Performance validation of the UgenTec CE-IVD FastFinder Assay Plugin for the TaqPath COVID-19, Flu A/B, RSV Combo Kit (CE-IVD)

Abstract

The UgenTec™ FastFinder assay plugin for the Applied Biosystems™ TaqPath™ COVID-19, Flu A/B, RSV Combo Kit is now available as a diagnostic device for the detection of SARS-CoV-2, the influenza A/B (Flu A and Flu B) viruses, and the respiratory syncytial virus (RSV). The FastFinder solution utilizes an assay-specific algorithm and decision mechanism to convert PCR raw data into test results. The diagnostic device utilizes the TaqPath COVID-19, Flu A/B, RSV Combo Kit workflow, with the exception that PCR-generated results are analyzed with the UgenTec FastFinder plugin instead of Applied Biosystems™ Pathogen Interpretive Software. This white paper describes the performance validation studies performed by UgenTec.

Introduction

With SARS-CoV-2 now part of the viral ecosystem, laboratories need to test for multiple respiratory viruses in a single patient sample. This helps to detect and differentiate viral infections like RSV and flu that present symptoms similar to those of COVID-19, enabling accurate diagnosis and proper patient care. The CE-IVD–marked TaqPath COVID-19, Flu A/B, RSV Combo Kit is a highly accurate, multiplex real-time PCR assay that can detect and differentiate between SARS-CoV-2, influenza A/B, and RSV in a single sample, enabling laboratories to expand their respiratory sample testing menu while keeping costs under control.

Workflow

The FastFinder Assay Plugin for the TaqPath COVID-19, Flu A/B, RSV Combo Kit is intended to be used in combination with the assay kit to convert PCR raw data into test results. An overview of the workflow is provided below (Figure 1). Note that the UgenTec plugin's instructions for use (IFU) must be followed to run the plugin.

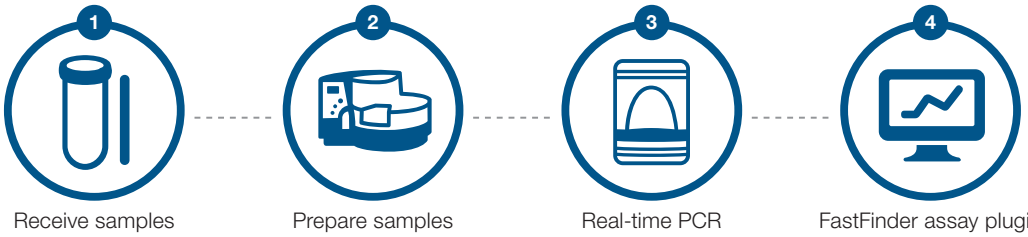

Figure 1. Workflow of the FastFinder Assay Plugin for the TaqPath COVID-19, Flu A/B, RSV Combo Kit.

Once nasopharyngeal (NP) swab 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, Flu A/B, RSV Combo Kit on one of the following real-time PCR instruments (step 3):

- Applied Biosystems™ 7500 Fast Real-Time PCR Instrument
- Applied Biosystems™ QuantStudio™ 5 Real-Time PCR Instrument, 96-well, 0.2-mL block
- Applied Biosystems™ QuantStudio™ 7 Flex Real-Time PCR Instrument, 384-well block

Upon completion of PCR, the raw data (SDS or EDS files) are transferred to the FastFinder Assay Plugin for data analysis (step 4). Please note that this step differs from the workflow of the TaqPath COVID-19, Flu A/B, RSV Combo Kit, which uses the Pathogen Interpretive Software CE-IVD Edition to analyze the data and generate a report.

The FastFinder analysis platform hosts, processes, and displays the results of assay-specific plugins. With the TaqPath COVID-19, Flu A/B, RSV Combo Kit and its corresponding assay plugin, the FastFinder platform transforms into a system for automated PCR analysis. The analysis workflow begins with a PCR raw data file from an assay-supported instrument and ends with a sample result.

