Fast Track DIAGNOSTICS
A Siemens Healthineers Company

IVD RxOnly
For *in vitro* diagnostic use
For use under Emergency Use Authorization Only

FTD™ SARS-CoV-2

Current Revision and Date	11416299_en Rev. E, 2022-01
Product Name	FTD SARS-CoV-2 (FTD-114-96) REF 11416302 Σ 96
Specimen Types	Upper respiratory specimens (such as anterior nasal and mid-turbinate nasal swabs, nasopharyngeal swabs, oropharyngeal swabs, nasopharyngeal wash/aspirate or nasal aspirate) and Bronchoalveolar lavage (BAL)
Processed Sample Volume	200 µL / 650 µL required (see details in the respective extraction sections)
Validated Systems	• NucliSENS® easyMAG® (bioMérieux) / Applied Biosystems® 7500 Real-Time PCR System (ThermoFisher Scientific) • VERSANT® kPCR Molecular System (Siemens Healthineers)

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Intended Use

FTD SARS-CoV-2 is a real-time polymerase chain reaction (RT-PCR) test intended for the qualitative detection of nucleic acid from SARS-CoV-2 in upper respiratory specimens (such as anterior nasal and mid-turbinate nasal swabs, nasopharyngeal swabs, oropharyngeal swabs, nasopharyngeal wash/aspirate or nasal aspirate) and bronchoalveolar lavage from individuals suspected of Coronavirus Disease 2019 (COVID-19) by their healthcare provider. Testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), U.S.C. §263a, that meet requirements to perform high complexity tests. Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in upper respiratory specimens and bronchoalveolar lavage during the acute phase of infection. Positive results are indicative of the presence of the SARS-CoV-2 RNA; clinical correlation with patient history and other diagnostic information is necessary to determine the patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease. Laboratories within the United States and its territories are required to report all results to the appropriate public health authorities. Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

FTD SARS-CoV-2 is intended for use by qualified laboratory personnel specifically instructed and trained in the techniques of RNA extractions and real-time PCR (*in vitro* diagnostic procedures). FTD SARS-CoV-2 is only for use under the Food and Drug Administration's (FDA) Emergency Use Authorization.

Summary and Explanation

On December 31st, 2019, the World Health Organization (WHO) was informed of multiple cases of pneumonia of unknown etiology detected in Wuhan City, Hubei Province of China. Soon, a new strain of coronavirus, SARS-CoV-2, observed for the first time in humans was identified to be the cause of this new disease, later called COVID-19. On January 30th, 2020, WHO declared SARS-CoV-2 as a Public Health Emergency of International Concern. Since its emergence, it has rapidly spread worldwide, causing a massive global outbreak, which has reached the status of a pandemic.

The first symptoms of the COVID-19 are not very specific. People may experience runny nose, headache, muscle pain and tiredness. Fever, cough and respiratory signs often occur 2 or 3 days later and can lead to severe pneumonia and death. The risk of developing more severe symptoms of COVID-19 are currently unknown; however, individuals with pre-existing conditions may be more at risk of developing severe symptoms. The duration of incubation is on average 5 days, with extremes of 2 to 12 days.[1]

FTD SARS-CoV-2 is an aid in the identification of COVID-19 disease by the detection of SARS-CoV-2 RNA extracted from upper respiratory specimens (such as anterior nasal and mid-turbinate nasal swabs, nasopharyngeal swabs, oropharyngeal swabs, nasopharyngeal wash/aspirate or nasal aspirate) and bronchoalveolar lavage (BAL) from individuals suspected of COVID-19 by their healthcare provider.

Principles of the Procedure

Method

FTD SARS-CoV-2 is a real-time reverse transcription-polymerase chain reaction (rRT-PCR) test for the detection of RNA from SARS-CoV-2 in human upper respiratory and BAL specimens.

Nucleic acids should be first extracted from the specimen types listed in the *Intended Use* section, with the addition of the FTD Internal Control (FTD IC).

The eluate with purified nucleic acids of SARS-CoV-2 is added to a master mix to enable the RT-PCR reaction using Applied Biosystems® 7500 Real-Time PCR Thermocycler (nucleic acids extracted with NucliSENS® easyMAG®) or the VERSANT® kPCR Molecular System. The master mix contains enzyme, buffer, deoxyribonucleotide triphosphate (dNTPs) and synthetic primers and probes specific for the targeted sequences. The advantage of using multiple primer/probe pairs in a single reaction mixture is to detect different targets in one reaction simultaneously.

In the presence of the target, primers and probes will hybridize to the specific sequence and allow amplification by the polymerase. The different probes include fluorescent dyes and quenchers close to each other, limiting the fluorescence emitted. However, during amplification, the polymerase extends the new strand and degrades the fluorescent dye-labeled probe using its exonuclease activity. This results in the separation of the fluorescent dye from the quencher, thereby allowing the emission of fluorescence.

The level of fluorescence increases with the generation of amplicons and is proportional to the amount of pathogen nucleic acid contained in the sample. The increased fluorescence level is reported as a cycle threshold (Ct) value by the real-time thermocycler.

The assay uses primer and probe sets that target N gene and ORF1ab region of SARS-CoV-2. The mix further includes a primer and probe set to detect a sequence in the genome of equine arteritis virus (EAV) that serves as an internal control (IC).

Reagents

Warnings and Precautions

CAUTION

Federal (USA) law restricts this device to sale by or on the order of a licensed healthcare professional.

- For use under an Emergency Use Authorization (EUA) only.
- For *in vitro* diagnostic use (IVD).
- For prescription use only.
- FTD SARS-CoV-2 has not been FDA cleared or approved but has been authorized for emergency use under an Emergency Use Authorization for use by laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), U.S.C. §263a, that meet requirements to perform high complexity tests.
- FTD SARS-CoV-2 has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens.

- The emergency use of the FTD SARS-CoV-2 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 Federal Food, Drug, and Cosmetic Act, 21 U.S.C. § 360bbb-3(b)(1), unless the declaration is terminated or authorization is revoked sooner.
- Laboratories within the United States and its territories are required to report all results to the appropriate public health authorities.

Safety data sheets (SDS) are available at www.siemens-healthineers.com/sds. Strict adherence to the following warnings and precautions are required when running FTD SARS-CoV-2.

WARNING

The FTD IC contains lysis buffer.

Internal Ctrl 96:

Hazardous ingredient: Maleic acid (0.1% [w/w])

WARNING

H317: May cause an allergic skin reaction.

P280: Wear protective gloves/protective clothing/eye protection/face protection.

P302+P352: IF ON SKIN: Wash with plenty of soap and water.

P333+P313: If skin irritation or rash occurs: Get medical advice/attention.

P362+P364: Take off contaminated clothing and wash it before reuse.

Handling Requirements

- The use of this product should be limited to personnel trained in the techniques of PCR.
- Use appropriate precautions required when handling all laboratory reagents.
- Treat contaminated materials as biohazardous.
- Wear personal protective apparel, including disposable gloves, throughout the assay procedure. Thoroughly wash hands after removing gloves and dispose of the gloves as biohazardous waste.
- To minimize risk of carryover contamination, regularly change gloves.
- Do NOT:
  - Eat, drink, smoke or apply cosmetics in areas where reagents or samples are handled.
  - Pipette by mouth.
  - Use reagents if reagents appear turbid or cloudy after bringing to specified temperature.
  - Use components beyond expiration date printed on kit label.
  - Interchange vial or bottle caps, as cross-contamination may occur.
- Avoid the use of sharp objects wherever possible. If skin or mucous membrane exposure occurs, immediately wash the area with copious amounts of water; seek medical advice.
- Use all pipetting devices and instruments with care and follow the manufacturers' instructions for calibration and quality control.
- Avoid contamination of reagents and samples. Use aerosol-resistant pipette tips and use a new tip every time a volume is dispensed.
- Do not mix reagents from different kit lots.
- Dispose of hazardous or biologically contaminated materials according to the practices of your institution. Discard all materials in a safe and acceptable manner in compliance with all country, state, local and regulatory requirements.

For patient samples only:

- Disinfect spills promptly with 0.5% sodium hypochlorite solution (1:10 v/v bleach) or equivalent disinfectant.
- For viral inactivation before extraction (or viscous sample), FTD recommends diluting the specimen (1:2) with VERSANT® Sample Preparation 1.0 Lysis Buffer. Additional Lysis Buffer is available for purchase.

REF	Contents
10634919	VERSANT® Sample Preparation 1.0 Lysis Buffer

NOTE: For more information, refer to the VERSANT® Sample Preparation 1.0 Reagents instructions for use.

For all reagents:

- Disinfect spills promptly using Microcide SQ®. Do not use bleach.

Storage and Handling

Store the components of this FTD product in its original packaging at -30°C to -10°C. Components are stable until the expiration date as stated on the outer box label.

Freeze product immediately after use. Reagents can sustain up to 10 freeze-thaw cycles.

Specimen Collection and Handling

This section describes the general industry practice for upper respiratory tract specimen handling and storage that will help ensure accurate test results.

This test is for use with extracted nucleic acids from upper respiratory specimens (such as anterior nasal and mid-turbinate nasal swabs, nasopharyngeal swabs, oropharyngeal swabs, nasopharyngeal wash/aspirate and nasal aspirate) and bronchoalveolar lavage of human origin.

Respiratory pathogen detection depends on the collection of high-quality specimens, their rapid transport to the laboratory, appropriate storage and treatment before laboratory testing. Transport the specimen to the laboratory immediately after collection and process/test as soon as possible. Label all specimens appropriately according to the laboratory's procedure. To protect the viral RNA from degradation, correct specimen handling is very important (as recommended by CDC[2]).

CAUTION

Handle all samples as if the samples contain potentially infectious agents.

Use universal precautions, such as wearing personal protective apparel, including disposable gloves. Thoroughly wash hands after removing gloves and dispose of the gloves as biohazardous waste.

Prior to sample collection, no special preparation of the patient is required. No pretreatment is required for sample storage.

Collecting the Specimen

Collect all specimens according to the standard technique from the laboratory or clinician. Refer to the CDC Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens from Patients Under Investigation (PUIs) for 2019 Novel Coronavirus.[3] Obtain swabs directly from the infection site in order to avoid contamination with surrounding microbiota.

IMPORTANT! Remel M4RT® transport medium is not recommended for use with FTD SARS-CoV-2.

Storing and Transporting the Specimen

Anterior nasal and mid-turbinate nasal swabs, nasopharyngeal and oropharyngeal swabs should be placed immediately in a sterile transport tube containing a viral transport medium or similar method.

Nasopharyngeal wash/aspirate, nasal aspirates (and the non-bacteriostatic saline used to collect these specimens) as well as BAL should be placed immediately into a sterile transport tube or dry container.

Specimens (upper respiratory or BAL) should be delivered promptly to the laboratory and can be refrigerated at temperatures of 2–8°C and/or shipped on ice packs for up to 72 hours after collection, as recommended by CDC.[3] Specimens may be frozen to -20°C or ideally -70°C and shipped on dry ice if further delays are expected.

It is important to avoid repeated freezing and thawing of specimens (as recommended by WHO[4]).

NOTES:

- Specimen storage and shipment advice are only recommendations. Check local regulations and institutional policy.
- Pack and label the specimen in compliance with your local and international regulations that cover the transport of clinical samples and etiological agents.

Procedure

Materials Provided

Each reagent contains additional volume for pipetting inaccuracy. Each kit includes FTD Internal Control (IC), FTD Negative Control (NC) and FTD Positive Control (PC) components. The box and each vial are labeled with a lot number.

Reagent	Composition	Description / Quantity	Storage
SCoV2 PP Mix 96	Synthetic oligonucleotides, buffer	PP mix for SARS-CoV-2 (N gene), SARS-CoV-2 (ORF1ab) and IC 1 x 144 µL	-30°C to -10°C
SCoV2 PC 96	Double-stranded synthetic DNA molecules, buffer, stabilizing agents	FTD PC 1 x 150 µL	
Negative Ctrl 96	Nuclease-free water	FTD NC 1 x 2000 µL	
Internal Ctrl 96	Double-stranded circular DNA molecules, buffer, <5.0% guanidinium hydrochloride, <0.1% maleic acid	FTD IC 1 x 350 µL	
25x RT-PCR Enz. 96	Enzymes, buffer, glycerol, Ribonuclease (RNase) inhibitors, stabilizing agents	25x RT-PCR Enzyme mix 1 x 96 µL	

2x RT-PCR Buff. 96	Tris-hydrochloride (Tris-HCl) buffer, Deoxyadenosine triphosphate (dATP), Deoxycytidine triphosphate (dCTP), Deoxyguanosine triphosphate (dGTP), Deoxythymidine triphosphate (dTTP), PCR adjuvants	2x RT-PCR Buffer 1 x 1200 µL

Legend: PP = Primer/probe, PC = Positive control, Ctrl = Control, Enz. = Enzyme, Buff. = Buffer

The maximum number of reactions produced by an FTD SARS-CoV-2 kit is dependent on the workflow:

Workflow	Software Version	Number of Reactions
NucliSENS® easyMAG® +	v2.1 or later	96
Applied Biosystems® 7500 Real-Time PCR System	v2.3 or later	
VERSANT® kPCR Molecular System		
VERSANT® kPCR Molecular System SP	v4.0 or later	88*
VERSANT® kPCR Molecular System AD with MiPLX Software Solution	v2.0 or later	

*Automated PCR plate setup with VERSANT® kPCR SP Module limits the total number of available reactions.

Materials Required but Not Provided

The kit has been validated with the NucliSENS® easyMAG® (bioMérieux)/Applied Biosystems® 7500 Real-Time PCR System (ThermoFisher Scientific), and the VERSANT® kPCR Molecular System (Siemens Healthineers).

The following material and reagents are required for extraction with the NucliSENS® easyMAG®:

REF	Contents
280133	NucliSENS® easyMAG®, Magnetic Silica Beads[a]
280134	NucliSENS® easyMAG®, Lysis Buffer[a]
280130	NucliSENS® easyMAG®, Extraction Buffer 1[a]
280131	NucliSENS® easyMAG®, Extraction Buffer 2[a]
280132	NucliSENS® easyMAG®, Extraction Buffer 3[a]
280135	NucliSENS® easyMAG®, Disposables[a]
N/A	Nuclease-free water

a. For more information about the materials listed in the table, refer to the manufacturer (bioMérieux).

The following material and reagents are required for extraction with the VERSANT® kPCR Molecular System SP (SP Module):

REF	Contents
04801677	VERSANT® Sample Preparation 1.0 Reagents Box 1[a]
04801685	VERSANT® Sample Preparation 1.0 Reagents Box 2[a]
10283255	96 Deep Well Plate 2 mL
10282930	Disposable Tips 300 µL
10282929	Disposable Tips 1 mL
10489008	Reagent Containers 4 x 200 mL & 2 x 60 mL
72.694.005	Sterile Sarstedt 2 mL skirted micro tubes[b]
N/A	Nuclease-free water

a. For more information on the VERSANT® Sample Preparation 1.0 Reagents, refer to the product instructions for use.

b. For more information, refer to the manufacturer (Sarstedt).

FTD Recommendations:

- Use an external RNA positive control (external RNA PC) with each run.
- Use one of the two listed external RNA PCs for diagnostic testing.

Supplier	REF	Contents
SeraCare	0505-0159	AccuPlex™ SARS-CoV-2 Molecular Controls Kit – Full Genome[a]
Exact Diagnostics	COV019CE	SARS-CoV-2 Positive Run Control[b]
	COV000CE	SARS-CoV-2 Negative Run Control[b,c]

a. For more information, refer to the manufacturer (SeraCare) instructions for use.

b. For more information, refer to the manufacturer (Exact Diagnostics) instructions for use.

c. Extract the SARS-CoV-2 Negative Run Control with the clinical samples. The SARS-CoV-2 Negative Run Control must not replace the FTD NC.

