WHO Emergency Use Assessment Coronavirus disease (COVID-19) IVDs PUBLIC REPORT

Product: FTD-114 SARS-CoV-2
EUL Number: EUL-0502-193-00
Outcome: Accepted

The EUL process is intended to expedite the availability of in vitro diagnostics needed in public health emergency situations and to assist interested UN procurement agencies and Member States in determining the acceptability of using specific products in the context of a Public Health Emergency of International Concern (PHEIC), based on an essential set of available quality, safety and performance data. The EUL procedure includes the following:

- Quality Management Systems Review and Plan for Post-Market Surveillance: desk-top review of the manufacturer's Quality Management System documentation and specific manufacturing documents;
- Product Dossier Review: assessment of the documentary evidence of safety and performance.

FTD-114 SARS-CoV-2, with product codes 11416300 and 11416284 , CE-mark regulatory version, manufactured by Fast Track Diagnostics Luxembourg S.à r.l., 29, rue Henri Koch, L-4354 Esch-sur-Alzette, Luxembourg was listed on 21 May 2020.

Report amendments and/or product changes

This public report has since been amended. Amendments may have arisen because of changes to the EUL product for which WHO has been notified and has undertaken a review. Amendments to the report are summarized in the following table, and details of each amendment are provided below.

Version	Summary of amendment	Date of report amendment
2.0	1. Fulfilment of determining the limit of detection with the WHO international standard post listing commitment for EUL and amended IFU. Corrections on the product name. 2. Addition of second kit size for FTD SARS-CoV-2 of 96rx (FTD SARS-CoV-2 (FTD-114-96), with product code 11416284.	12-Mar-2021

Intended use:

According to the claim of intended use from Fast Track Diagnostics Luxembourg S.à r.l., *"FTD SARS-CoV-2 is a semi-automated qualitative in vitro nucleic acid amplification test for the detection of ORF1ab and N gene of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) nucleic acids in nasopharyngeal and oropharyngeal swabs of patients with signs and symptoms of SARS-CoV-2 infection in conjunction with clinical and epidemiological risk factors, who are suspected of Coronavirus Disease 2019 (COVID-19). The kit is intended for professional use by laboratory personnel trained in real-time PCR at a level 2 biosafety laboratory or equivalent per local regulations.*

The test is intended as an aid in the diagnosis of infections caused by the new human coronavirus SARS-CoV-2.

For in vitro diagnostic use."

Specimen type that was validated:

Nasopharyngeal and oropharyngeal swab specimens.

Test kit contents:

Component	32 tests (product code 11416300)	96 tests (product code 11416284)
SCoV2 PP Mix	1 x 48 µL	1 x 144 µL
SCoV2 PC	1 x 150 µL	1 x 150 µL
Negative Ctrl	1 x 2000 µL	1 x 2000 µL
Internal Ctrl	1 x 128 µL	1 x 350 µL
25x RT-PCR Enz.	1 x 32 µL	1 x 96 µL
2x RT-PCR Buff	1 x 400 µL	1 x 1200 µL

Items required but not provided:

Specimen collection swabs and viral transport medium or Amies transport medium.

Extraction/Purification platform (systems):

Equipment: NucliSENS easyMAG (bioMérieux) (Software v2.1 or higher).

Extraction reagents:

- NucliSENS easyMAG, Magnetic Silica Beads (Part Number (P/N) 280133
- NucliSENS easyMAG, Lysis Buffer (P/N 280134)
- NucliSENS easyMAG, Extraction Buffer 1 (P/N 280130)

- NucliSENS easyMAG, Extraction Buffer 2 (P/N 280131)
- NucliSENS easyMAG, Extraction Buffer 3 (P/N 280132)
- NucliSENS easyMAG, Disposables (P/N 280135)
- Nuclease-free water.

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

Amplification and detection platform (system):

Thermo Fisher Scientific Applied Biosystems 7500 Real-Time PCR System (Software v2.3 or higher)

Storage:

Store all reagents FTD-SARS-CoV-2 at -30°C to -10°C

Shelf-life upon manufacture:

24 months.

