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VERSANT kPCR Molecular System

Automation of the FTD SARS-CoV-2 Assay

Guido Hennig, PhD
Ellen Sampson, MS, MBA
Global Scientific Marketing

siemens-healthineers.com/molecular

Siemens Healthineers Headquarters
Siemens Healthcare GmbH
Henkestr. 127
91052 Erlangen, Germany
Phone: +49 9131 84-0
siemens-healthineers.com

Legal Manufacturer
Fast Track Diagnostics
Luxembourg S.à.r.l.
29, rue Henri Koch
4354 Esch-sur-Alzette
Luxembourg

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SIEMENS Healthineers

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Introduction

Fast Track Diagnostics (FTD), a Siemens Healthineers company, has a portfolio of real-time PCR kits for single and multiplex detection of pathogenic targets for syndromic testing in infectious disease. These kits can be validated on a number of instruments commonly used in most molecular diagnostic laboratories, namely real-time PCR cyclers and sample-to-results platforms.*

This flexible approach can have considerable value, especially during a pandemic, when the integration of new assays into existing laboratory operations must be efficient and expedited without compromising the quality and accuracy of the patient results.

Validation of the FTD SARS-CoV-2 Assay with the Nuclisens EasyMag and Applied Biosystems 7500

FTD extended its portfolio of kits by developing and commercializing the FTD SARS-CoV-2 Assay during the COVID-19 pandemic. The FTD SARS-CoV-2 Assay is a real-time PCR assay intended to specifically detect the novel coronavirus SARS-CoV-2. The assay design targets two distinct but highly conserved SARS-CoV-2 genomic regions, ORF1ab and N gene, which are both detected in the FAM channel of the real-time PCR cycler (Figure 1). This dual-target design is robust and contributes to high analytical sensitivity. In contrast to other SARS-CoV-2 assays that employ a multiplex design, the dual-target approach potentially reduces the chance of inconclusive results and the need for repeat testing.[1]

The FTD SARS-CoV-2 Assay is CE-IVD–labeled for diagnostic use, authorized by the United States FDA under an Emergency Use Authorization (EUA) for use by authorized laboratories, and listed by the World Health Organization (WHO).†

The FTD SARS-CoV-2 Assay was validated for diagnostic use on the Nuclisens EasyMag system (bioMerieux) and the Applied Biosystems 7500 PCR thermocycler (Thermo Fisher Scientific). This validation demonstrated the FTD SARS-CoV-2 Assay to have excellent performance characteristics under both CE-IVD and EUA. (Table 1 and 2).[2]

Assay	Target Region	Detection Channel
SARS-Cov-2	N	FAM
	ORF1ab	FAM
	IC	Cy5

Figure 1. Dual-target design for FTD SARS-CoV-2 Assay.

**User responsible for validations.*

†CE-IVD labeled for diagnostic use in the EU. This test has not been FDA-cleared or approved. This test has been authorized by FDA under an EUA for use by authorized laboratories. This test has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens. This test is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostics for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Act, 21. U.S.C. § 360bbb- 3(b)(1), unless the authorization is terminated or revoked sooner.

Table 1. Performance characteristics of FTD SARS-CoV-2 Assay (CE-IVD) with the Nuclisens EasyMag system and Applied Biosystems 7500 thermocycler.

Characteristics	Results
Limit of detection	11.5 copies/reaction
Cross-reactivity	No in vitro cross-reactivity with 32 common human flora organisms and the pathogenic organisms causing respiratory infections, including six coronaviruses No in silico cross-reactivity to NCBI nucleotide collection database
Analytical specificity	100% using 50 negative clinical samples, 35 negative controls, and 34 non-template controls
Inclusivity	In silico analysis showed 100% detection rate on 901 full-length sequences from NCBI and GISAID databases
Precision	Repeatability and reproducibility with coefficient of variation (CV) <5%
Interference	No interference with 10 potential interfering substances in clinical samples
Clinical performance	CE-IVD: Diagnostic sensitivity of 100% (91.8–100, 95% CI) and diagnostic specificity of 100% (93.8–100, 95% CI) when compared to the Seegene ALLPLEX 2019-nCoV Assay

Table 2. Performance characteristics of FTD SARS-CoV-2 Assay (FDA EUA) with the Nuclisens EasyMag system and Applied Biosystems 7500 thermocycler.

