Performance validation of the UgenTec CE-IVD FastFinder Assay Plugin for the TaqPath COVID-19 CE-IVD RT-PCR Kit

Abstract

The UgenTec™ FastFinder assay plugin for the Applied Biosystems™ TaqPath™ COVID-19 CE-IVD RT-PCR Kit is now available as a diagnostic device for the detection of nucleic acid from SARS-CoV-2. The FastFinder solution utilizes an assay-specific algorithm and decision mechanism to convert raw PCR data into test results. The diagnostic device utilizes the TaqPath COVID-19 CE-IVD RT-PCR Kit workflow, with the exception that PCR-generated results are analyzed with the FastFinder plugin instead of the Applied Biosystems™ COVID-19 Interpretive Software. This white paper describes the performance validation studies performed by UgenTec.

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 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 from individuals suspected of having a SARS-CoV-2 infection. This test uses a multitarget design, which the European Centre for Disease Prevention and Control (ECDC) and the World Health Organization (WHO) have noted may be useful for identifying emerging variants [1,2].

The UgenTec FastFinder Analysis platform hosts, processes, and displays the results of assay-specific plugins. The FastFinder assay plugin for the TaqPath COVID-19 RT-PCR Kit is intended to be used in combination with the TaqPath kit and a supported RT-PCR instrument to convert PCR raw data into test results. Combining the TaqPath COVID-19 CE-IVD RT-PCR Kit with the assay plugin transforms the FastFinder platform into a system for automated PCR analysis.

Workflow

An overview of the workflow is provided below (Figure 1). Note that the instructions for use (IFU) provided by UgenTec must be followed to run the plugin.

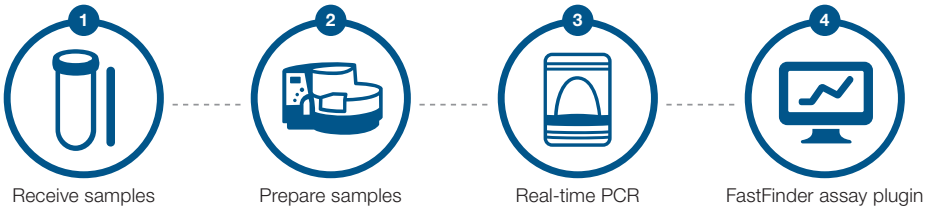

Figure 1. Workflow of the FastFinder assay plugin for the TaqPath COVID-19 CE-IVD RT-PCR Kit.

Once upper respiratory or bronchoalveolar lavage (BAL) specimens are received by the lab (step 1), nucleic acid is purified (step 2). The purified RNA is reverse-transcribed into cDNA, and then amplified using the TaqPath COVID-19 CE-IVD RT-PCR Kit and one of the following supported Applied Biosystems™ real-time PCR instruments (step 3):

- 7500 Real-Time PCR Instrument
- 7500 Fast Real-Time PCR Instrument
- 7500 Fast Dx Real-Time PCR instrument
- QuantStudio™ 5 Real-Time PCR Instrument (96-well, 0.2 mL; 96-well, 0.1 mL; and 384-well)
- QuantStudio™ 5 Dx Real-Time PCR Instrument (96-well, 0.2 mL)
- QuantStudio™ 7 Flex Real-Time PCR Instrument (384-well)

Upon completion of the PCR, the raw data (SDS or EDS files) are transferred to the FastFinder assay plugin for data analysis (step 4). Please note that step 4 differs from the workflow of the TaqPath COVID-19 RT-PCR Kit, which uses Applied Biosystems™ COVID-19 Interpretive Software to analyze the data and generate a report.

Interpretation rules for control validity and sample results were designed into the assay plugin based on the TaqPath COVID-19 CE-IVD RT-PCR Kit IFU. The rules engine contains a decision tree with criteria for defining a final result (detected, not detected, inconclusive, or invalid) with criteria established and defined in the TaqPath COVID-19 CE-IVD RT-PCR Kit IFU, including the C_q cutoff values. As an additional safeguard, the FastFinder plugin assigns samples as marked for manual review, blocking authorization and sign-out until a trained professional has confirmed or rejected the suggested sample status or C_q change.