General Laboratory Equipment and Consumables

- Adjustable micropipette capable of dispensing 1000 µL, 200 µL, 100 µL, 20 µL and 10 µL
- Disposable, aerosol-resistant pipette tips, sterile-package
- Disposable, powder-free gloves
- Vortex mixer
- Benchtop centrifuge
- Sample rack(s)
- Sample collection devices/material
- Bleach/Microcide (or other product according to laboratory cleaning procedure)
- 96-well PCR plates and plate sealers
- Tubes

Assay Procedures

Workflow Using the NucliSENS® easyMAG® System and the Applied Biosystems® 7500 Real-Time PCR System

Extraction Using the NucliSENS® easyMAG® System

Preparing the Sample

1. Thaw the FTD NC and the FTD IC.

2. Before use, ensure reagents have reached room temperature (15°C to 30°C); mix FTD NC, FTD IC and external RNA PC (by quick vortexing) and spin down briefly.
3. Prepare samples for extraction procedure (if applicable, thaw to room temperature).

Validated extraction volumes are shown below:

Type	Extraction Volume
Sample volume	200 µL
Elution volume	55 µL

4. Add samples, external RNA PC and FTD NC into the disposables.
5. Program instrument accordingly.
6. Select **DISPENSE LYSIS** for lysis buffer to dispense and to start the incubation step. During the incubation period, prepare beads as described in the NucliSENS® easyMAG® manual.
7. Once incubation is complete, add 2 µL FTD IC directly to the mix of lysis buffer and sample.
8. Add beads to each well of the disposable and perform extraction protocol.

WARNING

- Never add the FTD IC prior to addition of lysis buffer.
- Never add the FTD IC after extraction.
- Adding FTD IC to each of the samples, the external RNA PC and to the FTD NC is an important step to monitor nucleic acid extraction, as well as the inhibition of nucleic acid amplification.
- Do not extract FTD PC provided with the kit.

Real-Time PCR Using the Applied Biosystem® 7500

Preparing the Experiment

1. Before use, ensure reagents are completely thawed, mixed (by quick vortexing) and spun down briefly.

   Exception:

   - The 25x RT-PCR Enzyme must be stored at -30°C to -10°C or on a cooling block at all times.
   - The FTD PC must be thawed and stored at room temperature (15°C to 30°C) for 20 to 30 minutes. Vortex thoroughly before use.

The volume of each reagent required for 1, 10, 32, and 96 reactions are as follows:

Number of Reactions		**1**	**10**	**32**	**96**
	2x RT-PCR Buffer	12.5 µL	125 µL	400 µL	1200 µL
	Primer/Probe Mix	1.5 µL	15 µL	48 µL	144 µL
	25x RT-PCR Enzyme	1 µL	10 µL	32 µL	96 µL
	Total	**15 µL**	**150 µL**	**480 µL**	**1440 µL**

2. Prepare a separate 2 mL tube per primer/probe mix and label accordingly. Pipette the required amount of 2x RT-PCR Buffer based on the number of reactions.
3. Pipette the required amount of SCoV2 PP Mix in the corresponding tube containing 2x RT-PCR Buffer.
4. Master Mix Preparation:

   NOTES:

   - To obtain accurate volumes and to avoid wasting material, do not immerse the whole tip into the liquid when pipetting the 25x RT-PCR Enzyme.
   - Pipette liquid very slowly to prevent air bubbles.
   - Wipe tip against edge of the vessel to remove excess liquid outside the tip before dispensing.
   - Change tip after each pipetting step.

a. Pipette the required amount of 25x RT-PCR Enzyme in each of the tubes containing SCoV2 PP Mix and 2x RT-PCR Buffer.
b. Vortex master mix briefly and spin down.
c. Use master mix immediately and do not store after use.

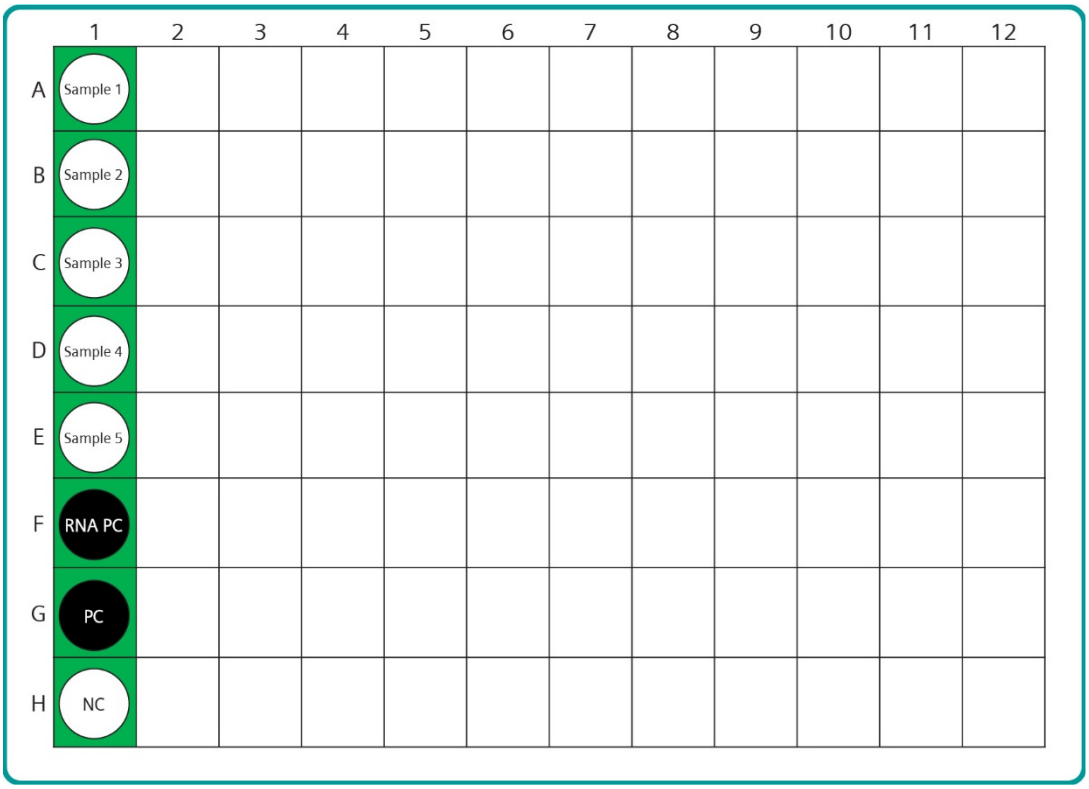
Prepare a 96-Well Plate for the Applied Biosystems® 7500

NOTE: The FTD PC, FTD NC and external RNA PC must be run on each plate to perform analysis.

NOTE: The external RNA PC is recommended but is not provided. For more information, refer to the *Materials Required but Not Provided* section on page 10.

Refer to Figure 1 for an example of the placement of patient samples and controls.

Figure 1: Samples and Controls – Plate Map Example



Legend: Green = SCoV2 master mix (A1–H1) • RNA PC = External Ribonucleic Acid Positive Control (F1) • PC = FTD Positive Control (G1) • NC = FTD Negative Control (H1)

To prepare a 96-well plate (compatible with the Applied Biosystems® 7500):

1. Pipette 15 µL of the SCoV2 master mix into wells A1 to H1.
2. Add 10 µL of the extracted samples into wells A1 to E1.
3. Add 10 µL of the extracted external RNA PC into well F1.

4. Add 10 µL of the FTD PC into well G1.
5. Add 10 µL of the extracted FTD NC into well H1.
6. Seal plate with appropriate adhesive film.
7. Gently vortex plate, then centrifuge briefly.
8. Place plate into the Applied Biosystems® 7500.

NOTE: For the use of the Applied Biosystems® 7500, refer to manufacturers' operating instructions.

Program the Thermocycler

The detection wavelengths for the dyes used in this kit are shown in the following table:

SCoV2 PP Mix and Thermocycler Detection Settings		
Pathogen	**Dye**	**Detection Wavelength (nm)[a]**
SARS-CoV-2	green	520
—	yellow	550
—	orange	610
IC (EAV)	red	670

[a] Detection wavelengths listed are from the Applied Biosystems® 7500. Wavelengths may vary for other thermocyclers.

NOTE: Both targets (N gene and ORF1ab) are labeled with the same dye and are detected in the same channel.

NOTE: Change setting for passive reference dye to **NONE** (by default, ROX dye is selected).

PCR Program

The programming steps for the thermocycler are shown in the following table:

Stage	Cycles	Acquisition	Temperature	Time
Hold	/	/	50°C	15 minutes
Hold	/	/	94°C	1 minute
Cycling	40	/	94°C	8 seconds
		Yes	60°C	1 minute

For customer support, contact your local technical support provider or distributor.

Completion of Run

Remove the sealed PCR plate according to the thermocycler manufacturers' instructions. Review the results and discard the PCR plate as biohazardous waste, according to local regulatory requirements.

Workflow Using the VERSANT® kPCR Molecular System

Extraction Using the VERSANT® kPCR Molecular System (SP Module)

Preparing the Sample

1. For viral inactivation before extraction (or viscous sample), FTD recommends diluting the specimen (1:2) into the VERSANT® Sample Preparation 1.0 Lysis Buffer before proceeding with the automated extraction process.
2. Thaw the FTD NC and the FTD IC.
3. Before use, ensure reagents have reached room temperature (15°C to 30°C); mix FTD NC, FTD IC and external RNA PC, gently vortex tubes, then centrifuge briefly.

   NOTE: Refer to the external RNA PC manufacturer's preparation instruction. The external RNA PC is recommended but is not provided. For more information, refer to the *Materials Required but Not Provided* section on page 10.
4. Prepare a 1:5 FTD IC dilution in a 2 mL Sarstedt skirted micro tube and label it accordingly.

   - The FTD IC dilution volume for the Internal Control Mix varies based on the number of samples run on the SP Module. Calculate the IC dilution volume using the following formula:

     IC dilution volume = (# of Samples x 11) + 125

   - Required volumes depending on the total number of samples per extraction are shown in the following table:

Samples per Extraction*	**10**	**32**	**64**	**87****	**96**
Internal Control	47 µL	95.4 µL	165.8 µL	216.4 µL	236.2 µL
Nuclease-free water	188 µL	381.6 µL	663.2 µL	865.6 µL	944.8 µL
Total IC Dilution Volume	**235 µL**	**477 µL**	**829 µL**	**1082 µL**	**1181 µL**

* Including external RNA PC and FTD NC.

** When using Dynamic Assay Preparation FTD1 (SP1.0 500-15-10), one FTD-SARS-CoV-2 kit is enough to run 88 PCR reactions (87 extracted samples [including external RNA PC and FTD NC] plus one non-extracted FTD PC).

5. Add 650 µL of FTD NC into a 2 mL Sarstedt skirted micro tube and label it accordingly.
6. Add 650 µL of extractable external RNA PC into a 2 mL Sarstedt skirted micro tube and label it accordingly.
7. Prepare samples for extraction procedure (if applicable, thaw to room temperature).

8. Transfer at least 650 µL of each sample into sterile polypropylene tubes that meet the size requirements for the sample carrier.

   NOTE: Sample tubes must meet the following requirements:

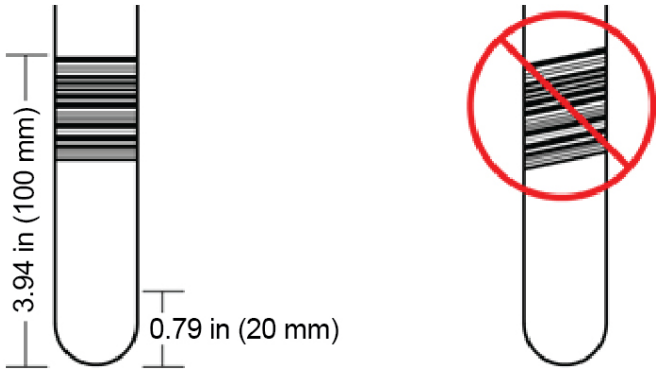
   - Do not use sample tubes with internal threads.
   - For the 32-position sample carrier, only use 0.43 x 2.36 in to 0.55 x 3.94 in (11 x 60 mm to 14 x 100 mm) sample tubes with an inner diameter greater than or equal to .35 in (9 mm).

9. Place barcode labels on all sample tubes. The system requires that all samples are uniquely identified with a barcode. See Figure 2 for guidance on placing the barcode on the sample tube.

   NOTES:

   - For a given run, ensure that each sample barcode is unique.
   - Position the barcode within a range of 0.79 to 3.94 in (20 to 100 mm) from the bottom of the sample tube.
   - Affix the barcode tightly to the sample tube in a vertical position.

Figure 2: Placement of the Barcode Label on the Sample Tube



10. Carefully remove the sample tube caps before placing the sample tubes onto the sample carriers.

NOTES:

- Ensure that the barcode on each of the sample tubes is correctly positioned so that it is clearly visible through the opening on the carrier.
- To reduce risk of cross-contamination, discard sample tube caps as you remove them.
- Use new caps when recapping the tubes.
- If any splashing of samples occurs, remove and discard your gloves and put on clean ones.

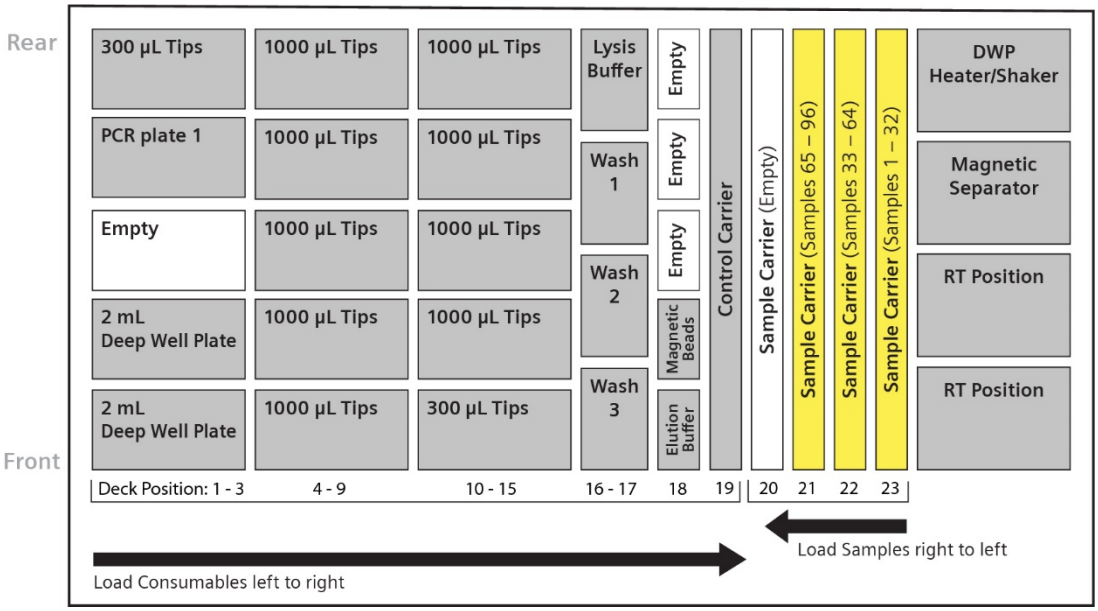
Loading the Autoload Tray

NOTES:

- Sample carriers are loaded from right to left, starting at Deck Position 23. Consumable carriers are loaded from left to right, starting at Deck Position 1 (see Figure 3).
- Push the carrier in until it touches the stop clips just beyond the autoload tray. Do not attempt to force the carrier past the stop clips.

1. Load the sample carriers onto the track starting at Deck Position 23 to Deck Position 21.
2. Load the consumable carriers onto the track starting at Deck Position 1–3. Refer to Figure 3.

Figure 3: VERSANT® kPCR SP Open Protocol Assay – Autoload Tray Layout



NOTES:

- Ensure that the barcodes on the sample carriers are facing to the right.
- Before starting the run, ensure that all the carriers are loaded in the correct positions on the autoload tray.