Characteristics	Results
Limit of detection	0.0023 TCID50/mL
Cross-reactivity	No in vitro cross-reactivity with 32 common human flora organisms and the pathogenic organisms causing respiratory infections, including six coronaviruses No in silico cross-reactivity to NCBI nucleotide collection database
Analytical specificity	100% using 50 negative clinical samples, 35 negative controls, and 34 non-template controls
Inclusivity	In silico analysis showed 100% detection rate on 901 full-length sequences from NCBI and GISAID databases
Precision	Repeatability and reproducibility with coefficient of variation (CV) <5%
Interference	No interference with 10 potential interfering substances in clinical samples
Clinical performance	EUA FDA: Positive percent agreement (PPA) of 100% (92–100, 95% CI) and negative percent agreement (NPA) of 100% (88.7–100, 95% CI) when compared to the Roche COBAS SARS-CoV-2 Assay

Testing for COVID-19 during a pandemic presents challenges for molecular labs

With COVID-19 testing during pandemic conditions, where manufacturers and laboratories experience limited supplies of sample extraction instruments and reagents, thermal cycler instruments, and associated consumables, it became imperative to be able to offer a total solution for COVID-19 testing from Siemens Healthineers. Providing labs not only with SARS-CoV-2 assays but also access to automated real-time PCR platforms that isolate high-quality nucleic acids from clinical samples, automate the PCR plate setup step, and generate data, all with accurate patient traceability, became essential for the timely delivery of actionable results. Furthermore, high-throughput capabilities with limited hands-on-time was especially critical during the COVID-19 pandemic.

Validation of the FTD SARS-CoV-2 Assay with the VERSANT kPCR Molecular System†

The VERSANT kPCR Molecular System comprises the VERSANT kPCR Sample Preparation Module (SP) and the VERSANT kPCR Molecular System AD‡ (AD). The SP, in conjunction with the VERSANT Sample Preparation 1.0 Reagents kit, is a universal solution that automates the extraction of both DNA and RNA from any human and/or pathogenic source.

The magnetic nanobeads with a precipitated nanolayer of silica on the surface are unique to Siemens Healthineers extraction technology and provide for the efficient purification of high-quality nucleic acids from a wide variety of sample types.[4,5]

With the increasing volume of COVID-19 testing and the need for a more automated workflow, the FTD SARS-CoV-2 Assay has now been validated with the VERSANT kPCR Molecular System.† The workflow for the VERSANT kPCR Molecular System is shown in Figure 2.

Samples obtained from patients to be tested for SARS-CoV-2 may first be pretreated by adding 350 µL of VERSANT Lysis Buffer to 350 µL of sample under a biosafety hood in order to mitigate any risk of aerosolization of potentially infectious SARS-CoV-2 virus during extraction (not shown). This pretreatment step is optional and is in accordance with CDC guidelines for the inactivation of SARS-CoV-2.[6]

The samples are loaded onto the SP instrument for automated extraction and PCR plate setup. A preprogrammed protocol, Dynamic Assay Preparation FTD 1 (SP 1.0 500-15-10), is used. This protocol automatically adds an internal control (IC)§ to all samples prior to nucleic acid extraction.

Viral RNA from the clinical samples, along with the IC, are extracted and eluted in a volume of 100 µL. In a final step, 15 µL of the FTD SARS-CoV-2 MasterMix is combined with 10 µL of the sample eluates and dispensed into a 96-well PCR plate. The software that supports the SP instrument also manages the pipetting of the positive control (PC) and the negative control (NC).

In addition, this software includes an option to collect and store the residual volume of the eluates in an eluate storage plate. This residual eluate may have application for other molecular analyses such as sequencing or may be used for subsequent PCR plate setups for use with other FTD respiratory kits. The PCR plate containing the eluates and FTD SARS-CoV-2 MasterMix is then sealed and transferred by the operator to the AD.

During this step, the nucleic acid from the sample is amplified and detected via RT-qPCR using a very fast 90-minute PCR cycling protocol. VERSANT MiPLX software (version 2.0) is used for data analysis including cycle thresholds (Ct) and an analysis report. Schematic details of the workflow are shown in Figure 2.

