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. A, 2020-05
Product Name	FTD SARS-CoV-2 (FTD-114-96) REF 11416302 Σ 96
Specimen Types	Upper respiratory specimens (such as nasal swabs, nasopharyngeal swabs, oropharyngeal swabs, nasopharyngeal wash/aspirate or nasal aspirate) and Bronchoalveolar lavage (BAL)
Processed Sample Volume	200 µL required

FTD SARS-CoV-2 was validated with the Applied Biosystems® 7500 Real-Time PCR System (ThermoFisher Scientific) and the NucliSENS® easyMAG® (bioMérieux).

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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 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), 42 U.S.C. §263a, 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. Laboratories within the United States and its territories are required to report all positive 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 trained 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 30, 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 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 addition of the internal control (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. 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 simultaneously detect different targets in one reaction.

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 in close proximity 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.
- Laboratories within the United States and its territories are required to report all positive 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 IC contains lysis buffer.

WARNING

FTD SARS-CoV-2 IC:

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

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

- Use of this product should be limited to personnel trained in the techniques of PCR.
- Take normal precautions required for handling all laboratory reagents.
- **For patient samples only:**
  - Disinfect spills promptly with 0.5% sodium hypochlorite solution (1:10 v/v bleach) or equivalent disinfectant.
- **For all reagents:**
  - Disinfect spills promptly using Microcide SQ. Do not use bleach.
- Regard 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.

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 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

Nasal, 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 pack 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

Table 1 details the components for FTD SARS-CoV-2.

Table 1: FTD SARS-CoV-2 Components

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 96 reactions: 1 x 144 µL	-30°C to -10°C
SCoV2 PC 96	Double-stranded synthetic DNA molecules, buffer, stabilizing agents	— 96 reactions: 1 x 150 µL	
Negative Ctrl 96	Nuclease-free water	— 96 reactions: 1 x 2000 µL	
Internal Ctrl 96	Double-stranded circular DNA molecules, buffer, <5.0% guanidinium hydrochloride, <0.1% maleic acid	— 96 reactions: 1 x 350 µL	
25x RT-PCR Enz. 96	Enzymes, buffer, glycerol, Ribonuclease (RNase) inhibitors, stabilizing agents	25x RT-PCR Enzyme mix 96 reactions: 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 96 reactions: 1 x 1200 µL	

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

IMPORTANT! The table above reflects the standard kit color scheme. Due to supplier issues during the COVID-19 crisis, individual tube cap colors may be substituted due to availability. Always check the labeling of the reagent prior to use.

Each vial contains additional volume for pipetting inaccuracy. The box and each vial are labeled with a lot number.

Reagents in the kits are sufficient for 96 reactions. Each kit includes IC, NC and PC components.

REF	Contents	Number of Reactions
11416302 (FTD-114-96)	FTD SARS-CoV-2	96

Materials Required but Not Provided

The kit has been validated with the Applied Biosystems® 7500 Real-Time PCR System (ThermoFisher Scientific) and the NucliSENS® easyMAG® (bioMérieux).

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

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

NOTE: Refer to the manufacturer (bioMérieux) for specific part number information.

FTD recommends use of an external RNA positive control (RNA PC) with each run, such as:

Supplier Part Number	Contents
0505-0126	AccuPlex™ SARS-CoV-2 Reference Material Kit

NOTE: Refer to the manufacturer (Seracare) for specific part number information.

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-packaged
- Disposable, powder-free gloves
- Vortex mixer
- Desktop centrifuge
- Sample rack
- Sample collection devices/material
- Bleach/Microcide (or other product according to laboratory cleaning procedure)
- 96-well PCR plates and plate sealers
- Tubes

Assay Procedure

Extraction Using the NucliSENS® easyMAG® System

To prepare the sample:

1. Thaw negative control (NC, white cap) and internal control (IC, dark blue cap).
2. Before use, ensure reagents have reached room temperature (15°C to 30°C); mix NC, IC and external RNA PC (by short vortexing) and spin down briefly.
3. Prepare samples for extraction procedure (thaw, if applicable, to room temperature).

Table 2 shows the validated extraction volumes.