Warnings/limitations:

Refer to the instructions for use (IFU)

Product dossier assessment

Fast Track Diagnostics Luxembourg S.à r.l. submitted a product dossier for the FTD-114 SARS-CoV-2 for detecting severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) as per the "*Instructions for Submission Requirements: In vitro diagnostics (IVDs) Detecting SARS-CoV-2 Nucleic Acid (PQDx_0347 version 4)"*. The information (data and documentation) submitted in the product dossier was reviewed by WHO staff and external technical experts (assessors) appointed by WHO.

Post listing Commitments for EUL:

As commitments to listing, the manufacturer is required to;

1. Review the limit of detection with the WHO international standard when available. The response that was submitted was acceptable and the issue was closed.
2. Submit Flex and robustness studies report at the end of the study by 30 October 2020. The submitted response was acceptable and the issue was closed.
3. Submit Stability studies interim report by 15 January 2021 and final report by 15 January 2023. The interim report was submitted and is under review.
4. Submit amended IFU by 18 December 2020. The IFU was submitted and issue was closed.

Risk benefit assessment conclusion: acceptable.

Quality Management Systems Review

To establish the eligibility for WHO procurement, Fast Track Diagnostics Luxembourg S.à r.l. was asked to provide up-to-date information about the status of their quality management system.

Based on the review of the submitted quality management system documentation by WHO staff, it was established that sufficient information was provided by Fast Track Diagnostics Luxembourg S.à r.l. to fulfil the requirements described in the "*Instructions for Submission Requirements: In vitro diagnostics (IVDs) Detecting SARS-CoV-2 Nucleic Acid (PQDx_347)*".

Quality management documentation assessment conclusion: acceptable.

Plan for Post-Market Surveillance

Post-market surveillance, including monitoring all customer feedback, detecting and acting on adverse events, product problems, non-conforming goods and processes is a critical component of minimizing potential harm of an IVD listed for emergency use.

The following post-EUL activities are required to maintain the EUL listing status:

1. Notification to WHO of any planned changes to a EUL product, in accordance with "*WHO procedure for changes to a WHO prequalified in vitro diagnostic" (document number PQDx_121*); and
2. Post-market surveillance activities, in accordance with " *Guidance for post-market surveillance and market surveillance of medical devices, including in vitro diagnostics"* (ISBN 978-92-4-001531-9)[1]

Fast Track Diagnostics Luxembourg S.à r.l. is also required to submit an annual report that details sales data and all categories of complaints in a summarized form. There are certain categories of complaints and changes to the product that must be notified immediately to WHO, as per the above-mentioned documents.

The manufacturer has committed to ensure that post-emergency use listing safety, quality and performance monitoring activities are in place which are in accordance with WHO guidance "*Guidance for post-market surveillance and market surveillance of medical devices, including in vitro diagnostics"*

Scope and duration of procurement eligibility

FTD-114 SARS-CoV-2, product codes 11416300 and 11416284 manufactured by Fast Track Diagnostics Luxembourg S.à r.l. is considered to be eligible for WHO procurement for 12 months from the day of listing. The assay may be used for the detection of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) RNA. This listing does not infer that the product meets WHO prequalification requirements and does not mean that the product is listed as WHO prequalified.

As part of the on-going requirements for listing as eligible for WHO procurement, Fast Track Diagnostics Luxembourg S.à r.l. must engage in post-market surveillance activities to ensure that the product continues to meet safety, quality and performance requirements. Fast Track Diagnostics Luxembourg S.à r.l. is required to notify WHO of any complaints, including adverse events related to the use of the product within 7 days.

WHO reserves the right to rescind eligibility for WHO procurement, if additional information on the safety, quality, performance during post-market surveillance activities, and if new data becomes available to WHO that changes the risk benefit balance.