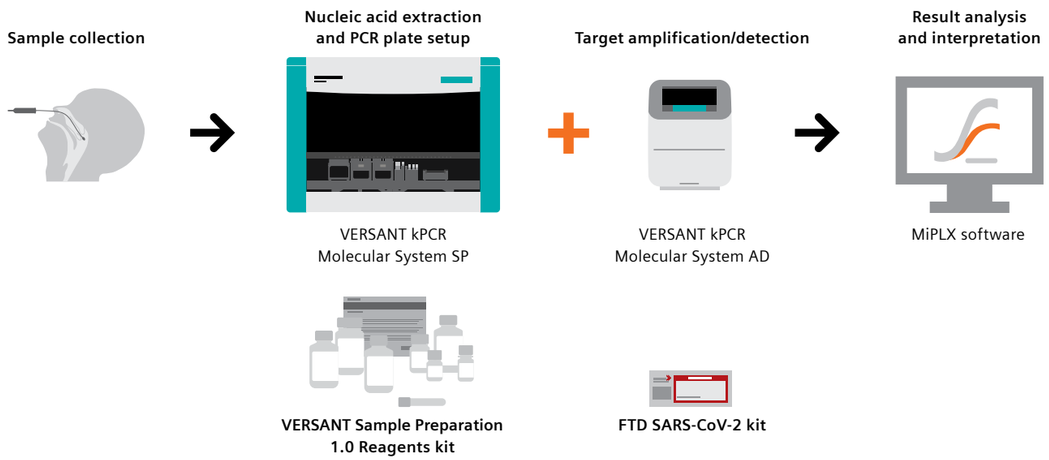

Figure 2. VERSANT kPCR Molecular System workflow for the FTD SARS-CoV-2 Assay.

†CE-IVD labeled for diagnostic use in the EU. This test has not been FDA-cleared or approved. This test has been authorized by FDA under an EUA for use by authorized laboratories. This test has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens. This test is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostics for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Act, 21. U.S.C. § 360bbb- 3(b)(1), unless the authorization is terminated or revoked sooner.
‡QUANTSTUDIO 5 DX real-time thermocycler (originally supplied by ThermoFisher) modified to run MiPLX software.
§The IC must be diluted 1:5 prior to adding it to the instrument deck.

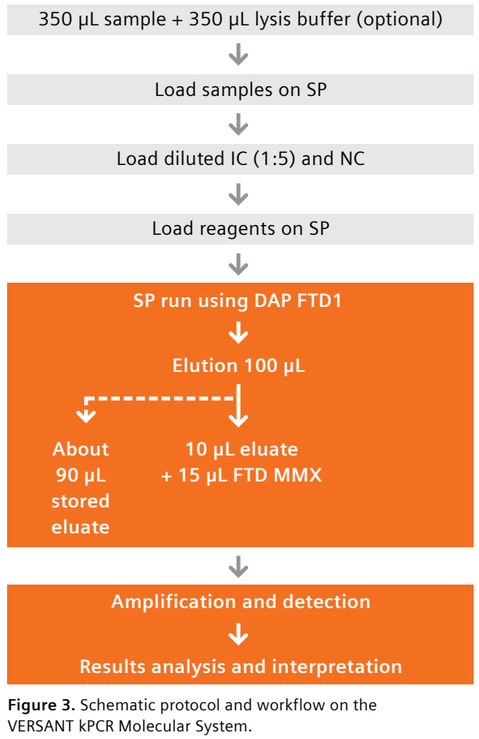

Figure 3. Schematic protocol and workflow on the VERSANT kPCR Molecular System.

The automated extraction and PCR plate setup require a maximum of 210 minutes for a full plate of 96 PCR reactions. The entire process, including sample handling, instrument setup, PCR cycling, and generation of results, takes about 5 hours and 30 minutes for 96 PCR reactions. A laboratory can process up to 192 PCR reactions in 9 hours using one VERSANT kPCR Molecular System (Table 3).

Analytical sensitivity (LoD)†

Twenty individual samples were prepared by spiking a concentration of 0.0023 TCID50/mL of SARS-CoV-2 virus culture into a simulated respiratory matrix (enriched transport media spiked with mucin, blood, and HeLA cells to mimic clinical samples).

Samples were prepared with three different types of transport media, including Universal Viral Transport (UVT, Becton Dickinson), Viral Transport Medium (VTM, in-house preparation based on CDC SOP #DSR-052-01), and ESWAB Liquid Amies medium (ESWAB, COPAN).

The limit of detection (LoD) of 0.0023 TCID50/mL, with an overall detection rate of greater than 95% and as previously determined on the EasyMag/ABI 7500 workflow, was confirmed on the VERSANT kPCR Molecular System (Table 4). Interestingly, the LoDs of the Roche COBAS SARS-CoV-2 assay (ORF1a gene target with 0.007 TCID50/mL and E gene target with 0.004 TCID50/mL) and Hologic Panther Fusion SARS-CoV-2 assay (0.01 TCID50/mL) were both reported to be less sensitive than the FTD SARS-CoV-2 Assay on the VERSANT kPCR Molecular System when using the same patient virus culture USA-WA1/2020.[7]

Table 3. Turnaround times (TAT) for FTD SARS-CoV-2 Assay with the VERSANT kPCR Molecular System.

Number of PCR Reactions	Time Required for Extraction and PCR Plate Setup	Time for PCR Run	Total Time
24	110 min	90 min	230 min[a]
48	140 min	90 min	260 min[a]
96	210 min	90 min	330 min[a]
192	2 x 210 min	2 x 90 min	<10 hours[b]

a. Sample handling, loading of consumables, preparation of internal control, positive control, negative control, master mix preparation: 30 minutes.
b. Second extraction run starts immediately after first extraction run has been completed and placed on the thermocycler.