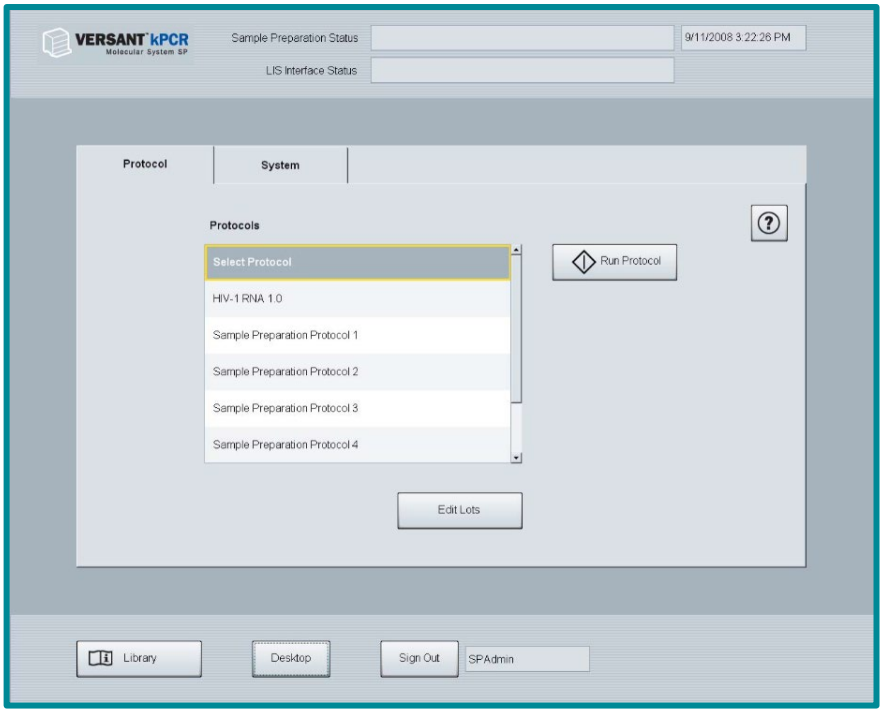
3. After loading the prepared carriers onto the autoload tray, start the run.

Entering Lot Information

To enter lot information, perform the following actions:

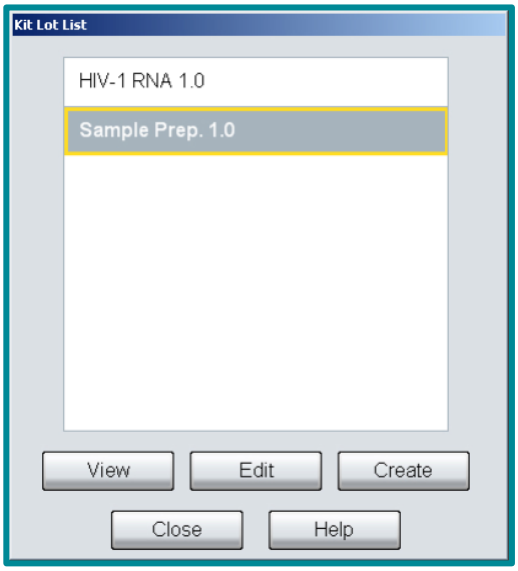
1. Select the Protocol tab at the main screen (see Figure 4).

Figure 4: Protocol Tab



2. If you are using a kit from a new lot of sample preparation reagents, enter the lot-specific information using the hand-held scanner:
   a. At the Protocol tab, select Edit Lots. The Kit Lot List window displays (see Figure 5).

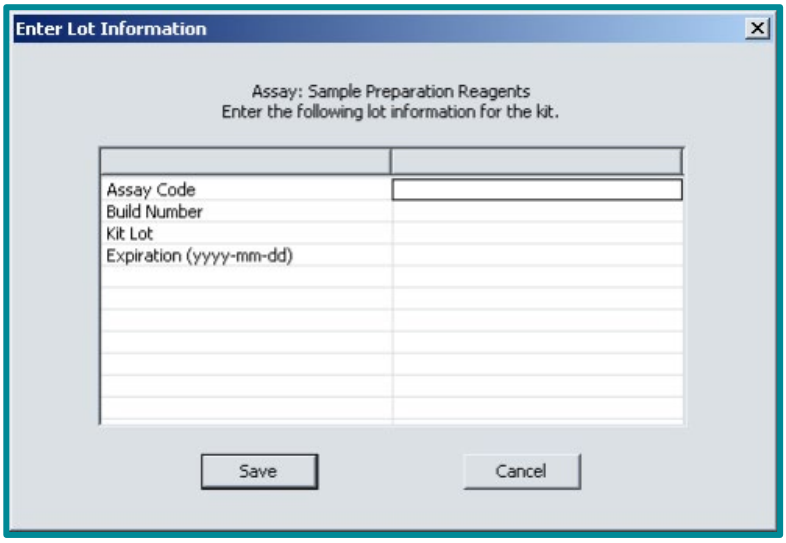
Figure 5: Kit Lot Window



b. Select Sample Prep 1.0 from the list.

c. Select Create. The Enter Lot Information window displays (see Figure 6).

Figure 6: Enter Lot Information Window



d. Place the cursor in the first row of the right column.

e. Scan the 2D barcode that is printed on the assay product insert supplement.

3. When you scan the 2D barcode, the form fields populate automatically. Verify the lot information entered.

NOTE: Scanning the 2D barcode is only required for new lots of reagent kits. However, as

a good practice, check the assay kit information before each run. Verify lot information as follows:

a. At the protocol tab, select Edit Lots.
b. Select Sample Prep 1.0 from the list.
c. Select View.
d. Verify that the information is correct for the assay kit lot you plan to run.
e. Select OK.

4. The system returns to the Kit Lot List window.
5. If the lot information is correct, select Close to return to the main system window and start your run.

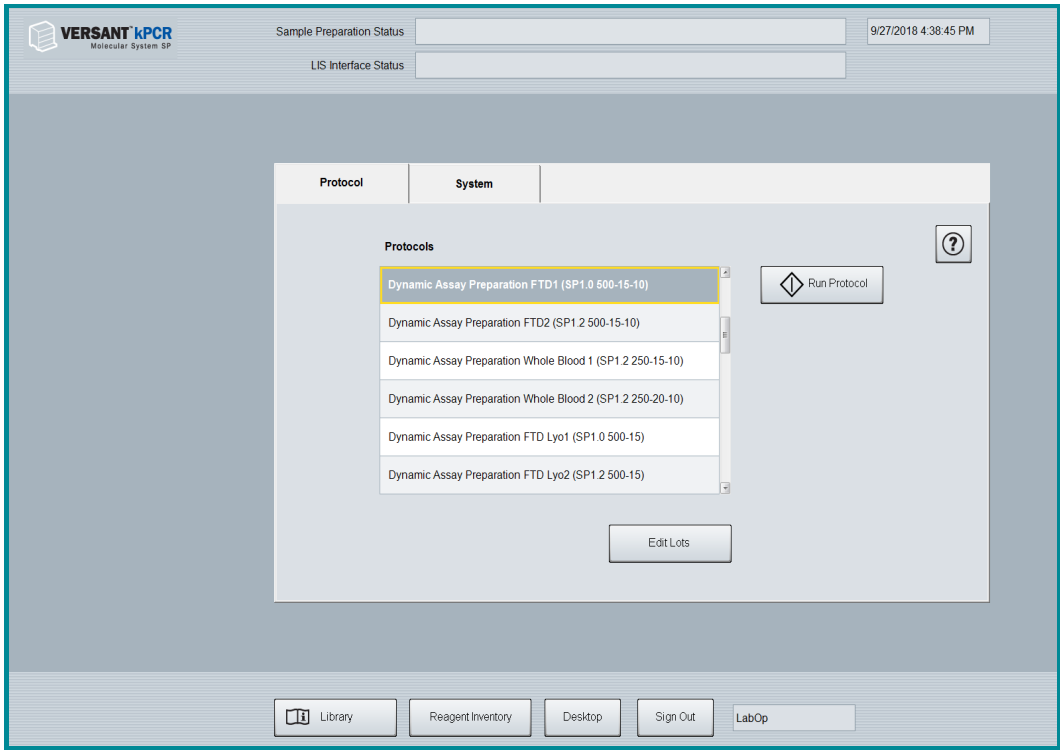
NOTE: If assay kit information is not correct, select Create and scan the 2D barcode as described above.

Starting the Run

To start the run, perform the following actions:

1. Select the Protocol tab at the main screen (see Figure 7).

Figure 7: Protocol Tab



2. Select Dynamic Assay Preparation FTD1 (SP1.0 500-15-10).
3. Click Run Protocol.

The SP Module verifies that it can initialize the iSWAP, the waste sensors, and the heater/shaker. If the system is unable to complete initialization, an error window displays.

Refer to the *VERSANT® kPCR Molecular System Sample Preparation (SP) Application Guide*[5] for more information about these errors.

4. After the system completes initialization, the user is prompted to empty the solid waste container.

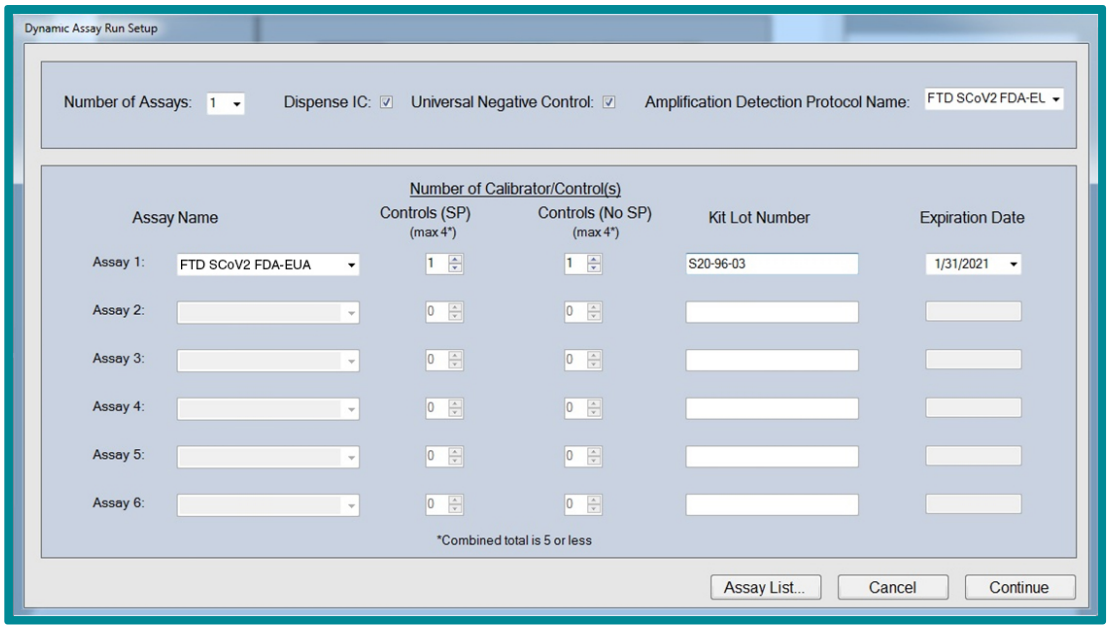
   Refer to the *VERSANT® kPCR Molecular System Sample Preparation (SP) Application Guide*[5] for instructions on emptying and reinstalling the solid waste container.

5. When the solid waste container is reinstalled correctly, the Install the Solid Waste Container window closes, and the Dynamic Assay Run Setup window displays.

Configuring a Dynamic Assay Run

When starting a run, the Dynamic Assay Run Setup window opens automatically and displays the information that was saved from the last run (see Figure 8). Use this window to configure a Dynamic Assay run.

Figure 8: Dynamic Assay Run Setup



1. In the Number of Assays list, select 1.
2. Select the Dispense IC checkbox. FTD IC is dispensed to all samples and controls extracted by the SP Module.

WARNING

- Never manually add FTD IC to the sample, before or after extraction.
- Adding FTD IC to each of the samples and to the FTD NC is an important step to monitor nucleic acid extraction, as well as the inhibition of nucleic acid amplification.

---

3. Select the Universal Negative Control checkbox. The FTD NC will be extracted along with the clinical samples on the SP Module.
4. In the Amplification Detection Protocol Name list, type or select a name in the box.
   - Type FTD SCoV2 FDA-EUA.
   - Subsequent runs of protocol; select FTD SCoV2 FDA-EUA from the drop-down.

CAUTION

The Amplification Detection Protocol Name on the SP Module must match the kPCR protocol name saved on the AD Module to link the Dynamic Assay Protocol information to the AD.

5. In the Assay Name drop-down, type or select an assay name next to each numbered assay.

   - Type FTD SCoV2 FDA-EUA.
   - Subsequent runs of assay; select FTD SCoV2 FDA-EUA from the drop-down.

   If a stored assay name is selected, the Controls, Kit Lot Number, and Expiration Date fields populate with the stored assay information, and the Dispense IC and Universal Negative Control fields display values from the last saved run.

CAUTION

If you are using the LIS, the assay name must match the dye channel name entered in the AD Module set up.

6. In the Number of Calibrator/Control(s) drop-down, select:
   a. 1 in the Control (SP) (external RNA PC).
   b. 1 in the Control (No SP). This FTD PC will be added directly to the PCR plate after the sample extraction process is complete.
7. (Optional) In the Kit Lot Number list, type the kit lot number.

8. In the Expiration Date list, select the expiration date for the lot.

   Expired reagents are highlighted in red on the Dynamic Assay Run Setup window and the VERSANT® kPCR Sample Preparation Run Report. The system does not enforce the expiration of the kit lot.

9. Click Continue.

Loading Samples

1. Ensure that the barcode on each of the samples is visible through the opening of the sample carrier. The barcode of each of the samples is read as the carriers are loaded.
2. Ensure that you have loaded all sample carriers on the autoload tray.
3. Click Load Samples.

   The system begins loading the sample carriers from the autoload tray onto the deck and reads all carrier IDs, sample barcodes and sample slot locations for sample tubes contained on the sample carrier.

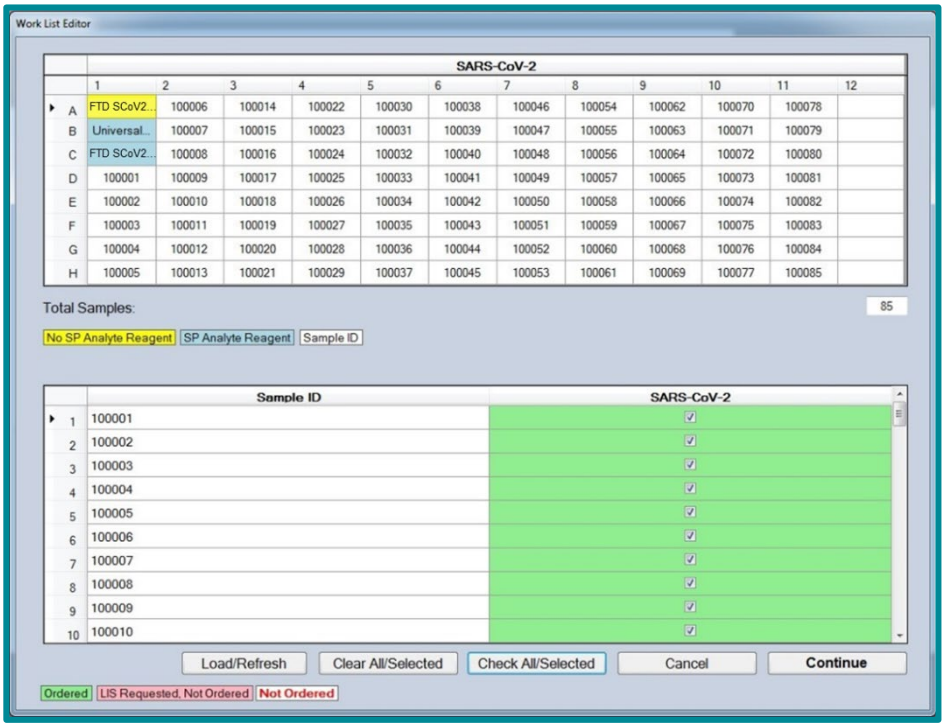
   If a barcode error occurs, refer to the *VERSANT® kPCR Molecular System Sample Preparation (SP) Application Guide*[5] for more information about manually entering barcodes.