If tracking control samples over time is desired, users can contact UgenTec to obtain the FastFinder Quality Control (QC) module. According to the FastFinder plugin IFU, the QC module can be used to assess assay precision or perform trend analysis, as it is designed to detect both random and systematic errors. Since the QC module is an independent software module in the platform, performance of the QC module was not in scope of this validation study.

The data analysis component of the plugin differentiates positive curves from negative curves and determines the C_q values of each PCR curve using a static model trained by machine learning technology. The FastFinder plugin uses the same C_q cutoff values for each assay target as the COVID-19 Interpretive Software, which are listed in the IFU of the TaqPath COVID-19 CE-IVD RT-PCR Kit. With the FastFinder plugin, it is possible to manually override the C_q cutoff values listed in the TaqPath COVID-19 CE-IVD RT-PCR Kit IFU and manually review results, which is not supported using the COVID-19 Interpretive Software, and would be considered off-label use of the assay. All changes in the FastFinder software are audit-trailed to maintain full data integrity.

Optionally, a cross-contamination and prevalence-driven contamination detection feature can be enabled in the “Lab Configuration” tab before analysis. This feature will mark samples with suspicious amplification (e.g., a weak-positive sample with a strong-positive neighbor or a cluster of positive samples) for manual review by adding an error notification. It should be noted that manual intervention is not permitted in the COVID-19 Interpretive Software.

Requirements for control presence and validity are designed into the assay plugin, and the user is responsible for performing a final review of the interpreted results. All errors must be resolved, either by confirming them or by changing the results before the analysis can be authorized. Please note that the COVID-19 Interpretive Software used with the TaqPath COVID-19 CE-IVD RT-PCR Kit does not support result changes.

All assay-specific information (device type, assay type, used channels and targets, algorithm, and decision tree) is fixed within the assay plugin. The plugin is self-contained and encrypted, offering full sample traceability across workflow steps. Upon final review and authorization, results may be exported as input for an external software system (e.g., LIMS).

Performance

Performance metrics such as limit of detection, sensitivity, specificity, accuracy, positive percent agreement (PPA), and negative percent agreement (NPA) were assessed by UgenTec as described below.

Limit of detection (LOD)

UgenTec used the data files of the original LOD study from Thermo Fisher to confirm comparable LOD of the FastFinder plugin to the COVID-19 Interpretive Software for the TaqPath COVID-19 CE-IVD RT-PCR Kit. The LOD reflects the lowest SARS-CoV-2 viral concentration (genomic copy equivalent, or GCE) that can be detected at least 95% of the time.

Pooled, contrived nasopharyngeal swab (NP) and bronchoalveolar lavage (BAL) samples were spiked with purified SARS-CoV-2 viral genomic RNA at the LOD established in the TaqPath COVID-19 CE-IVD RT-PCR Kit IFU. To confirm comparable LOD, each target must be detected in at least 19 out of 20 replicates (95%) prepared at or below the LOD. Contrived samples were processed through the TaqPath COVID-19 CE-IVD RT-PCR Kit workflow using the 7500 Fast Dx (96-well format) and QuantStudio 7 Flex (384-well format) real-time PCR instruments. UgenTec processed the resulting data files using each instrument-specific FastFinder assay plugin. SARS-CoV-2 viral RNA was detected in 100% of replicates for both sample types on both instruments (LOD results obtained from the same data files using the COVID-19 Interpretive Software are shown as reference) (Table 1). Therefore, UgenTec concluded that the FastFinder plugin's performance is comparable to the COVID-19 Interpretive Software with contrived NP and BAL samples at 1x LOD.

Table 1. Percent of positive samples detected from 20 replicates at 1x LOD on 7500 Fast Dx (96-well) and QuantStudio 7 Flex (384-well) systems by the FastFinder plugin and COVID-19 Interpretive Software.