[1] Available on the web page https://www.who.int/publications/i/item/guidance-for-post-market-surveillanceand-market-surveillance-of-medical-devices-including-in-vitro-diagnostics

Labelling

1.0 Labels

2.0 Instructions for Use (IFU)

1.0 Product labels

1.1 Product code 11416300 labels

Preparation of FTD SARS-CoV-2

Cat N°: FTD-114 -32

PCR mix preparation (1x)

PPmix (µL)	1.5
Buffer (µL)	12.5
Enzyme (µL)	1

TO PIPETTE (µL)

	32rxn	64rxn
PPmix	53	/
PC	200	/
NC	2200	/
IC	140	/
ENZ	36	/
BUF	440	/

IN IFU or ON BOX LABELS (µL)

	32rxn	64rxn
PPmi	48	/
PC	150	/
NC	2000	/
IC	128	/
ENZ	32	/
BUF	400	/

Components

SCoV2 PPmix (1x Duplex SARS-CoV-2 and EAV)
SCoV2 PC
Negative Control (Nuclease-free water)
Internal Control (IC Pool of MCMV, BMV, EAV and S.equi)
Enzyme (ENZ1)
Buffer (BUF1)

Box setting

Tube color	Label - Face A	Label - Face B
	FAST TRACK DIAGNOSTICS STORE:-30°C to -10°C	RGT: SCoV2 PP Mix LOT: EXP:
	FAST TRACK DIAGNOSTICS STORE:-30°C to -10°C	RGT: SCoV2 PC LOT: EXP:
	FAST TRACK DIAGNOSTICS STORE:-30°C to -10°C	RGT: Negative Ctrl LOT: EXP:
	FAST TRACK DIAGNOSTICS STORE:-30°C to -10°C	RGT: Internal Ctrl LOT: EXP:
	FAST TRACK DIAGNOSICS STORE:-30°C to -10°C	RGT: 25x RT-PCR Enz. LOT: EXP:
	FAST TRACK DIAGNOSTICS STORE:-30°C to -10°C	RGT: 2x RT-PCR Buff. LOT: EXP:

Tube color	Label - Face A	Label - Face B

FTD-114 -32 16 holes M paper box

*: make sure to have on the label of IC the following symbol

MADE IN LUXEMBOURG

Σ 32

REF 11416301 (FTD-114-32)

xxxxxxxx Rev. A

LOT SCoV2YY-32-00

YYYY-MM-DD

YYYY-MM-DD

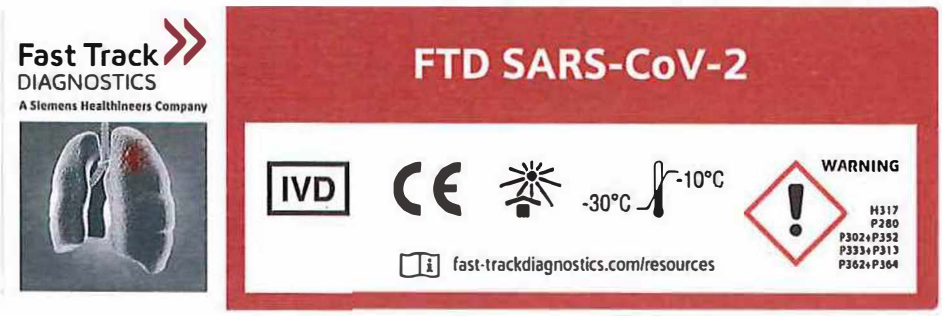
Fast Track Diagnostics Luxembourg S.à.r.l.; 29, rue Henri Koch; L-4354 Esch-sur-Alzette; Luxemburg

SCoV2 PP Mix: 114SC-YYMMDD00 (1x 48µL)
SCoV2 PC: 114SC-YYMMDD00 (1x 150µL)
Negative Ctrl: NC-YYMMDD00 (1x 2000µL)
Internal Ctrl: IC-YYMMDD00 (1x 128µL)
25x RT-PCR Enz.: E32-YYMMDD00 (1x 32µL)
2x RT-PCR Buff.: B400-YYMMDD00 (1x 400µL)

1.2 Product code 11416284 labels

Fast Track DIAGNOSTICS
A Siemens Healthineers Company

Top Label:



Component-side Labels:

Fast Track
DIAGNOSTICS
A Siemens Healthineers Company

Tube Labels :