   When all sample carriers are loaded, the Loading Samples Confirmation window displays, indicating the number of samples and carriers loaded.

4. If the number of samples indicated is correct, click Yes.

   The Work List Editor displays and shows the PCR Plate Map and the Work List (see Figure 9). The Work List includes the sample IDs of all samples loaded on the SP instrument. If the system is connected to the LIS, all test orders will be displayed in the Work List.

Figure 9: Work List Editor



Legend: A1 – FTD PC • B1 – Extracted FTD NC • C1 – Extracted external RNA PC

5. Click Check All/Selected to select all samples in the work list editor (right column).
6. Click Continue.

About the PCR Plate Map

The PCR Plate Map is populated with the control and sample IDs based on the Dynamic Assay Protocol Setup and the Work List selections in the LIS.

The PCR plate map is read-only, except for the names of the controls. You can modify the control well names to match the reagents that you are loading. The control name must be between 2 and 20 letters, numbers, and symbols and is case sensitive. The control name becomes the sample ID. The Universal Negative Control name cannot be changed.

The PCR plate map reflects the order that the controls and samples are loaded into the PCR plate. This is based on the number of SP and No SP controls selected for each assay. The plate is loaded in the following order:

About the Work List

The first column of the work list contains the sample IDs for all samples loaded on the system starting with position 1 of the sample rack loaded in track Position 23 on the deck. The adjacent column headers include the assay names that were defined in the Dynamic Assay Run Setup window.

Test order information from the LIS:

- If a sample ID is associated with an assay in the test order from the LIS, then the assay cell is checked, otherwise, it is unchecked.
- If a sample ID from the LIS cannot be accommodated on the PCR Plate due to a lack of available wells for an assay, each associated checkbox for that sample will be disabled and highlighted red.
- A sample ID with no assays selected will be highlighted in red.

The following table describes the actions you can perform for any sample in the worklist (including samples with test orders from the LIS).

To	Do
Select the required assay(s) for each sample.	Select the checkbox in the assay column next to the Sample ID.

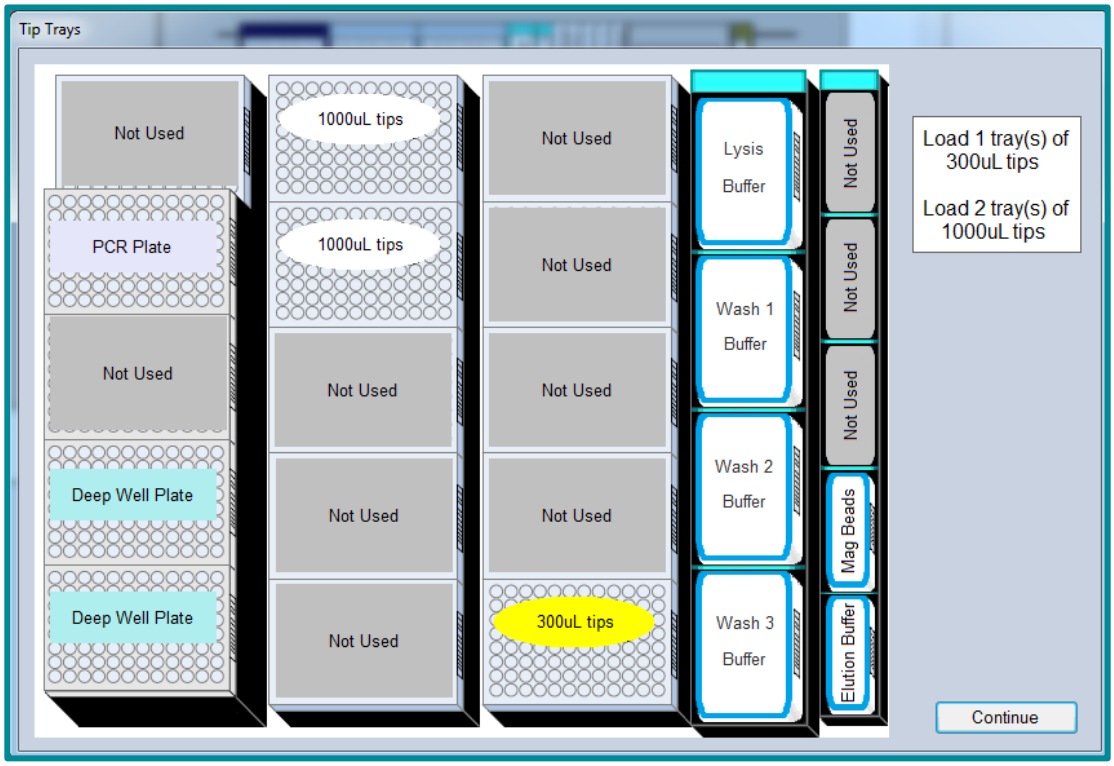
Reload the worklist with the initial sample information from the LIS.	Click Load/Refresh.
Clear all checkboxes in the worklist.	Click Clear All/Selected.
Select all checkboxes in the worklist.	Click Check All/Selected.
Abort the workflow, unload the racks from the deck to the autoload tray, and return to the Dynamic Assay Run Set Up window.	Click Cancel.
Continue the workflow from the SP Control Carrier Loading window and load the consumables.	Click Continue.

Note that the PCR plate map entries display as you select items in the worklist editor.

Loading Consumables and Reagents

After loading samples, click Continue in the Worklist Editor window, the Tip Trays window displays (see Figure 10).

Figure 10: Tip Trays



1. Fill the carriers with tips and plates.

   The Tip Tray window displays the locations for 300 µL and 1000 µL tips, deep-well plates and PCR plate based on the number of assays, controls and samples selected.

2. Load the Tip Tray Carriers onto the deck.
3. Prepare the VERSANT® SP 1.0 Reagents.
   a. Visually inspect each reagent for the presence of solids. If small amounts of solids are present, allow them to settle before pouring the reagent. Ensure the solids do not go into the troughs.
   b. Mix bottles containing Lysis Buffer, Wash Buffer 1, Wash Buffer 2, and Wash Buffer 3 by gently inverting 10 times.

      NOTE: Slight color changes may occur with the Lysis Buffer. These changes do not indicate a change in the quality of the buffer.

   c. Pour the entire volume of each reagent into a new, properly labeled, 200 mL trough. Minimize the formation of bubbles by pouring along the inside wall of the trough.

4. Load the Large Trough Carrier:

   - Lysis Buffer in Position 1 (nearest the carrier barcode)
   - Wash 1 in Position 2
   - Wash 2 in Position 3
   - Wash 3 in Position 4 (nearest the carrier handle)
   - Place the large trough carrier at deck Positions 16–17 on the autoload tray of the SP Module.

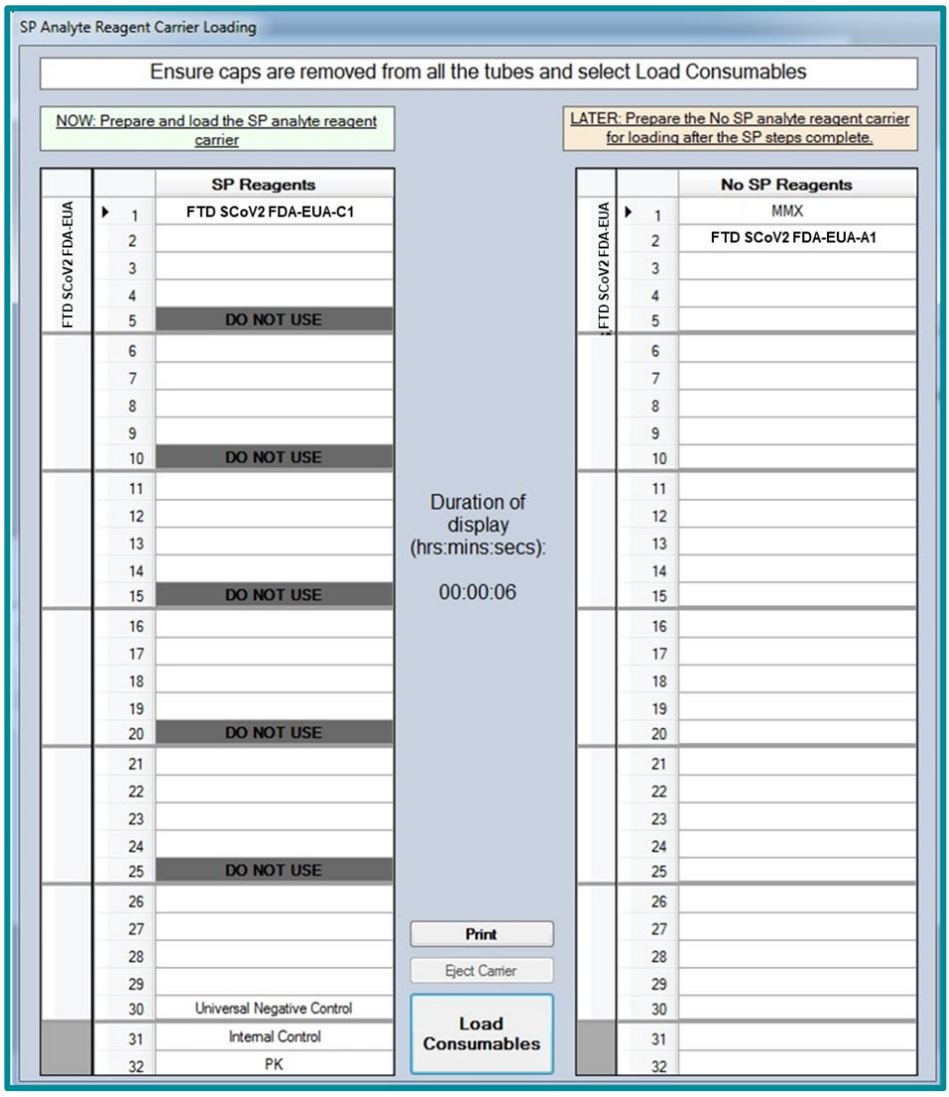
5. Load the Small Trough and Control Carriers:

   a. Mix the Magnetic Beads by vortexing the bottle for 60 seconds.
   b. Pour Magnetic Beads into a new, properly labeled, 60 mL trough.
   c. Mix the Elution Buffer by gently inverting the bottle 10 times.
   d. Pour the Elution Buffer into a new, properly labeled, 60 mL trough.
   e. Place Magnetic Beads in Position 4 and Elution Buffer in Position 5 in the small trough carrier as shown.
   f. Place the small trough carrier at deck Position 18 on the autoload tray of the SP Module.

6. Click Continue.

The SP Analyte Reagent Carrier Loading window displays (see Figure 11).

Figure 11: SP Analyte Reagent Carrier Loading Window



7. Add SP reagents as indicated:
   a. Take out the barcode label (0007) from the SP 1.0 Reagent Box 2 (Proteinase K [PK] bag) and place it on a new, clean 5 mL tube (provided in the same bag).
   b. Mix Proteinase K by gently inverting the bottle 10 times.
   c. Pour the entire volume of Proteinase K into the barcoded 5 mL tube.
   d. Place the barcoded 5 mL tube into Position 32 of the control carrier. Ensure that the barcode is positioned vertically so that it is clearly visible through the opening in the carrier.
   e. Place the FTD IC dilution into Position 31.
   f. Place the FTD NC into Position 30.

g. Place the external RNA PC into Position 1.

8. Load control carrier onto deck Position 19 on the autoload tray of SP Module.
9. Click Print to print a copy of the SP/No SP Analyte Reagent Carrier Map Report.
10. Ensure that caps are removed from all tubes and troughs and click Load Consumables.

The system performs the following activities:

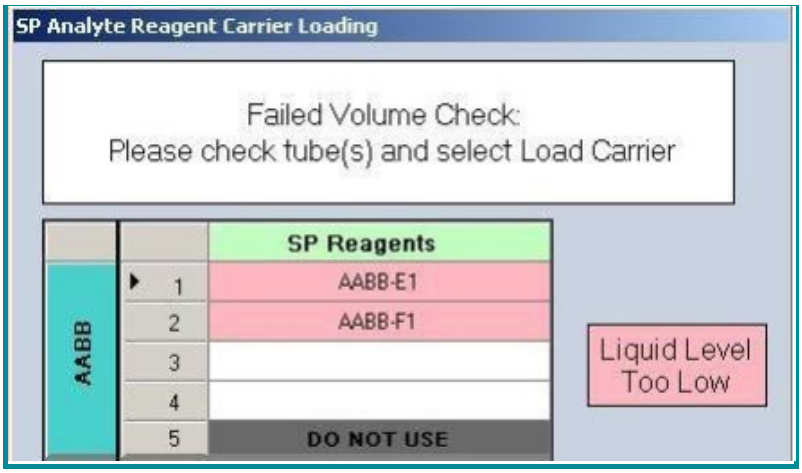
- Checks that the consumable carriers (Tip Carrier, Plate Carrier, Control Carrier, and Sample Preparation carriers) are available on the autoload tray.
- Loads the Tip Carrier.
- Loads the Sample Preparation Reagents Carrier and confirms adequate volume is present.
- Loads the Control Carrier.
- Confirms adequate volume is present in the Controls and IC.
- Confirms adequate volume is present for PK.

About Liquid Volume Levels

If the system does not confirm adequate volume is available in the PK, IC or master mix, or if it does not detect liquid in the Controls (No SP):

- The system ejects the Control Carrier from the deck.
- The SP Analyte Reagent Carrier Loading window displays the `Liquid Level Too Low warning` (see Figure 12).
- The corresponding reagent position is highlighted.

Figure 12: SP Analyte Reagent Carrier Loading Window with Liquid Level Warning



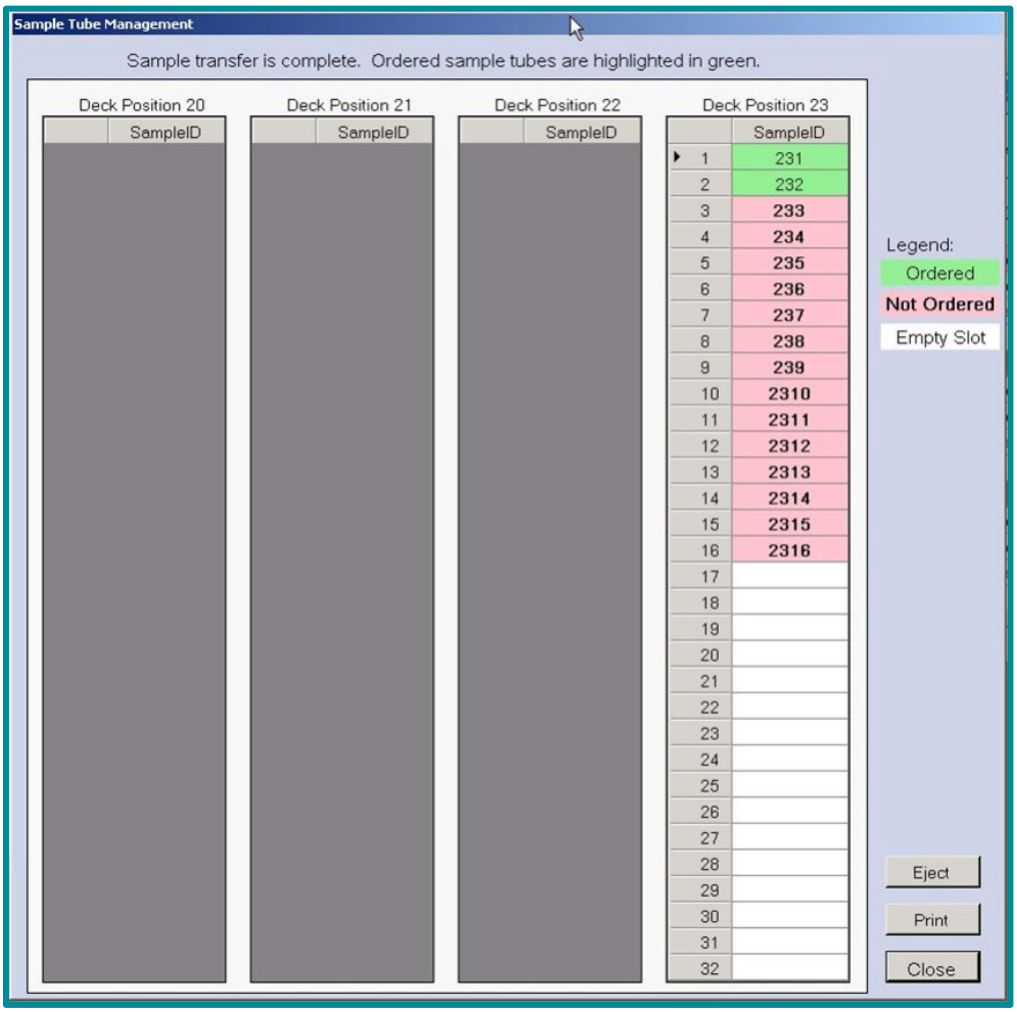
To continue the run, fill the control with adequate volume and then click Load Consumables.