Specimen type	LOD	FastFinder plugin		COVID-19 Interpretive Software	
		7500 Fast Dx system (96-well)	QuantStudio Flex system (384-well)	7500 Fast Dx system (96-well)	QuantStudio 7 Flex system (384-well)
BAL	10 GCE/reaction, 250 GCE/mL	100%	100%	100%	100%
NP	10 GCE/reaction, 250 GCE/mL	100%	100%	100%	100%

Clinical evaluation

UgenTec used the data files of the original clinical evaluation study from Thermo Fisher to evaluate the performance of the FastFinder plugin for the TaqPath COVID-19 CE-IVD RT-PCR Kit. In this study, a total of 450 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 FastFinder Assay Plugin for the TaqPath COVID-19 CE-IVD RT-PCR Kit.

Four samples deemed inconclusive with the cobas SARS-CoV-2 Test were removed from analysis.

PPA and NPA were calculated for the TaqPath COVID-19 CE-IVD RT-PCR Kit using the FastFinder plugin relative to the cobas SARS-CoV-2 comparator test with confidence intervals calculated using the Clopper-Pearson method. The results are summarized in Tables 2 and 3:

Table 2. Detection of SARS-CoV-2 in clinical samples using TaqPath COVID-19 CE-IVD RT-PCR Kit with the FastFinder plugin vs. a comparator method.

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

Table 3. PPA and NPA of the TaqPath COVID-19 CE-IVD RT-PCR Kit using the FastFinder plugin vs. cobas SARS-CoV-2 Test.

	Concordance	Percentage	95% Confidence interval
PPA	171/172	99.4%	96.8%–100.0%
NPA	261/274	95.3%	92.0%–97.4%

Sensitivity, specificity, and accuracy of the FastFinder plugin versus COVID-19 Interpretive Software

The same 450 retrospective upper respiratory samples used in the clinical evaluation study were utilized to determine accuracy, sensitivity, and specificity by directly comparing results from the TaqPath COVID-19 CE-IVD RT-PCR Kit using the FastFinder Assay Plugin and COVID-19 Interpretive Software. Results obtained using the TaqPath COVID-19 CE-IVD RT-PCR Kit, which uses the COVID-19 Interpretive Software, were set as true positive and true negative (Table 4).

The FastFinder plugin validation studies performed by UgenTec demonstrated that the sensitivity, specificity, and accuracy are 100% when compared to the COVID-19 Interpretive Software (Table 5). The FastFinder Assay Plugin reported the same results as the COVID-19 Interpretive Software for 449 of the 450 samples tested. One sample that tested positive for SARS-CoV-2 with the TaqPath COVID-19 CE-IVD RT-PCR Kit had an inconclusive result using the FastFinder Assay Plugin and was removed from the calculation.

Sensitivity, specificity, and accuracy

Sensitivity is the ability of the test to correctly identify true positives, whereas specificity refers to the ability to identify true negatives. The accuracy of a test is its ability to differentiate between true positive and true negative samples. The following formulas* were used to calculate sensitivity, specificity, and accuracy [1]:

Sensitivity = (TP)/(TP+FN)

Specificity = (TN)/(TN+FP)

Accuracy = (TP+TN)/(TP+TN+FP+FN)

* TP: true positive; FP: false positive; TN: true negative; FN: false negative.

Table 4. Detection of SARS-CoV-2 in clinical samples with the FastFinder plugin vs. COVID-19 Interpretive Software.

Detection of SARS-CoV-2	Comparator: TaqPath COVID-19 CE-IVD Kit with COVID-19 Interpretive Software		
TaqPath COVID-19 kit CE-IVD with FastFinder plugin	Positive	Negative	Total
Positive	186	0	186
Negative	0	263	263
Total	186	263	449

Table 5. Accuracy, sensitivity, and specificity of the FastFinder plugin vs. COVID-19 Interpretive Software.