RGT:SCoV2 PP Mix 96
LOT:114SC96-YYMMDD00
EXP:MM-YYYY

FAST TRACK
DIAGNOSTICS
STORAGE:-30Cto-10C
144ul

RGT:SCoV2 PP Mix 96
LOT:114SC96-YYMMDD00
EXP:MM-YYYY
144µl

Storage: -30°C to -10°C

RGT:SCoV2 PC 96
LOT:114PC96-YYMMDD00
EXP:MM-YYYY

FAST TRACK
DIAGNOSTICS
STORAGE:-30Cto-10C
150ul

RGT:SCoV2 PC 96
LOT:114PC96-YYMMDD00
EXP:MM-YYYY
150µl

Storage: -30°C to -10°C

RGT:Negative Ctrl 96
LOT:NC96-YYMMDD00
EXP:MM-YYYY
2000µl

Storage: -30°C to -10°C

RGT:Internal Ctrl 96
LOT:IC96-YYMMDD00
EXP:MM-YYYY
350µl

Storage: -30°C to -10°C

RGT:25x RT-PCR Enz. 96
LOT:E96-96-YYMMDD00
EXP:MM-YYYY

FAST TRACK
DIAGNOSTICS
STORAGE:-30Cto-10C
96ul

RGT:25xRT-PCR Enz.96
LOT:E96-96-YYMMDD00
EXP:MM-YYYY
96µl

Storage: -30°C to -10°C

RGT:2x RT-PCR Buff. 96
LOT:B1200-96-YYMMDD00
EXP:MM-YYYY

FAST TRACK
DIAGNOSTICS
STORAGE:-30Cto-10C
1800ul

RGT:2xRT-PCR Buff.96
LOT:B1200-96-YYMMDD00
EXP:MM-YYYY
1200µl

Storage: -30°C to -10°C

2.0 Instructions for use[2]

[2] English version of the IFU was the one that was assessed by WHO. It is the responsibility of the manufacturer to ensure correct translation into other languages.

2.1 Product code 11416300 IFU

Fast Track DIAGNOSTICS

A Siemens Healthineers Company

IVD CE

FTD™ SARS-CoV-2

Current Revision and Date	11416297_en Rev. B, 2020-12		
Product Name	FTD SARS-CoV-2 (FTD-114-32)	REF 11416300	Σ 32
Specimen Types	Nasopharyngeal and Oropharyngeal swabs		
Processed Sample Volume	200 µL required		
Validated System	NucliSENS® easyMAG® (bioMérieux) / Applied Biosystems® 7500 Real-Time PCR System (ThermoFisher Scientific)		

Table of Contents

Intended Use

FTD SARS-CoV-2 is a semi-automated qualitative *in vitro* nucleic acid amplification test for the detection of ORF1ab and N gene of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) nucleic acids in nasopharyngeal and oropharyngeal swabs of patients with signs and symptoms of SARS-CoV-2 infection in conjunction with clinical and epidemiological risk factors, who are suspected of Coronavirus Disease 2019 (COVID-19). The kit is intended for professional use by laboratory personnel trained in real-time PCR at a level 2 biosafety laboratory or equivalent per local regulations.

The test is intended as an aid in the diagnosis of infections caused by the new human coronavirus SARS-CoV-2.

For *in vitro* diagnostic use.

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 severity of clinical signs requires that approximately 20% of patients remain in hospital and 5% require admission to intensive care. The most serious forms are observed mainly in people who are vulnerable because of their age (over 70) or associated diseases. However, the infection can also be asymptomatic or paucisymptomatic (*i.e.*, causing little or no clinical manifestations) in 30% to 60% of infected subjects. The duration of incubation is on average 5 days, with extremes of 2 to 12 days.[1]

The serial interval (*i.e.*, time between one person developing symptoms of a disease and a second person becoming infected and developing symptoms) is approximately 4 days. Thus, the serial interval of COVID-19 is shorter than its median incubation period. This suggests that a substantial proportion of secondary transmission may occur prior to illness onset.[2,3] This “silent transmission” makes this pandemic hard to contain and more likely to spread very quickly.

FTD SARS-CoV-2 is an aid in the identification of COVID-19 disease by the detection of SARS-CoV-2 ribonucleic acid (RNA) extracted from nasopharyngeal and oropharyngeal swabs of patients suspected of COVID-19 infection.

