-19 kit.

	Reference standard		
TaqPath COVID-19 test	Positive	Negative	**Total**
Positive	177	0	**177**
Negative	0	261	**261**
Total	**177**	**261**	**438**

Clinical sensitivity and specificity calculations			
Clinical sensitivity	177/177	100.0%	95% CI, 97.9–100%
Clinical specificity	261/261	100.0%	95% CI, 98.6–100%

For the majority of samples evaluated using the TaqPath COVID-19 CE-IVD RT-PCR Kit, the C_t values of the detected genes were in close proximity, with an average ΔC_t of ~1.4 between the highest and lowest C_t values within the same sample.

For two samples, the N and S genes were detected, but not the *orf1ab* gene. Both samples had C_t values in the low 30s for the detected targets, indicating a low viral RNA concentration.

The S gene was not detected in a total of 39 samples positive for SARS-CoV-2. A majority of the samples had C_t values in the high viral titer range for *orf1ab* and N genes, 4 samples had C_t values in the medium range, and 2 samples had C_t values in the low range. The TaqPath COVID-19 CE-IVD RT-PCR Kit uses a multi-target design, to compensate for emerging SARS-CoV-2 variants and mutations. One reason for the S gene target failure (SGTF) is the presence of the *69-70del* mutation in the S gene. Using the S.delH69V70 assay of the TaqMan SARS-CoV-2 Mutation Panel, a PCR genotyping assay, we confirmed the presence of the *69-70del* mutation in 37 of the 39 samples with the observed SGTF phenotype (one specimen

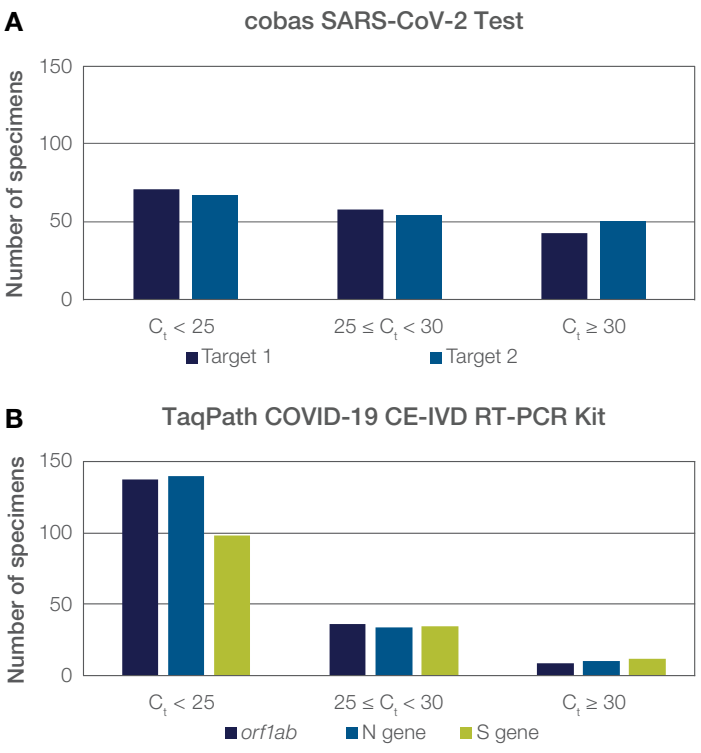

Figure 2. Overview of C_t values. (A) C_t values from 172 specimens that tested positive with the cobas SARS-CoV-2 Test. **(B)** C_t values from 184 specimens that tested positive for SARS-CoV-2 with the TaqPath COVID-19 CE-IVD RT-PCR Kit. In 2 specimens, the *orf1ab* gene was not detected. In 39 specimens, the S gene was not detected.

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was not included in the test and one specimen failed to amplify due to low viral titer). The *69-70del* mutation is present in the Alpha (B.1.1.7) variant of concern, as well as other variants. Because of this, the SGTF of the TaqPath COVID-19 CE-IVD RT-PCR Kit may signal the presence of the *69-70del* mutation and thus potentially the Alpha (B.1.1.7) variant.

The Alpha variant of concern was reported in the majority of cases by mid-March in Germany [1]. From the samples collected in February 2021, we observed the SGTF phenotype in ~21% of the SARS-CoV-2 positive specimens. Further investigation is required to confirm whether isolates harboring the *69-70del* mutation represent the Alpha variant of concern.

Conclusions

The TaqPath COVID-19 CE-IVD RT-PCR Kit supports a wide range of qPCR instruments such as all Applied Biosystems 7500 systems (7500, 7500 Fast, 7500 Fast Dx), the QuantStudio 5 system (in 96-well, 0.1 mL and 0.2 mL blocks, and 384-well block format), and the QuantStudio 7 Flex system (384-well block format). The turnaround time from sample to result is about 3 hours for 94 samples in a 96-well format and an estimated 6.5 hours for 379 samples in a 384-well format. Please note that these turnaround times include time to receive samples, which may vary between labs; we assumed 45 minutes to receive 94 samples and 100 minutes to receive 379 samples in our calculations. Estimated times are based on the use of one KingFisher Flex Purification System with a processing time of 25 minutes for one 96-well plate and a total of 30 minutes to prepare the PCR run.

The TaqPath COVID-19 CE-IVD RT-PCR Kit is also designed to allow staggering of sample batches to support high-throughput sample testing. For example, laboratories equipped with one KingFisher Flex Purification System and two qPCR instruments (in 96-well format) can achieve testing of up to ~2,800 samples in 24 hours (with 2 full-time employees per 8-hour shift).

The TaqPath COVID-19 CE-IVD RT-PCR Kit displayed excellent clinical performance with a PPA of 99.4% and a NPA of 95.3% to the comparator. After resolving discordant samples using NGS, the specificity and sensitivity of the TaqPath COVID-19 CE-IVD RT-PCR Kit was calculated to be 100% for both. (Note: discordant results that could not be resolved were excluded from the sensitivity and specificity calculations.)

References

1. van Loon W, Rössig H, Burock S, et al. (2021) Emergence of SARS-CoV-2 B.1.1.7 Lineage at Outpatient Testing Site, Berlin, Germany, January-March 2021. *Emerg Infect Dis.* 27(7):1931-1934. doi:10.3201/eid2707.210845

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