Performance metric	Percent	95% Confidence interval
Sensitivity	100%*	97.9%–100%
Specificity	100%*	98.6%–100%
Accuracy	100%*	99.2%–100%

* One sample that tested positive for SARS-CoV-2 with the TaqPath COVID-19 CE-IVD RT-PCR Kit had a “void” result using the FastFinder Assay Plugin and was removed from the calculation.

Bridging studies to supported RT-PCR instruments

To validate performance of all supported PCR instrument-specific assay plugins, UgenTec performed a bridging study using data files from each supported instrument from the original bridging studies done by Thermo Fisher. The study utilized eluates from pooled negative NP samples formulated to varying concentrations of SARS-CoV-2 RNA relative to the LOD. Three replicates of each concentration (9x, 3x, 1x, 0.33x, and 0.11x LOD) were prepared on each plate.

All samples were run on each PCR instrument using both the COVID-19 Interpretive Software and the FastFinder plugin. The lowest concentration that resulted in 3/3 positive calls on each instrument on both software analysis programs is listed in Table 6. Each line in Table 6 represents an individual run file; some instruments had multiple run files. Each run contained 3 negative controls (NC), and all 3 NCs were negative by both types of analysis software on every instrument tested.

Results in Table 6 demonstrate that the FastFinder plugins for the bridged PCR instruments can detect SARS-CoV-2 at concentrations close to the LOD. The FastFinder plugin detected 3/3 positive results at lower concentrations than the COVID-19 Interpretive Software in 7/12 of the data files that were analyzed. According to the FastFinder plugin's IFU, it is possible that samples around the LOD will be flagged by the FastFinder plugin for manual review. In the bridging study, 38/216 results were flagged for review. None of the 38 flagged results were changed, however, one curve without a flag was changed from "not detected" to "detected" in an uncertain well. It should be noted that such manual intervention is not supported using the COVID-19 Interpretive Software.

Table 6. Validation of various RT-PCR instruments: lowest concentration for positive calls in 3/3 replicates.

Instrument	FastFinder plugin	COVID-19 Interpretive Software
7500 Fast Dx system	0.33x LOD	0.33x LOD
	0.33x LOD	0.33x LOD
	0.33x LOD	0.33x LOD
	0.33x LOD	1x LOD
7500 Fast system	0.33x LOD	0.33x LOD
	0.33x LOD	1x LOD
	1x LOD	1x LOD
7500 system	0.33x LOD	1x LOD
	0.33x LOD	1x LOD
QuantStudio 5 Fast system	0.11x LOD	0.33x LOD
QuantStudio 5 system	0.33x LOD	1x LOD
QuantStudio 5 Dx system	0.33x LOD	1x LOD

Summary

UgenTec now offers CE-IVD–marked FastFinder assay plugins for the TaqPath COVID-19 CE-IVD RT-PCR Kit. This decision-support software has been validated to analyze and interpret the results of the TaqPath COVID-19 CE-IVD Kit generated on six widely used RT-PCR instruments. The validation study performed by UgenTec demonstrates comparable performance between the FastFinder plugin and the COVID-19 Interpretive Software. The FastFinder assay plugin for the TaqPath COVID-19 CE-IVD RT-PCR Kit is now available as a diagnostic device to detect SARS-CoV-2 to enable efficient and accurate diagnosis, helping to ensure proper patient care.

References

1. https://www.ecdc.europa.eu/sites/default/files/documents/SARS-CoV-2-variant-multiple-spike-protein-mutations-United-Kingdom.pdf
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3. H. M. N. A. E. A. G. Baratloo A, "Part 1: Simple Definition and Calculation of Accuracy, Sensitivity and Specificity.," Emergency, vol. 3, no. 2, pp. 48–49, 2015.

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For more information on Thermo Fisher Scientific products, visit **thermofisher.com/ugentecform**

For more information on UgenTec FastFinder software, visit **ugentec.com/create-account/thermofisher**

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