Table 2: Validated Extraction Volumes

Type	Volume
Sample volume	200 µL
Elution volume	55 µL

4. Add samples, external RNA PC and NC into the disposables.
5. Program machine accordingly.
6. Select **DISPENSE LYSIS** for lysis buffer to dispense and to start the incubation step. During incubation period, prepare beads as described in the NucliSENS® easyMAG® manual.
7. Once incubation finishes, add 2 µL 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 IC prior to addition of lysis buffer.
- Never add the IC after extraction.
- Adding IC to each of the samples, the external RNA PC and to the NC is an important step to monitor nucleic acid extraction, as well as the inhibition of nucleic acid amplification.
- Do not extract positive control provided with the kit.

Real-Time PCR Preparation

Preparation of an experiment for the Applied Biosystems® 7500

To prepare the experiment:

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.
   - Positive Control: Thaw PC and store at room temperature (15°C to 30°C) for 20 to 30 minutes. Vortex PC thoroughly before use.

Table 3: Volume of Reagents Required for 1, 10, 32 and 96 Reactions

Number of Reactions	1	10	32	96
2x RT PCR Buffer	12.5 µL	125 µL	400 µL	1,200 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**	**1,440 µL**

2. Prepare a separate 1.5 mL tube per primer/probe mix and label accordingly. Pipette the required amount of 2x RT-PCR buffer based on the number of reactions (see Table 3).
3. Pipette the required amount of SCoV2 PP Mix in the corresponding tube containing 2x RT-PCR buffer (see Table 3).
4. **Master Mix Preparation:**

   NOTES:

   - In order 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 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 (see Table 3).
   b. Vortex master mix briefly and spin it down.
   c. Use master mix immediately and do not store after use.

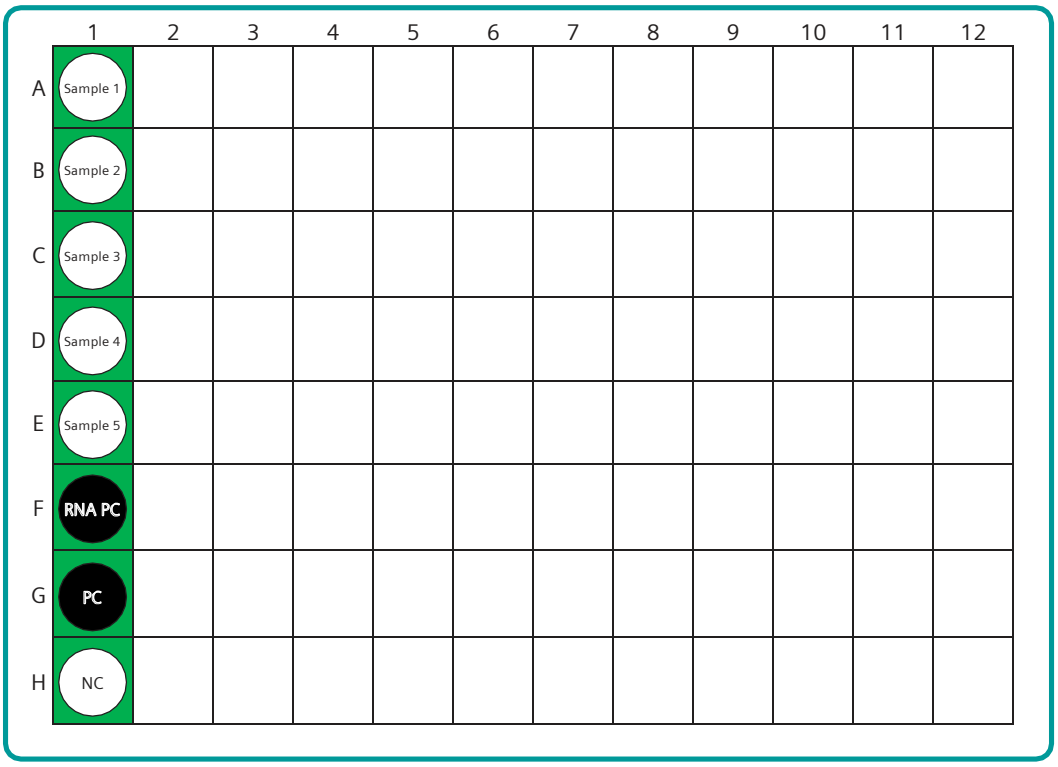
Prepare a 96-Well Plate for the Applied Biosystems® 7500

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

NOTE: The RNA PC is recommended, but is not provided. Refer to the *Materials Required but Not Provided* section on page 9 for more information.