Interpretation rules for control validity and sample results were designed into the assay plugin according to the TaqPath COVID-19, Flu A/B, RSV Combo 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 IFU of the assay using the predefined C_q cutoffs. 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. The QC module can be used to assess assay precision or perform trend analysis, as it is able 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 the 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 Pathogen Interpretive Software, which are listed in the IFU of the TaqPath COVID-19, Flu A/B, RSV Combo Kit. Please note that with FastFinder plugin, it is possible to manually override the C_q cutoff values listed in the TaqPath COVID-19, Flu A/B, RSV Combo Assay IFU, which is not supported by the Pathogen 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. Please note that manual intervention is not supported by the Pathogen 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 Pathogen Interpretive Software used with the TaqPath COVID-19, Flu A/B, RSV Combo Assay 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. Plugins are self-contained and encrypted. The assay plugin offers 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 Scientific to confirm comparable LOD of the FastFinder plugin to the Pathogen Interpretive Software for the TaqPath COVID-19, Flu A/B, RSV Combo Kit. The LOD reflects the lowest SARS-CoV-2, influenza A, influenza B, and RSV A and RSV B viral concentrations (genomic copy equivalents (GCE) or TCID50/mL, as indicated) that can be detected at least 95% of the time.

Pooled, contrived NP specimens were spiked with concentrations of virus (live or inactivated) from SARS-CoV-2, influenza A, influenza B, RSV A or RSV B at the LOD established for the TaqPath COVID-19, Flu A/B, RSV Combo Kit. 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, Flu A/B, RSV Combo Kit workflow using the 7500 Fast Real-Time PCR Instrument. UgenTec processed the resulting data files using the FastFinder Assay Plugin. All viruses were detected in at least 95% of replicates (results obtained from the same data files using the Pathogen Interpretive Software are shown as reference) (Table 1). Therefore, UgenTec concluded that the FastFinder plugin performs comparable to the Pathogen Interpretive Software with contrived samples at 1X LOD.

Table 1. LOD of viral targets.

Virus	Subtype/ lineage	Strain/isolate	LOD		% positive by FastFinder plugin	% positive by Pathogen Int. Software
SARS-CoV-2	N/A	USA-WA1/2020	50 GCE/mL	8.24×10^{-3} TCID50/mL	100%	100%
Influenza A (Perth)	H3N2	A/Perth/16/2009	350 GCE/mL	2.21×10^{-2} TCID50/mL	100%	100%
Influenza A (Brisbane)	H1N1	A/Brisbane/59/2007	384 GCE/mL	1.23×10^{-3} TCID50/mL	95%	95%
Influenza B (Florida)	Yamagata	B/Florida/04/2006	1,250 GCE/mL	1.47×10^{-1} TCID50/mL	100%	95%
Influenza B (Wisconsin)	Yamagata	B/Wisconsin/01/2010	350 GCE/mL	4.24×10^{-3} TCID50/mL	100%	95%
RSV A	N/A	A/2006	200 GCE/mL	1.36×10^{-2} TCID50/mL	95%	95%
RSV B	N/A	B/3/2015 Isolate #2	200 GCE/mL	1.31×10^{-2} TCID50/mL	100%	95%

Clinical evaluation

UgenTec used the data files of the original clinical evaluation study from Thermo Fisher to evaluate the performance of the FastFinder Assay Plugin for the TaqPath COVID-19, Flu A/B, RSV Combo Kit using the following archived NP specimens:

- 55 NP positive/65 NP negative for **SARS-CoV-2**
- 55 NP positive/114 NP negative for **influenza A/B**
- 55 NP positive/125 NP negative for **RSV**

Samples were extracted using the Applied Biosystems™ MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit (Applied Biosystems™ TaqPath™ COVID-19 Combo Kit Advanced and TaqPath™ COVID-19, Flu A/B, RSV Combo Kit) or the bioMérieux™ NucliSENS™ easyMAG™ platform (Lyra™ assays). All samples were then tested using the FastFinder Assay Plugin for TaqPath COVID-19, Flu A/B, RSV Combo Kit using the 7500 Fast instrument and the following target-specific comparator tests (performed according to their respective IFU):

- **SARS-CoV-2** compared against the TaqPath COVID-19 Combo Kit Advanced
- **Influenza A and B** compared against the Quidel™ Lyra™ Influenza A + B Assay
- **RSV A and B** compared against the Quidel™ Lyra™ RSV + hMPV Assay

PPA and NPA were calculated relative to each comparator test (Table 3).