After all consumables load, a window displays a timer that indicates when the Control (No SP) Reagents will need to be loaded.

The system begins the sample extraction process.

When all samples have been transferred to the Deep Well plate, the Sample Tube Management window displays the following message (see Figure 13): `Sample transfer is complete. Ordered sample tubes are highlighted in green.`

Figure 13: Sample Tube Management Window



The window remains open for 15 minutes. If you do not select one of the following options, the window automatically closes and the carriers are not ejected.

Option	Description
Eject	Eject the sample carriers from the deck.
Print	Print the Sample Tube Management window.

Close	Close the window.

Preparation of Master Mix and Positive Control (No SP Control)

NOTE: Prepare the master mix and the FTD PC (No SP) right before the end of the extraction.

Figure 14: SP Analyte Reagent Carrier Loading Window

1. Before use, ensure reagents are completely thawed, mixed (by short vortexing) and spun down briefly.

   Exception:

- The 25x RT-PCR Enzyme must be stored at -30°C to -10°C or on a cooling block at all times.
- The FTD PC must be thawed and stored at room temperature (15°C to 30°C) for 20 to 30 minutes. Transfer the FTD PC into a 2 mL Sarstedt skirted micro tube. Vortex thoroughly before use.

2. Prepare a separate 2 mL Sarstedt skirted micro tube for the SCoV2 master mix and label accordingly. Pipette the required amount of 2x RT-PCR Buffer, SCoV2 Primer/Probe mix and 25x RT-PCR Enzyme based on the number of reactions.

WARNING

Disregard the master mix volume displayed in the SP Analyte Reagent Carrier Loading window.

Always calculate the master mix input volume using the information provided in this section of the instructions for use.

a. The input volume for the master mix varies based on the number of samples run on the SP Module. Calculate the input volumes using the following formula:

Master mix input volume = 15.4 x (# of Samples + 5)

b. After calculating the master mix input volume, calculate the volumes of the respective master mix components:
   - 2x RT-PCR Buffer volume = 12.5/15 x Master mix input volume
   - Primer/Probe Mix volume = 1.5/15 x Master mix input volume
   - 25x RT-PCR Enzyme volume = 1/15 x Master mix input volume

   Example volumes depending on the number of reactions are shown in the following table:

Number of Reactions	**32**	**64**	**88***	**96**
2x RT-PCR Buffer	474.8 µL	885.5 µL	1193.5 µL	1296.2 µL
Primer/Probe Mix	57.0 µL	106.3 µL	143.2 µL	155.5 µL
25x RT-PCR Enzyme	38.0 µL	70.8 µL	95.5 µL	103.7 µL
Total	**569.8 µL**	**1062.6 µL**	**1432.2 µL**	**1555.4 µL**

* When using Dynamic Assay Preparation FTD1 (SP1.0 500-15-10), one FTD-SARS-CoV-2 kit is enough to run 88 PCR reactions (including one FTD PC).

NOTES:

- To obtain accurate volumes and avoid wasting material, do not immerse the whole tip into the liquid when pipetting the 25x RT-PCR Enzyme.
- Pipette liquid very slowly to prevent air bubbles.
- Wipe tip against edge of vessel to remove excess liquid outside the tip before dispensing.
- Change tip after each pipetting step.

3. Vortex master mix briefly and spin down.
4. Use master mix immediately and do not store after use.

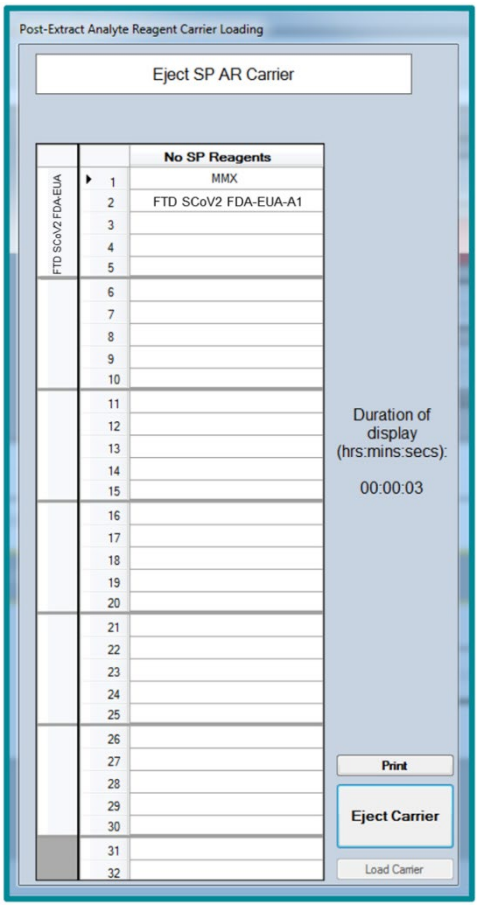
Loading the Control (No SP) Carrier

The system audibly and visually prompts the user to load the Controls (No SP) Control Carrier.

WARNING

When the system prompts, immediately replace the Controls (SP) reagents in the Control Carrier with the Controls (No SP) and the master mix reagents.

Figure 15: Post-Extract Analyte Reagent Carrier Loading Window



1. Click Eject Carrier to eject the Control Carrier from the instrument.
2. Remove the Control Carrier from the autoload tray, remove the reagents from the carrier, and replace them with the master mix and Controls (No SP) reagents. Ensure the caps are removed from all tubes before starting the run.
3. Click Load Consumables.

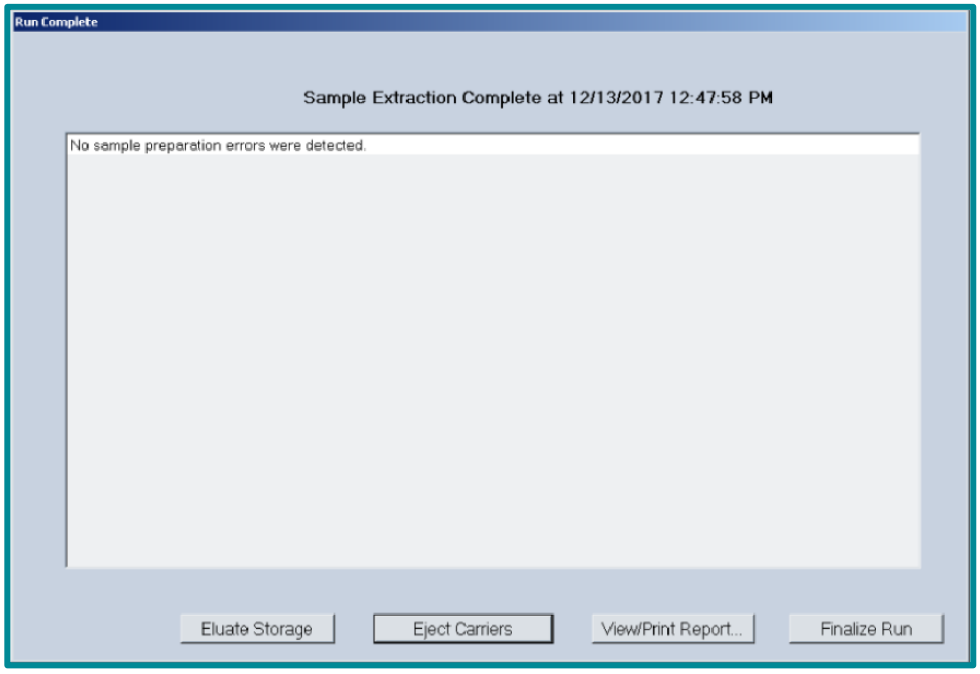
   The system performs the following actions:

   - Loads the Control Carrier.
   - Confirms adequate liquid is present in both the master mix and in the Controls (No SP).

Completing the SP Module Run

When a sample preparation run completes, the SP Software audibly beeps and displays the Run Complete window (see Figure 16).

Figure 16: Run Complete Window



- When the Run Complete dialog box displays without errors, complete the run.

- If the Run Complete dialog box indicates that sample preparation errors occurred during the run, note the error message and complete the run. Refer to the run report to determine where the sample preparation errors occurred.
- If the Run Complete dialog box indicates that a plate map file could not be generated, you cannot complete the run. Refer to the *SP Module Troubleshooting* section in the *VERSANT® kPCR Molecular System Sample Preparation (SP) Application Guide*[5] for more information about possible errors and solutions.

NOTE: FTD does not recommend using an Eluate Storage Plate for retesting with the FTD SARS-CoV-2 kit. Always re-extract the respective clinical samples before retesting.

To complete the run:

1. Verify that the autoload tray is clear and perform one of the following actions:
   - Click View/Print Report… to preview the run report file and check the results before ending the run. The system closes the Run Complete window and displays the Sample Preparation Run Report, unloads all carriers, and ends the run.
   - Click Eject Carriers to end the run without viewing the run report file. The system closes the Run Complete dialog box, unloads all carriers, and ends the run.
   - Click Finalize Run to eject carriers, finish the run, and return to the VERSANT® kPCR main screen.

2. After the run completes, immediately remove the PCR plates from the SP Module, immediately seal the PCR plate with an AD Module compatible cover, gently vortex plate, then centrifuge briefly.
3. Clean the SP Module.

Real-Time PCR Using the VERSANT® kPCR Molecular System AD (AD Module)

The VERSANT® kPCR Molecular System AD is a QuantStudio™ 5 Dx real-time thermocycler (originally supplied by ThermoFisher) modified to run the MiPLX Software Solution (Siemens Healthineers).

Running a PCR on the AD Module

To run a kPCR experiment based on a Dynamic Assay protocol, transport the sealed PCR plate to the AD Module.

NOTE: Always begin an amplification and detection run promptly on the AD Module after completing the SP Module run.

Refer to the *Load the Sealed PCR Plate* and *Unload the Sealed PCR Plate* sections in the *VERSANT® kPCR Molecular System AD (QuantStudio™ 5 Dx Real-Time) User Manual*.[6]

Importing the Plate Map

During sample preparation on the SP Module, the system generates a plate map that indicates the position of all the samples along with their corresponding sample IDs.

To manually transfer a plate map to the AD Module before importing the plate on the AD Module, the plate map from the SP Module must be loaded into a folder on the computer running the MiPLX Software Solution.

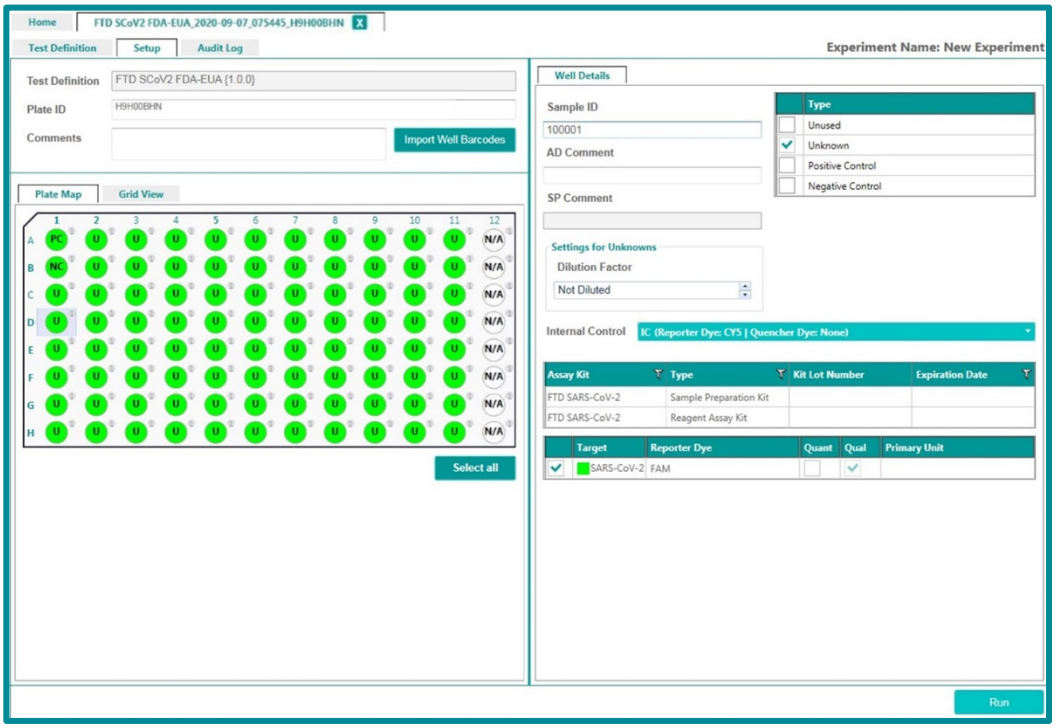
NOTE: The plate map file (example: H990KJYC.map) may be found on the SP computer in C:\VersantkPCR\Platemap. The plate map file may be transferred to C:\MapFiles on the AD computer.

To import the plate map:

1. Open the MiPLX Software Solution.
2. Select **Import Plate Map**.
3. Manually enter or scan the run ID. The run ID is the barcode number on the PCR plate (example: H990KJYC).
4. Click Ok to continue.
   - The plate map displays and has been imported into the VERSANT® kPCR Molecular System AD Software. The system automatically assigns the Sample IDs for all wells based on the plate map created on the SP Module.
   - The Amplification Detection Protocol, FTD SCoV2 FDA-EUA, parameters are automatically loaded to the MiPLX Software Solution.

View the complete Sample ID for a well by clicking the individual well and then clicking **Well Details** (see Figure 17).

Figure 17: Well Details



WARNING

Do not change the operating system settings that were configured by the Siemens service representative. These settings can change the character set available for Sample IDs and result in the use of invalid characters.

Do not edit the Sample IDs associated with the imported plate map file. The Sample ID used for the SP Module run must remain the same for the AD Module run. The Sample ID on Printed Reports may be truncated and LIS errors may occur if the Sample ID is revised.

- The system identifies each well as Unknown.

- If applicable, the system indicates any errors that occurred during sample preparation on the SP Module.