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 = Ribonucleic acid Positive Control (F1) • PC = Positive Control (G1) • NC = 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 RNA PC into well F1.
4. Add 10 µL of the PC into well G1.
5. Add 10 µL of the extracted 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: Refer to manufacturers' operating instructions for use of the Applied Biosystems® 7500.

Program the Thermocycler

Table 4 lists the detection wavelengths for the dyes used in this kit.

Table 4: Detector Programming

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 chart below details the programming steps for the thermocycler.

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 more information on how to program the thermocycler, go to www.fast-trackdiagnostics.com.

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.

Quality Control

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

- Negative Control (NC)
- Positive Control (PC)
- Internal Control (IC)

In addition, FTD recommends the use of an external RNA positive control (RNA PC) such as the AccuPlex™ SARS-CoV-2 Reference Material Kit (Material number 0505-0126).

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

The 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 (PC), which is added to each RT-PCR run. It monitors the RT-PCR process and performance of the primers and probes.

The external RNA PC is processed as a sample (extraction and RT-PCR). It confirms the validity of the extraction, reverse transcription and amplification. To obtain a low positive control, the content of the positive vial should be diluted to obtain a final concentration of 700 copies per milliliter (cop/mL). Be aware that the AccuPlex™ SARS-CoV-2 Reference Material must be extracted prior to RT-PCR according to the instructions from the Extraction Using the NucliSENS® easyMAG® System section on page 12. The AccuPlex™ SARS-CoV-2 Reference Material Kit content must not substitute the positive control (PC) and negative control (NC) reagents provided with the SARS-CoV-2 kit.

Criteria for a Valid Run

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

1. NC shall not show any amplification traces other than the one for the IC. The IC must fall below a Ct of 33. Manually inspect the 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. PC must show a positive (*i.e.*, exponential) amplification trace for SARS-CoV-2. The 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 IC with a Ct less than 33. If IC is negative or shows a Ct value greater than 33 in a SARS-CoV-2 negative sample, the result is invalid. If 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.

Results

Interpretation of Results

Table 5 details the possible results with FTD SARS-CoV-2.

Table 5: Result Interpretation of Clinical Samples and Controls

Sample/Control	SARS-CoV-2	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.
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.
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.

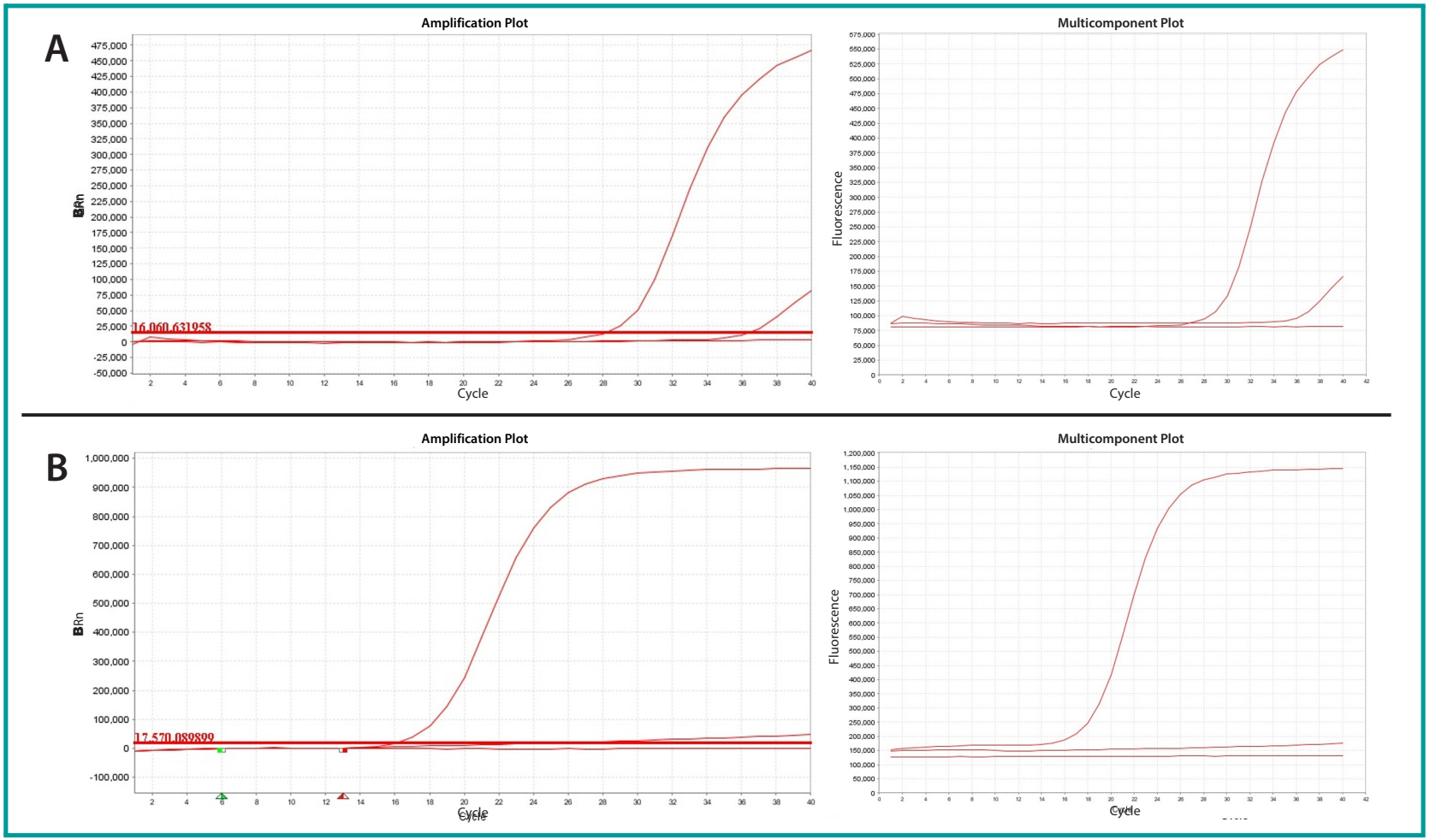
The IC must be positive in each extracted material that is not positive for SARS-CoV-2.