Table 4. LoD of the FTD SARS-CoV-2 Assay on the VERSANT kPCR Molecular System.†

Pathogen	Medium	LoD (TCID50/mL)	Replicates	Total Detected	Detection Rate (%)	Mean Ct	Std Dev
SARS-CoV-2	UVT[a]	0.0023	20	19	95	36.4	0.7
	VTM[b]		20	20	100	36.5	0.9
	ESWAB[c]		20	19	95	37.1	1.0

a Universal Viral Transport (Becton Dickinson).
b Viral Transport Medium (in-house preparation based on CDC SOP #DSR-052-01).
c ESWAB Liquid Amies medium (COPAN).

Clinical Performance†

Clinical performance was assessed by comparing the FTD SARS-CoV-2 Assay on the VERSANT kPCR Molecular System with the Hologic SARS-CoV-2 Assay on the Panther Fusion System.[7] A total of 109 nasopharyngeal swabs, including 60 positive and 49 negative specimens, were collected from male and female patients with signs and symptoms of an upper respiratory tract infection. The same set of clinical samples were tested for SARS-CoV-2 with both the FTD and Hologic assays and systems.

The positive percent agreement was 100% (CI 94.0–100) and the negative percent agreement was 93.9% (CI 83.5–97.9) (Table 5). Overall result concordance was 97%. Interestingly, two of the three discordant samples that were positive by FTD SARS-CoV-2 Assay and negative by the Hologic SARS-CoV-2 Assay were confirmed as positive by sequencing.

Table 5. Clinical performance of the FTD SARS-CoV-2 Assay on the VERSANT kPCR Molecular System.†

Comparison of FTD SARS-CoV-2 Assay with Hologic SARS-CoV-2 Assay (n = 109)			
		Hologic SARS-CoV-2	
		Positive	**Negative**
FTD SARS-CoV-2	Positive	60	3**
	Negative	0	46
Positive percent agreement	100% (60/60) (95% confidence interval: 93.98, 100)		
Negative percent agreement	93.88% (46/49) (95% confidence interval: 83.48, 97.90)		

***2 of the 3 discordant samples that were positive by FTD SARS-CoV-2 and negative by the Hologic SARS-CoV-2 assay were confirmed as positive by sequencing.*

Reproducibility††

A reproducibility study was performed with three independent SP and three AD instruments over three days with one run per day for each paired SP and AD system. Each run consisted of 12 samples representing three sample replicates for two concentration levels (at LoD of 0.0023 TCID50/mL and at 3x LoD of 0.0069 TCID50/mL) and two different sample matrices UVT and VTM.

An overall detection rate of 94% (17/18) was achieved at the LoD of 0.0023 TCID50/mL, and 100% detection was achieved at 3x LoD of 0.0069 TCID50/mL for each system (Table 6). These data show that the VERSANT kPCR Molecular System workflow can generate reproducible and consistent results even at very low virus concentrations around the limit of detection and when interchanging the SP and AD instruments.

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†† Data and documentation on file at Siemens Healthineers.

Table 6. Reproducibility of the FTD SARS-CoV-2 Assay on the VERSANT kPCR Molecular System.††

VERSANT kPCR Molecular System	Day	UVT LoD‡‡ Detected	VTM LoD‡‡ Detected	UVT 3x LoD‡‡ Detected	VTM 3x LoD‡‡ Detected
System A	1	3	2	3	3
	2	3	3	3	3
	3	3	3	3	3
	Detection Rate	94% (17/18)		100% (18/18)	
System B	1	3	3	3	3
	2	3	3	3	3
	3	2	3	3	3
	Detection Rate	94% (17/18)		100% (18/18)	
System C	1	3	3	3	3
	2	3	3	3	3
	3	2	3	3	3
	Detection Rate	94% (17/18)		100% (18/18)	

‡‡Spiked SARS-CoV-2 virus concentration in respective medium.
LoD: concentration at limit of detection; UVT: Universal Viral Transport (Becton Dickinson), VTM: Viral Transport Medium (in-house preparation based on CDC SOP #DSR-052-01).

Summary

The VERSANT kPCR Molecular System, comprising a VERSANT kPCR Sample Preparation instrument (SP) for automated extraction and PCR plate setup and VERSANT kPCR Molecular System AD (AD) for amplification and detection, along with the FTD SARS-CoV-2 Assay provides an optimal testing solution and workflow option for molecular laboratories that need increased automation for COVID-19 testing.

This Siemens Healthineers complete solution combines automation with high quality and efficient recovery of nucleic acids from COVID-19 clinical samples while delivering optimal assay performance and accurate results with a turnaround time that is actionable and timely for patient management.

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