Table 3 shows the PPA and NPA for the same data set processed using the TaqPath COVID-19, Flu A/B, RSV Combo Kit using the Pathogen Interpretive Software as a reference. The results were identical, with the exception of a single sample* that was negative by both the Lyra Influenza A + B Assay and the TaqPath COVID-19, Flu A/B, RSV Combo Kit using the Pathogen Interpretive Software, but was automatically flagged for manual review using the FastFinder plugin. For this reason, the flu A/B NPA was slightly lower for the FastFinder plugin (95.6%) than the Pathogen Interpretive Software (96.5%).

* In case of an uncertain result call, FastFinder software automatically raises the result for manual review, to alert the trained professional to confirm or reject a result that was marked as uncertain. Such samples can only be authorized for review after a manual intervention.

Table 2. Results of performance evaluation versus comparator methods.

Influenza	Comparator: Lyra Influenza A + B Assay			COVID-19	Comparator: TaqPath COVID-19 Combo Kit Advanced			RSV	Comparator: Lyra Influenza RSV + hMPV Assay		
FastFinder	**Positive**	**Negative**	**Total**	**FastFinder**	**Positive**	**Negative**	**Total**	**FastFinder**	**Positive**	**Negative**	**Total**
Positive	55	5	60	**Positive**	54	0	54	**Positive**	54	9	63
Negative	0	109	109	**Negative**	1*	65	66	**Negative**	1	116	117
Total	55	114	169	**Total**	55	65	120	**Total**	55	125	180

Table 3. PPA and NPA for FastFinder plugin and the Pathogen Interpretive Software versus comparator methods.

	Target:	Flu A/B	COVID-19	RSV A/B
FastFinder plugin	**PPA**	100% (93.5%–100.0%)	98.2% (90.3%–100.0%)	98.2% (90.3%–100.0%)
	NPA	95.6% (90.1%–98.6%)	100% (94.5%–100.0%)	92.8% (86.8%–96.7%)
Pathogen Interpretive Software	**PPA**	100% (93.5%–100.0%)	98.2% (90.3%–100.0%)	98.2% (90.3%–100.0%)
	NPA	96.5% (91.3%–99.0%)	100% (94.5%–100.0%)	92.8% (86.8%–96.7%)

Sensitivity, specificity, and accuracy of FastFinder plugin versus Pathogen Interpretive Software

UgenTec demonstrated the sensitivity, specificity, and accuracy for the FastFinder Assay Plugin for the TaqPath COVID-19, Flu A/B, RSV Combo Kit using the clinical evaluation data files and comparing results generated by the FastFinder plugin to those from the Pathogen Interpretive Software (Table 4). Results obtained using the Pathogen Interpretive Software were set as true positive and true negative.

The validation studies performed by UgenTec on the FastFinder platform demonstrated that the accuracy, sensitivity, and specificity are nearly 100% for all three targets when compared to the Pathogen Interpretive Software (Table 4).

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. Sensitivity, specificity, and accuracy of the FastFinder plugin vs. Pathogen Interpretive Software.

		Viral target		
		Flu A/B	COVID-19	RSV
Performance metric	Sensitivity	100% (94.0%–100%)	100% (93.4%–100%)	100% (94.3%–100%)
	Specificity	99.2% (95.5%–100%)	100% (94.6%–100%)	100% (96.9%–100%)
	Accuracy	99.4% (96.9%–100%)	100% (97%–100%)	100% (98.0%–100%)

Table 5. Validation of FastFinder plugins for the QuantStudio 5 and QuantStudio 7 Flex systems. Lowest concentration of viral targets detected in at least 95% of replicates.