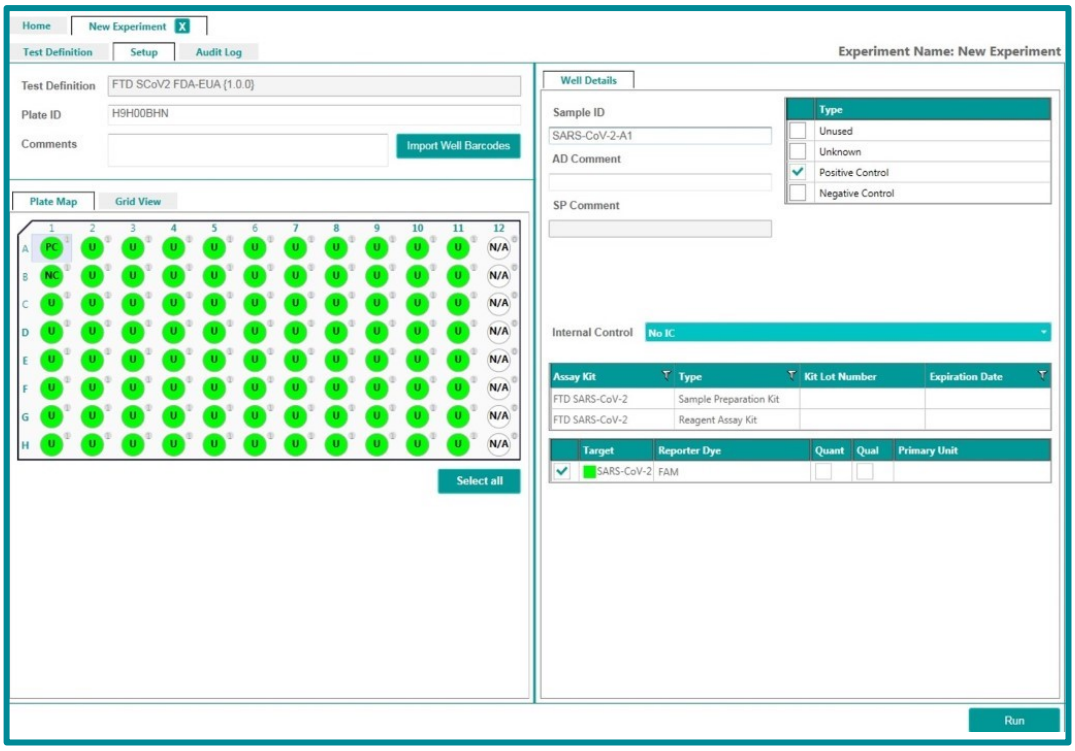
Entering Assay Kit Information

For Dynamic Assay Protocols, after importing a plate map into the AD Module, use the Assay Kit Information feature to enter assay reagent information. Otherwise, the system assumes that the assay reagent kit lot information is the same as the sample preparation reagent kit lot information (such as Kit Lot Number and Expiration Date).

Manually assign well types before starting the run (see Figure 18 through Figure 19):

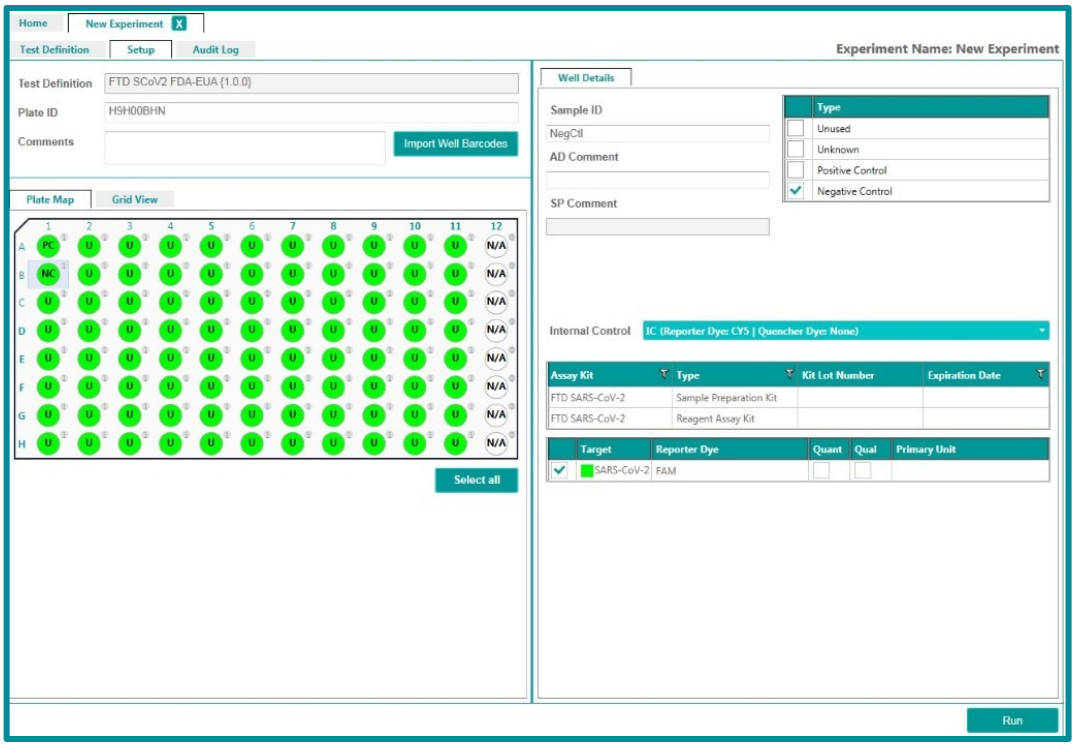
1. Select Well A1 (FTD PC).
2. Select Positive Control under Type.
3. Set Internal Control to No IC.

Figure 18: FTD PC Well Assignment



4. Select Well B1 (FTD NC).
5. Select **Negative Control** under Type.
6. Set Internal Control to **IC** (**Reporter Dye: CY5 | Quencher Dye: None**).

Figure 19: FTD NC Well Assignment



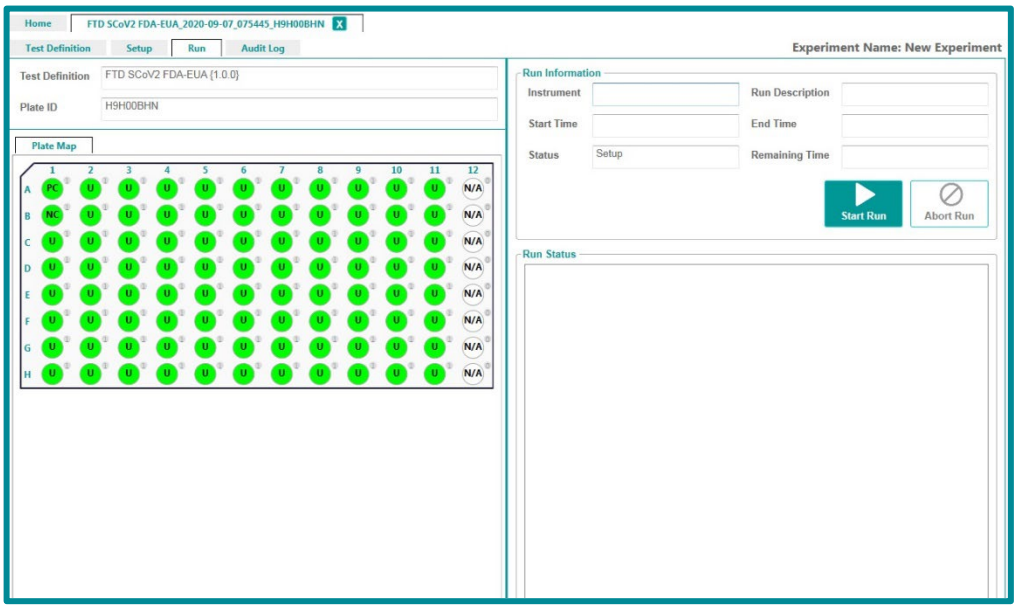
7. Select Well C1 (external RNA PC).
8. Select **Unknown Type** under Type (default setting).
9. Click **Run**.

NOTE: After starting the run, the MiPLX Software Solution automatically saves the .edp file in a default folder. When the run is over, the customer can Export as .edp and select a preferred folder.

Starting a Run on the AD Module

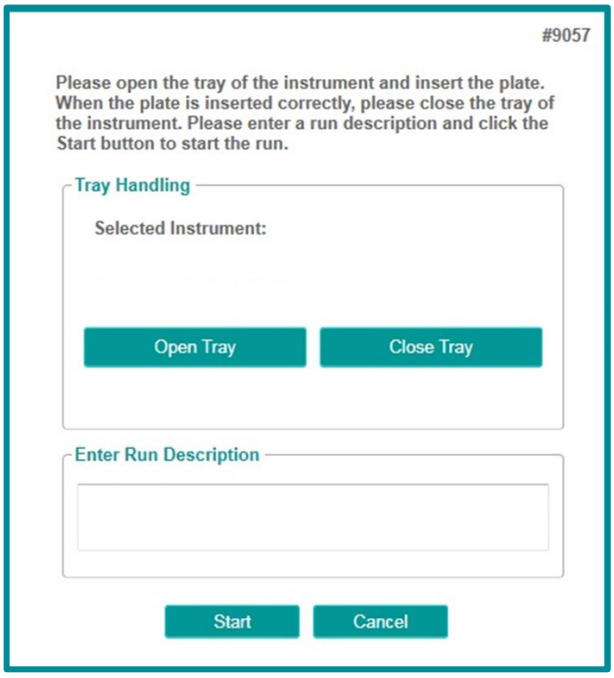
1. Click Start Run (see Figure 20).

Figure 20: Start Run



2. Click Open Tray (see Figure 21).

Figure 21: Tray Handling



3. Insert the plate on the cycler block.
4. Click Close Tray (see Figure 21).
5. Optional: Enter Run Description.
6. Click Start (see Figure 21).

Completing the Run on the AD Module

FTD highly recommends comparing the results from the run versus the Sample Preparation Run Report to detect any results that were not cleared or not selected from data gathering due to sample preparation errors.

To review the results:

1. Select the Results tab in the experiment window to display the analysis section of the software.
2. To view results, select Text Report tab from the right display window.
3. In the Text Report, review the results.

Adjusting the Threshold and Baseline

The MiPLX Software Solution sets the Threshold and Baselines to Auto, but it is recommended to check all amplification traces to determine if a manual adjustment of the Threshold and Baselines is required.

WARNING

Settings manually changed on the AD module are only applied to the run and cannot be saved for future runs.

To prevent reporting incorrect patient results, confirm that the appropriate manual Threshold and Baseline settings are used in each run.

To change the Threshold setting in the MiPLX Software Solution:

1. Click Re-Analyze.
2. Select Test Definition tab.
3. In the left Test Definition pane, select the target to apply the new threshold (SARS-CoV-2 in Assay Targets and/or IC in Internal Control Targets).
4. Expand Threshold determination factors in the Data Analysis Settings section.
5. Click Edit next to Auto Threshold and select Manual.
6. In the Manual Threshold field, click Edit and enter the appropriate value. Click Ok.
7. Click Apply to confirm the Threshold settings.

The Threshold setting must be set to Manual before changing the Baseline setting. To change the Baseline setting in the MiPLX Software Solution:

1. Click Re-Analyze.
2. Select Test Definition tab.
3. In the left Test Definition pane, select the target to apply the new baseline (SARS-CoV-2 in Assay Targets or IC in Internal Control Targets).
4. Expand Baseline determination factors in the Data Analysis Settings section.
5. Click Edit next to Auto Baseline and select Manual.
6. Click Edit next to Start Cycle and enter the appropriate value. Click Ok.
7. Click Edit next to Stop Cycle and enter the appropriate value. Click Ok.
8. Click Apply to confirm Baseline settings.

After clicking Apply, the MiPLX Software Solution will re-analyze the updated baseline and/or threshold data. The operator should review patient results after performing the adjustment.

WARNING

All other settings in the MiPLX Software Solution should not be manually adjusted.

Exporting Data to the Laboratory Information System (LIS)

If your system connects to an LIS, you can export your kPCR protocol data directly to the LIS.

Refer to the *Export Data to Laboratory Information System (LIS)* section in the *VERSANT® kPCR Molecular System AD (QuantStudio™ 5 Dx Real-Time) User Manual*.[6]

Quality Control

The FTD SARS-CoV-2 test includes the following controls:

- FTD NC
- FTD PC
- FTD IC

In addition, FTD recommends the use of an external RNA positive control (external RNA PC).

The assay uses the equine arteritis virus (EAV) as an IC, which is introduced into each sample and the FTD NC during the extraction process. The IC is extracted, processed and amplified simultaneously with each sample to monitor the extraction process and to allow the identification of PCR inhibition.

The FTD NC is processed as a sample (extraction and RT-PCR). It confirms the absence of contamination.

The FTD SARS-CoV-2 kit contains a positive control (FTD PC), which is added to each RT-PCR run. It monitors the RT-PCR process and performance of the primers and probes.

To obtain a low positive control, the content of the external RNA PC should be diluted. AccuPlex™ SARS-CoV-2 Molecular Controls Kit – Full Genome (SeraCare) should be tested at a final concentration of 350 copies per milliliter (cop/mL) and SARS-CoV-2 Positive Run Control (Exact Diagnostics) should be tested at a final concentration of 700 cop/mL. The external RNA PC and SARS-CoV-2 Negative Run Control must not substitute the FTD PC and FTD NC provided with the FTD SARS-CoV-2 kit.

Criteria for a Valid Run

The run is considered valid and patient results are reportable if all the following conditions are met:

1. FTD NC shall not show any amplification traces other than the one for the FTD IC. The FTD IC must fall below a Ct of 33. Manually inspect the FTD NC for unspecific amplification detected in the green detection channel. If there is a potential contamination (appearance of a curve in the green detection channel), results obtained are not interpretable and the whole run (including extraction) must be repeated.

2. FTD PC must show a positive (*i.e.*, exponential) amplification trace for SARS-CoV-2. The FTD PC must fall below a Ct of 33.
3. All samples, that do not show SARS-CoV-2 amplification, must show a positive amplification trace for the FTD IC with a Ct less than 33. If the FTD IC is negative or shows a Ct value greater than 33 in a SARS-CoV-2 negative sample, the result is invalid. If the FTD IC is negative or shows a Ct value greater than 33 in a SARS-CoV-2 positive sample, the run is valid.
4. External RNA PC must show a positive (*i.e.*, exponential) amplification trace for SARS-CoV-2. Amplification traces must be manually reviewed for amplification and run validity.

Results

Interpretation of Results

NOTE: MiPLX Software Solution does not support automated interpretation of the external RNA PC.

The results of interpretation of clinical samples and controls are shown below:

Sample/ Control	SARS-CoV-2	FTD IC	Overall Result	Interpretation
Patient Sample	Negative	Ct < 33	Valid	SARS-CoV-2 not detected.
	Negative	Ct ≥ 33	Invalid	There was an error during extraction/PCR. Retest the sample.
	Negative	Not detected	Invalid	There was an error during extraction/PCR. Retest the sample.
	Positive	Ct < 33	Valid	SARS-CoV-2 detected.
	Positive	Ct > 33	Valid	SARS-CoV-2 detected.
	Positive	Not detected	Valid	SARS-CoV-2 detected.
FTD NC	Negative	Ct < 33	Valid	Run is valid.
	Negative	Ct ≥ 33	Invalid	There was an error during extraction/PCR. Run is invalid.
	Negative	Not detected	Invalid	There was an error during extraction/PCR. Run is invalid.
FTD PC	Ct < 33	Not applicable	Valid	Run is valid.
	Ct ≥ 33	Not applicable	Invalid	There was an error during PCR. Run is invalid.
	Not detected	Not applicable	Invalid	There was an error during PCR. Run is invalid.

The results will be reported as a cycle threshold (Ct) unit.

If criteria listed in the *Criteria for a Valid Run* section are met, any patient sample displaying an exponential trace shall be considered as positive for the pathogen targeted by the kit. An absence of an exponential trace indicates an absence or undetectable load of nucleic acid.