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 2 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 2: 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/or sudden changes. Contact the equipment manufacturer or Fast Track Diagnostics for advice on how to correctly set up the baseline.

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), 42 U.S.C. §263a, to perform high complexity tests.
- 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).
- 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 decision. 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.

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/emergency-situations-medical-devices/emergency-use-authorizations#covid19ivd

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 will 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 will 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 prior to initiating testing.

D. Authorized laboratories using the FTD SARS-CoV-2 test will have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.

E. Authorized laboratories will 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: +011-352 281098-217 / 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 will 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] For ease of reference, this letter will refer to, "Laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, 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 and the Applied Biosystems® 7500 (ThermoFisher Scientific) real-time thermocycler.

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, lot number 323999, 1.7e5 median tissue culture infectious dose per milliliter [TCID50/mL]) was serially diluted in simulated respiratory matrix (SRM).

A preliminary LoD was determined testing replicates of 2-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 TCID50/mL) was tested. Twenty-three separate spiked samples were prepared by spiking the LoD concentration of the virus culture into SRM. Each sample was extracted once (200 µL sample input, 55 µL elution volume) and tested in singlicate with 3 different lots of PP mix. The results of the LoD confirmation study are summarized in Table 6.

Table 6: Results of LoD Confirmation

PP Mix Lot	LoD (TCID50/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: TCID50/mL = Median tissue culture infectious dose per milliliter, PP = Primer and probe, Ct = Cycle threshold, SD = Standard deviation

The LoD at 0.0023 TCID50/mL of FTD SARS-CoV-2 in simulated respiratory matrix, as determined by the Probit analysis, was experimentally confirmed with an overall detection rate of 97.85%.

Inclusivity – Analytical Sensitivity

The inclusivity *in silico* analysis was performed on all sequences available on the National Center for Biotechnology Information (NCBI) GenBank and GISAID databases. 1048 sequences (114 from GenBank and 934 from GISAID – sequences downloaded on 19 March 2020) were aligned against FTD SARS-CoV-2 primers and probes.

After excluding nonrelevant sequences, 901 sequences were determined to be appropriate for further analysis. *In silico* analysis concluded that FTD SARS-CoV-2 will detect all analyzed SARS-CoV-2 sequences in the NCBI GenBank (n=96) and in GISAID (n=805) databases.

FTD SARS-CoV-2 N gene assay detected all sequences from the GenBank database with a maximum of 1 mismatch (3 sequences) and 100% of all sequences obtained from the GISAID database with a maximum of 1 mismatch (11 sequences). The SARS-CoV-2 ORF1ab assay detected all sequences from the GenBank database without any mismatches and 100% of all sequences obtained from the GISAID database with a maximum of 1 mismatch (8 sequences). None of these mismatches were located at a critical position that would cause detection issues and are not predicted to impact assay performance.