QuantStudio 5 system (96-well, 0.2 mL)		
Target virus	FastFinder plugin	Pathogen Interpretive Software
SARS-CoV-2	100% positive at 0.33x LOD	100% positive at 1x LOD
Flu A (H1N1)	100% positive at 1x LOD	100% positive at 3x LOD
Flu B (Florida)	100% positive at 1x LOD	100% positive at 1x LOD
RSV A	100% positive at 1x LOD	100% positive at 1x LOD
RSV B	95% positive at 0.33x LOD	100% positive at 1x LOD

QuantStudio 7 Flex system (384-well)		
Target virus	FastFinder plugin	Pathogen Interpretive Software
SARS-CoV-2	100% positive at 0.33x LOD	100% positive at 3x LOD
Flu A (H1N1)	100% positive at 3x LOD	100% positive at 3x LOD
Flu B (Florida)	100% positive at 1x LOD	100% positive at 1x LOD
RSV A	100% positive at 1x LOD	100% positive at 1x LOD
RSV B	100% positive at 1x LOD	95% positive at 1x LOD

Bridging studies to the QuantStudio 5 and QuantStudio 7 Flex systems

To validate performance of all supported PCR instrument–specific assay plugins, UgenTec performed a bridging study for the QuantStudio 5 Real-Time PCR System (96-well, 0.2 mL) and the QuantStudio 7 Flex Real-Time PCR System (384-well).

The study utilized the data files of the original bridging study performed by Thermo Fisher. In this study, eluates from contrived NP samples were formulated at varying concentrations of the viral target relative to the previously determined LOD on the 7500 Fast Real-Time PCR Instrument. Twenty replicates of each target virus were prepared at 3x LOD, 1x LOD, 0.33x LOD, and 0.17x LOD to determine the lowest concentration that could be detected at least 95% (19/20) of the time. To confirm known negative samples result in a negative call, three replicates of a negative specimen devoid of analyte were included on each plate. All samples were

run on the QuantStudio 5 Real-Time PCR System (96-well, 0.2 mL) and QuantStudio 7 Flex Real-Time PCR System (384-well), and results were analyzed using the FastFinder Assay Plugin with the corresponding instrument-specific plugin. As a reference, the same data were also evaluated using the Pathogen Interpretive Software. The lowest concentration of each virus that was detected in at least 95% of replicates is listed in Table 5.

The acceptance criteria to conclude comparable performance between instrument-specific plugins was achieved. At least one test level yielded ≥95% positivity on each instrument. In addition, all negative samples produced a negative result for all five viral targets on both instruments and both software analysis methods. Results in Table 5 demonstrate that the FastFinder plugins for the QuantStudio 5 Real-Time PCR System (96-well, 0.2 mL) and QuantStudio 7 Flex Real-Time PCR System (384-well) can detect each target at concentrations near the LOD, with performance similar to that of the Pathogen Interpretive Software.

According to the plugin IFU, it is possible that samples around the LOD will be flagged by the FastFinder software for manual review. In the bridging study, 77/1,176 results were flagged for review. Of these, 74 samples were confirmed positive and 3 sample results were changed (2 were changed to negative, and 1 was changed to inconclusive). It should be noted that such manual intervention is not supported using the Pathogen Interpretive Software.

Summary

UgenTec now offers the CE-IVD-marked FastFinder Assay Plugin for the TaqPath COVID-19, Flu A/B, RSV Combo Kit. This decision-support software has been validated to analyze and interpret the results of the TaqPath COVID-19, Flu A/B, RSV Combo Kit generated on the 7500 Fast, QuantStudio 5 (96-well, 0.2 mL), and QuantStudio 7 Flex real-time PCR instruments from nasopharyngeal swabs. The validation study performed by UgenTec demonstrates comparable performance between FastFinder software and the Pathogen Interpretive Software. The FastFinder Assay Plugin for the TaqPath COVID-19, Flu A/B, RSV Combo Kit is now available as a diagnostic device to detect and differentiate SARS-CoV-2, influenza A and influenza B (flu A/B) viruses, and respiratory syncytial virus (RSV) from a single sample to enable efficient and accurate diagnosis, helping to ensure proper patient care.

Reference

1. 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.

CE IVD

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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