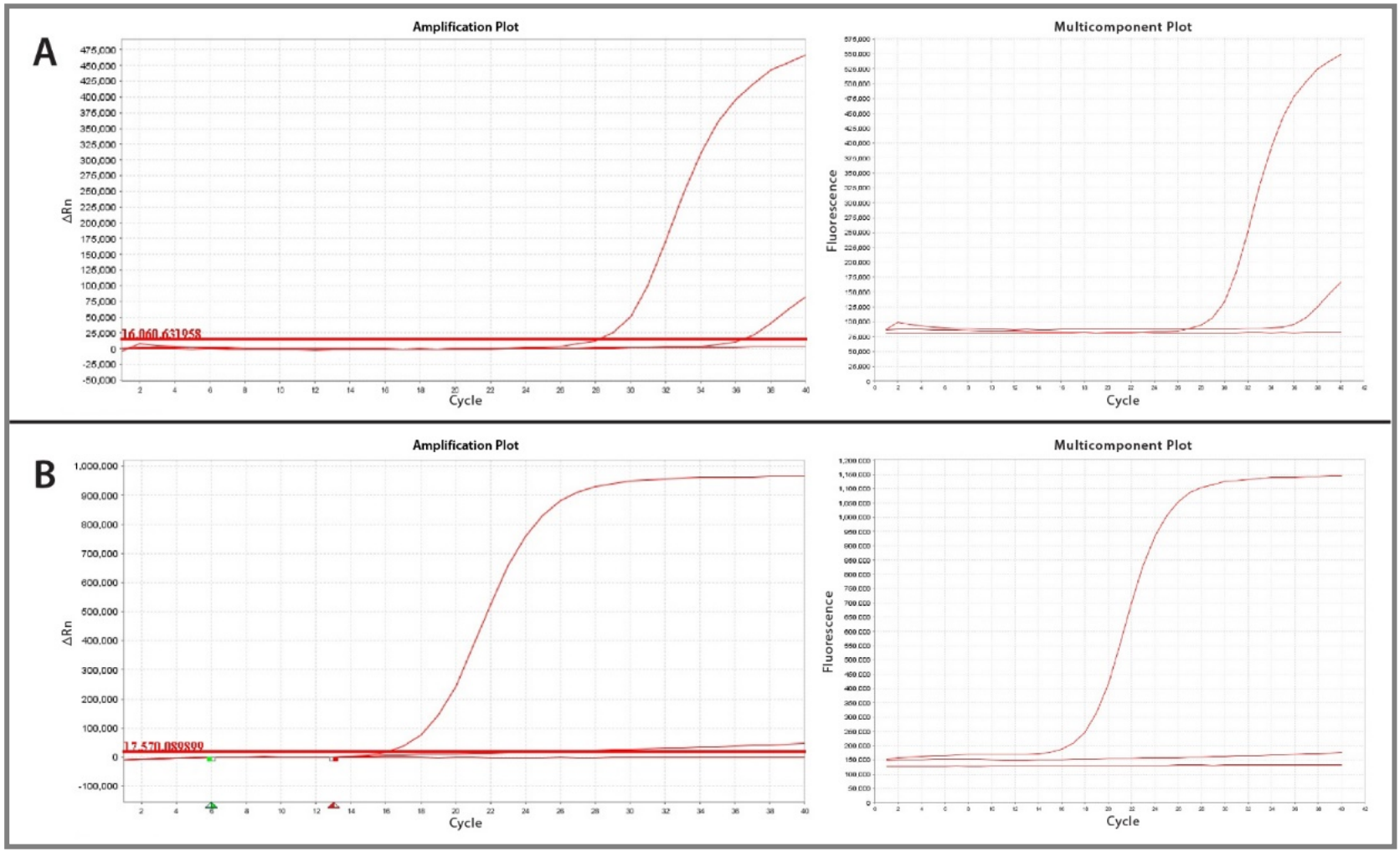
The FTD IC must be positive in each extracted material that is not positive for SARS-CoV-2.

IMPORTANT! Pay attention to the section below for important information regarding baseline settings and multicomponent plots.

Baseline Setting and Multicomponent Plot

The amplification curve baseline is one of the parameters that can affect PCR results. In case the baseline is incorrectly set, a Ct value can be displayed even if no real amplification occurred. Figure 22 illustrates the difference between a real amplification (A) and an incorrect baseline setting conveyed with a Ct value even if no amplification occurred (B).

Figure 22: Comparison between Real Amplification Signal (A) and Incorrect Baseline Setting (B)



Always check the signal displays in the Multicomponent Plot and the baseline is correctly set before concluding that an amplification trace is exponential. The Multicomponent Plot displays the complete spectral contribution of each dye and helps to review reporter dye signal for spikes, dips and sudden changes. Contact the equipment manufacturer or Fast Track Diagnostics for advice on how to set up the baseline correctly.

Limitations

- The use of the FTD SARS-CoV-2 as an *in vitro* diagnostic under the FDA Emergency Use Authorization (EUA) is limited to laboratories that are certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), U.S.C. §263a, that meet requirements to perform high complexity tests.
- FTD SARS-CoV-2 can be used only with the specimens listed in the Intended Use statement. Other specimen types have not been evaluated with this assay.
- The sample storage and shipment instructions provided are recommendations. Verification and validation data for specimen collection and handling (transport and storage) are not available.
- Remel M4RT® transport medium is not recommended for use with FTD SARS-CoV-2.
- This kit is a qualitative kit that does not provide a quantitative value for the detected pathogens in the specimen. There is no correlation between the Ct values obtained and the amount of pathogens in the specimen collected.
- Other parameters can lead to false positive, negative or invalid results related to patient conditions (use of antiviral therapy, patient age, patient history of respiratory infections, presence of symptoms and the stage of infection).
- The use of this kit should be limited to personnel trained in the technique of RT-PCR and in the use of FTD kits.
- The performance of the kit has been verified and validated using the procedures provided in the instructions for use only. Modifications to these procedures may alter the performance of the test.
- The performance of this kit has been evaluated for use with human specimen material only.
- This test shall not be the only element consulted for diagnosis or treatment decisions. A specimen not detected cannot be presumed to be negative for this pathogen since results are dependent on several variables, as explained above.
- Reliable results of this test require appropriate specimen collection as well as appropriate specimen and kit transport and storage and processing procedures. Failure to follow these procedures will produce incorrect results, leading to false-positive and negative values or invalid results.
- Low levels of viruses can be detected below the limit of detection, but results may not be reproducible.
- Mutations within the regions of the targets for the virus detected by the kit may occur. As a consequence, primer and probe combinations may fail to detect the presence of this virus.
- The performance of this test was established based on the evaluation of a limited number of clinical specimens. Clinical performance has not been established with all circulating variants but is anticipated to be reflective of the prevalent variants in circulation at the

time and location of the clinical evaluation. Performance at the time of testing may vary depending on the variants circulating, including newly emerging strains of SARS-CoV-2 and their prevalence, which change over time.

Conditions of Authorization for the Laboratory

The Fast Track Diagnostics™ SARS-CoV-2 test Letter of Authorization, along with the authorized Fact Sheet for Healthcare Providers, the authorized Fact Sheet for Patients and authorized labeling are available on the FDA website:

https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/in-vitro-diagnostics-euas.

To assist clinical laboratories using the FTD SARS-CoV-2 test, the relevant Conditions of Authorization are listed below:

A. Authorized laboratories[1] using the FTD SARS-CoV-2 test must include with test result reports, all authorized Fact Sheets. Under exigent circumstances, other appropriate methods for disseminating these Fact Sheets may be used, which may include mass media.
B. Authorized laboratories using the FTD SARS-CoV-2 test must use the FTD SARS-CoV-2 test as outlined in the FTD SARS-CoV-2 Instructions for Use. Deviations from the authorized procedures, including the authorized instruments, authorized extraction methods, authorized clinical specimen types, authorized control materials, authorized other ancillary reagents and authorized materials required to perform the FTD SARS-CoV-2 test are not permitted.
C. Authorized laboratories that receive the FTD SARS-CoV-2 test must notify the relevant public health authorities of their intent to run the test before initiating testing.
D. Authorized laboratories using the FTD SARS-CoV-2 test must have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.
E. Authorized laboratories must collect information on the performance of the test and report to DMD/OHT7-OIR/OPEQ/CDRH (via email: CDRH-EUA-Reporting@fda.hhs.gov) and FTD Technical Support (tel: 1-877-229-8601 / email: support-ftd.team@siemens-healthineers.com) any suspected occurrence of false positive or false negative results and significant deviations from the established performance characteristics of the test of which they become aware.
F. All laboratory personnel using the test must be appropriately trained in RT-PCR techniques and use appropriate laboratory and personal protective equipment when handling this kit and use the test in accordance with the authorized labeling.
G. FTD, its authorized distributor(s) and authorized laboratories using the FTD SARS-CoV-2 test must ensure that any records associated with this EUA are maintained until otherwise notified by FDA. Such records will be made available to FDA for inspection upon request.

[1] The letter of authorization refers to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meet requirements to perform high complexity tests as "authorized laboratories."

Performance Characteristics

Performance characteristics show the analytical and clinical performance data of FTD SARS-CoV-2. The analytical performance (analytical sensitivity, inclusivity and analytical specificity) was evaluated using the NucliSENS® easyMAG® (bioMérieux) extraction system with the Applied Biosystems® 7500 (ThermoFisher Scientific) PCR System and the VERSANT® kPCR Molecular System (Siemens Healthineers).

Limit of Detection – Analytical Sensitivity

Limit of detection (LoD) studies determine the lowest detectable concentration of SARS-CoV-2, at which greater or equal to 95% of all (true positive) replicates test positive.

To determine the LoD, a cultured virus (quantified) of an isolate from a US patient (USA-WA1/2020, vendor Zeptometrix, catalog number 0810587CFHI) was serially diluted in a simulated respiratory matrix (SRM).

A preliminary LoD was determined testing replicates of two-fold serial dilutions of quantified cultured SARS-CoV-2 virus. The preliminary LoD data were used for the Probit (PROBability unITs) Regression Analysis. Based on results provided by Probit analysis, an LoD confirmation study was performed. The LoD concentration from the Probit analysis (0.0023 $TCID_{50}$/mL) was

confirmed using the NucliSENS® easyMAG® extraction system / Applied Biosystems® 7500 real-time thermocycler and the VERSANT® kPCR Molecular System.

NucliSENS® easyMAG® / Applied Biosystems® 7500

Twenty-three separate spiked samples were prepared by spiking the LoD concentration of the virus culture into SRM (in ESwab™ Liquid Amies medium [COPAN]). Each sample was extracted once (200 µL sample input, 55 µL elution volume) and tested in singlicate with three different lots of PP mix. The results of the LoD confirmation study are summarized below:

PP Mix Lot	LoD ($TCID_{50}$/mL)	Tested	Total Detected	Detection Rate (%)	Mean Ct	SD
1	0.0023	23	22	95.7	38.0	0.9
2	0.0023	23	23	100.0	37.4	0.9
3	0.0023	23	22	95.7	37.4	0.8

Legend: $TCID_{50}$/mL = Median tissue culture infectious dose per milliliter, PP = Primer and probe, Ct = Cycle threshold, SD = Standard deviation

The LoD at 0.0023 $TCID_{50}$/mL of FTD SARS-CoV-2 in a simulated respiratory matrix, as determined by the Probit analysis, was experimentally confirmed with an overall detection rate of 97.85%.

VERSANT® kPCR Molecular System

Twenty separate spiked samples were prepared by spiking the LoD concentration of the virus culture into SRM. Samples were prepared in SRM in three different types of transport media, including Universal Viral Transport (UVT, Becton Dickinson), Viral Transport Medium (VTM) prepared in-house according to CDC Protocol DSR-052-01, and Eswab™ Liquid Amies medium (COPAN). Samples were diluted to the LoD at 0.0023 $TCID_{50}$/mL, as determined by the Probit Regression Analysis. Each sample was pretreated in a 1:2 dilution (350 µL sample with 350 µL VERSANT® Sample Preparation 1.0 Lysis Buffer). Samples were then extracted using the DAP FTD1 (SP1.0 500-15-10) protocol (500 µL input volume, 100 µL elution volume) and tested in singlicate. The results of the LoD confirmation study are summarized below:

Medium	LoD ($TCID_{50}$/mL)	Tested	Total Detected	Detection Rate (%)	Mean Ct	SD
SRM in UVT	0.0023	20	19	95.0	36.4	0.7
SRM in VTM	0.0023	20	20	100.0	36.5	0.9
SRM in Eswab™	0.0023	20	19	95.0	37.1	1.0

Legend: $TCID_{50}$/mL = Median tissue culture infectious dose per milliliter, PP = Primer and probe, Ct = Cycle threshold, SD = Standard deviation

The LoD at 0.0023 $TCID_{50}$/mL of FTD SARS-CoV-2 in a simulated respiratory matrix in three different transport media was experimentally confirmed with an overall detection rate of greater than or equal to 95%.

Inclusivity – Analytical Reactivity

Inclusivity is the capacity of an assay to detect several strains or serovars of species, several species of a genus, or a similar grouping of closely related organisms.

Inclusivity was assessed using an *in silico* analysis done on all sequences available at the time of the analysis for the target organism in the NCBI Nucleotide collection and the Global Initiative on Sharing All Influenza Data (GISAID) database (https://www.gisaid.org/).

A total of 87191 sequences were downloaded: 7753 from the GenBank database (02 July 2020) and 79438 from the GISAID database (24 August 2020). SARS-CoV-2 primers and probes were mapped to the sequences to check for potential matches producing amplicons. Incomplete sequences or sequences from animal hosts were excluded from the GISAID and GenBank analysis; that led to the exclusion of 892 sequences from the N gene assay and 207 sequences from the ORF1ab assay.

- The sequence alignment showed that the SARS-CoV-2 N gene assay showed no mismatch with 99.21% and 1 mismatch with 0.79% of the analyzed sequences from the GenBank database.
- The sequence alignment showed that the SARS-CoV-2 N gene assay showed no mismatch with 99.21% and 1 mismatch with 0.79% of the analyzed sequences from the GISAID database.
- The sequence alignment showed that the SARS-CoV-2 ORF1ab assay showed no mismatch with 99.49% and 1 mismatch with 0.51% of the analyzed sequences from the GenBank database.
- The sequence alignment showed that the SARS-CoV-2 ORF1ab assay showed no mismatch with 99.49% and 1 mismatch with 0.51% of the analyzed sequences from the GISAID database.

Due to the dual-target approach, only 9 of all the sequences that presented mismatches show a mismatch in both assays. None of these mismatches were located at a critical position that would cause detection issues and are not predicted to impact assay performance.

An *in silico* analysis of the SARS-CoV-2 primers and probe sets was performed against the variant UK, South Africa, Brazil, and California strains. The *in silico* analysis predicts that assay performance is unlikely to be impacted by the currently circulating escape variants.

Cross-Reactivity – Analytical Specificity

In Silico Analysis

A total of 39 bacterial/viral/fungal strains have been analyzed *in silico*. NCBI BLAST tool was used to check for cross-reactivity of the different primers and probes of the SARS-CoV-2 assay against the non-redundant nucleotide database. BLAST tool search default parameters were used except for the "organism." The search was limited to using the taxonomy ID (taxid/txid) of the respective pathogen. Each primer and probe were compared against all available genome sequences of a certain taxid.