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. Results are shown in Table 7.

Table 7: In Silico Cross-Reactivity Results[a]

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*[b]	NCBI:txid445 exclude NCBI:txid446	83%	< 80%
Bacillus anthracosis (Anthrax)	NCBI:txid1392	< 80%	< 80%

Table 7: In Silico Cross-Reactivity Results[a] (Continued)

Pathogen	Taxonomy ID Included in BLAST Search	In Silico Analysis Result N gene Primer/Probe	In Silico Analysis Result ORF1ab Primer/Probe
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%
Streptoococcus 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%
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 pneumniae	NCBI:txid83558	< 80%	< 80%

Table 7: In Silico Cross-Reactivity Results[a] (Continued)

Pathogen	Taxonomy ID Included in BLAST Search	In Silico Analysis Result N gene Primer/Probe	In Silico Analysis Result ORF1ab Primer/Probe
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] 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.

[b] 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™ medium (COPAN). In addition, 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.

Table 8 lists all tested organisms and the respective tested concentration. For some organisms the concentration was unknown, they were previously tested 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 indicated.

Table 8: List of Pools or Individual Pathogens Tested In Vitro

Pool ID / Sample ID	Interfering Organism	Tested Concentration	Unit	Result
P1	Adenovirus 71	1.13E+06	TCID50/mL	No cross-reactivity
	Human Parainfluenza virus 2	1.13E+06	TCID50/mL	No cross-reactivity
	Enterovirus	2.00E+06	TCID50/mL	No cross-reactivity
	Rhinovirus	1.13E+07	TCID50/mL	No cross-reactivity
	Streptococcus pneumoniae	1.24E+08	cop/mL	No cross-reactivity

Table 8: List of Pools or Individual Pathogens Tested In Vitro (Continued)

Pool ID / Sample ID	Interfering Organism	Tested Concentration	Unit	Result
P2	Human Metapneumovirus (hMPV) A	6.82E+05	TCID50/mL	No cross-reactivity
	Parainfluenza virus 3	1.13E+06	TCID50/mL	No cross-reactivity
	Parainfluenza virus 4	2.00E+05	TCID50/mL	No cross-reactivity
	Human Metapneumovirus (hMPV) B	1.56E+05	TCID50/mL	No cross-reactivity
	Parainfluenza virus 1	2.00E+05	TCID50/mL	No cross-reactivity
P3	Human coronavirus OC43	1.41E+04	TCID50/mL	No cross-reactivity
	Human coronavirus NL63	8.39E+03	TCID50/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
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	TCID50/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 salivaris*	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

Table 8: List of Pools or Individual Pathogens Tested In Vitro (Continued)

Pool ID / Sample ID	Interfering Organism	Tested Concentration	Unit	Result
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 given by supplier or tested with FTD Respiratory pathogens 21 (CE-IVD).

Legend: TCID50 = 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 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 the FDA-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 criteria for a valid run (IC failure). Results are displayed in Table 9.

Table 9: Positive and Negative Percent 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.

Troubleshooting

Table 10 describes a non-exhaustive list of control errors that a user may observe with FTD SARS-CoV-2 and suggested corrective actions.

Table 10: Control Errors

Observation	Possible Cause	Corrective Action
Positive control or external RNA positive control does not amplify	Incorrect programming of the thermocycler temperature profile.	Compare temperature profile to IFU.
	Incorrect configuration of the PCR reaction.	• Confirm reagents were added in the correct sequence; repeat the PCR, if necessary. • Check calibration of 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 internal control	PCR conditions do not comply with 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 IC was inhibited or the extraction of the IC was inadequate.	
Amplification in the negative control	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 PC, pipette the positive control last. • Decontaminate the workspace and instruments after each use.

If the problem persists, note the error and contact technical support, go to www.fast-trackdiagnostics.com.

Technical Assistance

For customer support, please contact your local technical support provider or distributor or refer to the Technical Support section of the Fast Track Diagnostics website at www.fast-trackdiagnostics.com.

References

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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 May 2020.
4. World Health Organization (WHO). Laboratory testing for coronavirus disease 2019 (COVID-19) in suspected human cases: Interim guidance, 2 March 2020.

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

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