The results showed that in a few cases, the analyzed pathogens have more than 80% homology with any of the primers/probes designed for FTD SARS-CoV-2. Among those are SARS-CoV, *Legionella sainthelensi*, *Legionella spiritensis*, *Staphylococcus epidermidis*, and *Streptococcus salivarius*. These pathogens have been analyzed in more detail in a sequence alignment. No potential unintended cross-reactivity is expected based on this *in silico* analysis. The results* of the *in silico* analysis are summarized below:

Pathogen	Taxonomy ID Included in BLAST Search	***In Silico*** Analysis Result N gene Primer/Probe	***In Silico*** Analysis Result ORF1ab Primer/Probe
Influenza C virus	NCBI:txid11552	< 80%	< 80%
Parechovirus	NCBI:txid138954	< 80%	< 80%
Candida albicans	NCBI:txid5476	< 80%	< 80%
Corynebacterium diphtheriae	NCBI:txid1717	< 80%	< 80%
Legionella non-*pneumophila*[a]	NCBI:txid445 exclude NCBI:txid446	83%	< 80%
Bacillus anthracosis (Anthrax)	NCBI:txid1392	< 80%	< 80%
Moraxella catarrhalis	NCBI:txid480	< 80%	< 80%
Neisseria elongata	NCBI:txid495	< 80%	< 80%
Neisseria meningitidis	NCBI:txid487	< 80%	< 80%
Pseudomonas aeruginosa	NCBI:txid287	< 80%	< 80%
Staphylococcus epidermidis	NCBI:txid1282	83%	< 80%
Streptococcus salivarius	NCBI:txid1304	< 80%	91%
Leptospira	NCBI:txid171	< 80%	< 80%
Chlamydia psittaci	NCBI:txid83554	< 80%	< 80%
Coxiella burnetii (Q-Fever)	NCBI:txid777	< 80%	< 80%

Staphylococcus aureus	NCBI:txid1280	< 80%	< 80%
Streptococcus pyogenes	NCBI:txid1314	< 80%	< 80%
Pneumocystis jirovecii	NCBI:txid42068	< 80%	< 80%
Human coronavirus 229E	NCBI:txid11137	< 80%	< 80%
Human coronavirus OC43	NCBI:txid31631	< 80%	< 80%
Human coronavirus HKU1	NCBI:txid290028	< 80%	< 80%
Human coronavirus NL63	NCBI:txid277944	< 80%	< 80%

* Results denote the percent coverage given by BLAST analysis for all primers and probes, in case homology was greater than 80%, it shows the highest value.

Pathogen	Taxonomy ID Included in BLAST Search	***In Silico*** Analysis Result N gene Primer/Probe	***In Silico*** Analysis Result ORF1ab Primer/Probe
SARS-coronavirus	NCBI:txid694009 exclude HCoV-19 (taxid:2697049)	90%	95%
MERS-coronavirus	NCBI:txid1335626	< 80%	< 80%
Adenovirus (*e.g.*, C1 Ad. 71)	NCBI:txid10509	< 80%	< 80%
Human Metapneumovirus (hMPV)	NCBI:txid162145	< 80%	< 80%
Human Parainfluenza virus 1–4	NCBI:txid12730, NCBI:txid11216, NCBI:txid1979160, NCBI:txid1979161	< 80%	< 80%
Influenza A virus	NCBI:txid11320	< 80%	< 80%
Influenza B virus	NCBI:txid11520	< 80%	< 80%
Enterovirus A–D (*e.g.*, EV68)	NCBI:txid138948, NCBI:txid138949, NCBI:txid138950, NCBI:txid138951	< 80%	< 80%
Human Respiratory syncytial virus	NCBI:txid11250	< 80%	< 80%
Rhinovirus	NCBI:txid147711, NCBI:txid147712, NCBI:txid463676	83%	< 80%
Chlamydia pneumoniae	NCBI:txid83558	< 80%	< 80%
Haemophilus influenzae	NCBI:txid727	< 80%	< 80%
Legionella pneumophila	NCBI:txid446	< 80%	< 80%

Mycobacterium tuberculosis	NCBI:txid1773	< 80%	< 80%
Streptococcus pneumoniae	NCBI:txid1313	< 80%	< 80%
Bordetella pertussis	NCBI:txid520	< 80%	< 80%
Mycoplasma pneumoniae	NCBI:txid2104	< 80%	< 80%

[a] Among the *Legionella* non-*pneumophila* strains, *Legionella sainthelensi* and *Legionella spiritensis* revealed a homology greater than 80% for given primers and probe.

In addition, the concerned organisms have been tested *in vitro* for potential cross-reactivity and no amplification was observed (see *In Vitro* Analysis section).

In Vitro Analysis

A total of 32 bacterial/viral RNA/DNA cultures or samples have been tested *in vitro* for cross-reactivity. A total of 5 pools were generated by spiking a maximum of 5 organisms into Eswab™ Liquid Amies medium (COPAN). Also, a pool of 5 nasal fluid patient samples was generated. Each pool was extracted in triplicate and tested with FTD SARS-CoV-2. Six cultures have been extracted in triplicates and tested separately without pooling. A culture was not available for human coronavirus HKU1, but a patient sample was extracted and tested. In addition, 6 genomic previously-acquired RNA/DNA samples were tested for PCR.

For some organisms, the concentration was unknown, they were tested previously by RT-PCR using FTD Respiratory pathogens 21 (CE-IVD) and Ct values are indicated. For others, the Ct values given by the vendor are shown. The results of all test organisms and the respective tested concentration pools tested *in vitro* are summarized below:

Pool ID / Sample ID	Interfering Organism	Tested Concentration	Unit	Result
P1	Adenovirus 71	1.13E+06	$TCID_{50}$/mL	No cross-reactivity
	Human Parainfluenza virus 2	1.13E+06	$TCID_{50}$/mL	No cross-reactivity
	Enterovirus	2.00E+06	$TCID_{50}$/mL	No cross-reactivity
	Rhinovirus	1.13E+07	$TCID_{50}$/mL	No cross-reactivity
	Streptococcus pneumoniae	1.24E+08	cop/mL	No cross-reactivity

P2	Human Metapneumovirus (hMPV) A	6.82E+05	$TCID_{50}$/mL	No cross-reactivity
	Parainfluenza virus 3	1.13E+06	$TCID_{50}$/mL	No cross-reactivity
	Parainfluenza virus 4	2.00E+05	$TCID_{50}$/mL	No cross-reactivity
	Human Metapneumovirus (hMPV) B	1.56E+05	$TCID_{50}$/mL	No cross-reactivity
	Parainfluenza virus 1	2.00E+05	$TCID_{50}$/mL	No cross-reactivity
P3	Human coronavirus OC43	1.41E+04	$TCID_{50}$/mL	No cross-reactivity
	Human coronavirus NL63	8.39E+03	$TCID_{50}$/mL	No cross-reactivity
	Influenza B virus	Unknown (Ct 20.4)[a]	N/A	No cross-reactivity
P4	*Chlamydophila pneumoniae*	2.01E+04	cop/mL	No cross-reactivity
	Haemophilus influenzae	5.71E+02	CFU/mL	No cross-reactivity
	Legionella pneumophila	1.00E+03	CFU/mL	No cross-reactivity
	Bordetella pertussis	1.43E+03	CFU/mL	No cross-reactivity

Pool ID / Sample ID	Interfering Organism	Tested Concentration	Unit	Result
P5	*Mycoplasma pneumoniae*	Unknown (Ct 21)[a]	N/A	No cross-reactivity
	Human Respiratory syncytial virus (HRSV-A)	3.14E+06	PFU/mL	No cross-reactivity
	Human Respiratory syncytial virus (HRSV-B)	3.26E+05	$TCID_{50}$/mL	No cross-reactivity
P6	Nasal Fluid Pool	N/A	N/A	No cross-reactivity
S7	*Pneumocystis jirovecii*	1.64E+08	cop/mL	No cross-reactivity
S8	*Streptococcus salivarius*	5.91E+07	cop/mL	No cross-reactivity
S9	*Staphylococcus epidermidis*	8.05E+06	cop/mL	No cross-reactivity

S10	*Legionella sainthelensi*	2.29E+08	cop/mL	No cross-reactivity
S11	*Legionella spiritensis*	4.08E+08	cop/mL	No cross-reactivity
S12	*Streptococcus pyogenes*	6.82E+08	cop/mL	No cross-reactivity
S13	*Mycobacterium tuberculosis*	1.00E+06	cop/mL	No cross-reactivity
S14	*Influenza* A virus	1.00E+06	cop/mL	No cross-reactivity
S15	SARS-coronavirus	Unknown (Ct 18)[a]	N/A	No cross-reactivity
S16	MERS-coronavirus	Unknown (Ct 29)[a]	N/A	No cross-reactivity
S17	Human coronavirus 229E	Unknown (Ct 28)[a]	N/A	No cross-reactivity
S18	Human coronavirus HKU1	Unknown (Ct 14)[a]	N/A	No cross-reactivity

[a] Ct value is given by the supplier or tested with FTD Respiratory pathogens 21 (CE-IVD).

Legend: $TCID_{50}$ = Median tissue culture infectious dose, cop/mL = copies per milliliter, CFU/mL = Colony-forming unit per milliliter, PFU/mL = Plaque-forming unit per milliliter

Clinical Performance

The performance of FTD SARS-CoV-2 was evaluated using the NucliSENS® easyMAG® extraction system / Applied Biosystems® 7500 real-time thermocycler and the VERSANT® kPCR Molecular System.

NucliSENS® easyMAG® / Applied Biosystems® 7500

The performance of FTD SARS-CoV-2 was established using 80 nasopharyngeal swabs, collected from male and female adult patients with signs and symptoms of an upper respiratory infection.

A total of 44 positive specimens and 36 negative specimens were tested with FTD SARS-CoV-2 using the NucliSENS® easyMAG® extraction method and the Applied Biosystems® 7500 Real-Time PCR System. The clinical performance study was evaluated by comparing FTD SARS-CoV-2 results to an FDA EUA-authorized RT-PCR test. During the analyses, 6 negative samples were excluded; 1 was excluded because of a protocol deviation and 5 did not pass the criteria for a valid run (FTD IC failure). The results are shown below:

Agreements between FTD SARS-CoV-2 and FDA EUA RT-PCR using Nasopharyngeal Swabs (n=74)		FDA EUA RT-PCR Test	
		Positive	Negative
FTD SARS-CoV-2	Positive	44	0
	Negative	0	30
Positive Percent Agreement	100% (44/44) (95% Confidence Interval: 91.97, 100)		
Negative Percent Agreement	100% (30/30) (95% Confidence Interval: 88.65, 100)		

The results showed 100% positive percent agreement (95% Confidence Interval: 91.97–100) and 100% negative percent agreement (95% Confidence Interval: 88.65–100) between FTD SARS-CoV-2 and the FDA-authorized RT-PCR test for the detection of SARS-CoV-2 in nasopharyngeal swabs.

VERSANT® kPCR Molecular System

The performance of FTD SARS-CoV-2 was determined using 109 nasopharyngeal swabs, collected from male and female patients with signs and symptoms of an upper respiratory infection.

A total of 60 positive specimens and 49 negative specimens, as determined using an FDA EUA-authorized RT-PCR test, were tested with FTD SARS-CoV-2 using the VERSANT® kPCR Molecular System. The clinical performance study was evaluated by comparing FTD SARS-CoV-2 results to the FDA EUA RT-PCR test. Each sample was pretreated in a 1:2 dilution (350 µL sample with 350 µL VERSANT® Sample Preparation 1.0 Lysis Buffer) before extraction. The results are shown below:

Agreements between FTD SARS-CoV-2 and FDA EUA RT-PCR test using VERSANT® kPCR Molecular System (n=109)		FDA EUA RT-PCR Test	
		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 3 discordant samples (positive by FTD SARS-CoV-2) were positive by sequencing.

The results showed 100% positive percent agreement (95% Confidence Interval: 93.98–100) and 93.88% negative percent agreement (95% Confidence Interval: 83.48–97.90) between FTD SARS-CoV-2 and the FDA EUA-authorized RT-PCR tests for the detection of SARS-CoV-2 in nasopharyngeal swabs. Two of the three discordant results (positive with FTD SARS-CoV-2) were also positive using sequencing methods.

FDA SARS-CoV-2 Reference Panel Testing

The evaluation of sensitivity and MERS-CoV cross-reactivity was performed using reference material (T1), blinded samples and a standard protocol provided by the FDA. The study included a range finding study and a confirmatory study for LoD on the VERSANT® kPCR Molecular System. Blinded sample testing was used to establish specificity and to corroborate the LoD. The results are summarized in the table below.

Reference Materials Provided by FDA	Specimen Type	Product LoD	Cross-Reactivity
SARS-CoV-2	NP	540 NDU/mL	N/A
MERS-CoV		N/A	ND

Legend: NP = Nasopharyngeal, NDU/mL = RNA nucleic acid-based amplification test (NAAT) detectable units per milliliter, N/A = Not applicable, ND = Not detected

Troubleshooting

This section describes a non-exhaustive list of control errors that a user may observe with FTD SARS-CoV-2 and suggested corrective actions.

Observation	Possible Cause	Corrective Action
FTD PC or external RNA PC control does not amplify	Incorrect programming of the thermocycler temperature profile.	Compare temperature profile to IFU.
	Incorrect configuration of the PCR run.	• Confirm reagents were added in the correct sequence; repeat the PCR, if necessary. • Check the calibration of the pipettes.
	Incorrect handling of the positive controls.	Inadequate or no vortexing, or control was not adequately thawed at room temperature.
	Storage conditions for one or more product components did not comply with the instructions or the FTD kit has expired.	Check storage conditions and expiration date on the kit box. Discard the kit if necessary.
Weak or no signal of the FTD IC	PCR conditions do not comply with the protocol.	Ensure extraction and amplification workflow was performed as described. Repeat analysis, if necessary. In case the problem persists, consider the presence of interfering material in your samples.
	Amplification of FTD IC was inhibited or the extraction of the FTD IC was inadequate	
Amplification in the FTD NC	Contamination during PCR plate set up or during extraction.	• Repeat PCR plate set up with new reagents, samples and controls. • Repeat extraction procedure with new reagents.

	• To avoid contamination from the FTD PC, pipette the positive control last. • Decontaminate the workspace and instruments after each use.

For customer support, contact your local technical support provider or distributor.

Technical Assistance

For customer support, contact your local technical support provider or distributor.

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References

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2. Center for Disease Control (CDC) website: https://www.cdc.gov/urdo/downloads/SpecCollectionGuidelines.pdf. Accessed March 2021.
3. CDC. Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens from Patients Under Investigation (PUIs) for 2019 Novel Coronavirus. https://www.cdc.gov/coronavirus/2019-nCoV/guidelines-clinical-specimens.html. Accessed March 2021.
4. World Health Organization (WHO). Laboratory testing for coronavirus disease 2019 (COVID-19) in suspected human cases: Interim guidance, 19 March 2020.
5. Siemens Healthineers. SMN 11222579, *VERSANT® kPCR Molecular System Sample Preparation (SP) Application Guide*.
6. Siemens Healthineers. SMN 11536891, *VERSANT® kPCR Molecular System AD (QuantStudio™ 5 Dx Real-Time PCR) User Manual*.

Definition of Symbols

This section describes all symbols used to convey product labeling description, use or handling information on components or unit-of-sale packaging.

Symbol	Definition	Symbol	Definition
IVD	*In vitro* diagnostic medical device	Σ n	Contains sufficient for <*n*> tests
REF	Catalog number	LOT	Batch code
	Manufacturer		Use-by date
	Date of manufacture		Keep away from sunlight
RxOnly	Prescription device (US Only) Applies only to United States IVD assays. CAUTION: Federal (USA) law restricts this device to sale by or on the order of a licensed healthcare professional	**YYYY-MM-DD**	Date format (Year-Month-Day)
	Consult instructions for use	**YYYY-MM**	Date format (Year-Month)
	Caution/Warning		Store upright
	Temperature limit		Irritant
		MADE IN LUXEMBOURG	Made in Luxembourg

Legal Information

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Appendix A: Additional Label

For Applied Biosystems® 7500 Real-Time PCR System

Please print the "Emergency Use Only" label below and place it on the front panel of Applied Biosystems® 7500 Real-Time PCR System. If the instrument is labeled as Research Use Only, please cover it with the "Emergency Use Only" label below. The instruments should retain this label throughout the EUA use of the FTD SARS-CoV-2.

Emergency Use Only

This instrument is authorized for use with FTD