Pathogens

New human coronavirus SARS-CoV-2

Coronaviruses belong to a large family of encapsulated viruses with a positive-sense RNA genome, which can cause illness in animals and humans. The genera Alphacoronavirus (AlphaCoV) and Betacoronavirus (BetaCoV) contain seven known human coronaviruses that can cause mild respiratory tract infections, but also more severe infections like severe acute respiratory syndrome (SARS), Middle East respiratory syndrome (MERS) and COVID-19.

SARS-CoV-2, the causative agent of COVID-19, belongs to the genus Betacoronavirus and was firstly identified in humans in January 2020. The transmission route is still under investigation. Although bats are likely reservoir hosts for SARS-CoV-2, there is still ongoing research investigating if pangolins (*Manis javanica*) could be considered as possible intermediate hosts for this novel human virus.[4] Human-to-human transmission occurs via droplets or direct contact.[5]

FTD SARS-CoV-2 detects two different genomic regions specific for SARS-CoV-2 (see *Performance Characteristics – Inclusivity* section on page 24).

Principles of the Procedure

Method

FTD SARS-CoV-2 is a real-time transcription-polymerase chain reaction (RT-PCR) test for the detection of pathogens in human samples.

Nucleic acids should be first extracted from the specimen types listed in the *Intended Use* section, with 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. 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 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 includes a primer and probe set to detect a sequence in the genome of equine arteritis virus (EAV) that serves as an internal control (FTD Internal Control, FTD IC). The FTD 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 FTD Negative Control (FTD NC) is also 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 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.

Reagents

Warnings and Precautions

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.

WARNING

Internal Ctrl:

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 nasopharyngeal and oropharyngeal swabs 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 due to the sensitivity of several pathogens to external influences. 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[6]).

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.

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

Nasopharyngeal Swabs

For nasopharyngeal swabs, insert the swab through the nares parallel to the palate until resistance is encountered or the distance is equivalent to that from the ear to the nostril of the patient indicating contact with the nasopharynx. Once inserted, gently turn the swab to obtain infected cells and place into the appropriate container (as recommended by CDC[6]).

Oropharyngeal Swabs

For oropharyngeal swabs, insert the swab into the posterior pharynx and tonsillar areas. Rub the swab gently over both tonsillar pillars and posterior oropharynx and avoid touching the tongue, teeth, and gums. Place then into the appropriate container (as recommended by CDC[6]).

Storing and Transporting the Specimen

Nasopharyngeal and oropharyngeal swabs should be transported in a viral transport medium or Amies transport medium. Specimens which can be delivered promptly to the laboratory can be refrigerated and shipped at temperatures of 2–8°C (according to manufacturer instructions). 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[7]).

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	Synthetic oligonucleotides, buffer	PP mix for SARS-CoV-2 (N gene), SARS-CoV-2 (ORF1ab) and IC 32 reactions: 1 x 48 µL	-30°C to -10°C
SCoV2 PC	Double-stranded synthetic DNA molecules, buffer, stabilizing agents	FTD Positive Control (FTD PC) 32 reactions: 1 x 150 µL	
Negative Ctrl	Nuclease-free water	FTD Negative Control (FTD NC) 32 reactions: 1 x 2000 µL	
Internal Ctrl	Double-stranded circular DNA molecules, buffer, <5.0% guanidinium hydrochloride, <0.1% maleic acid	FTD Internal Control (FTD IC) 32 reactions: 1 x 128 µL	
25x RT-PCR Enz.	Enzymes, buffer, glycerol, Ribonuclease (RNase) inhibitors, stabilizing agents	25x RT-PCR Enzyme mix 32 reactions: 1 x 32 µL	
2x RT-PCR Buff.	Tris-hydrochloride (Tris-HCl) buffer, Deoxyadenosine triphosphate (dATP), Deoxycytidine triphosphate (dCTP), Deoxyguanosine triphosphate (dGTP), Deoxythymidine triphosphate (dTTP), PCR adjuvants	2x RT-PCR Buffer 32 reactions: 1 x 400 µL	

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

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 32 reactions. Each kit includes IC, NC and PC components.

REF	Contents	Number of Reactions
11416300 (FTD-114-32)	FTD SARS-CoV-2	32

Materials Required but Not Provided

The kit was validated with the bioMérieux extraction platform, NucliSENS® easyMAG® (Software v2.1 or higher) and the ThermoFisher Scientific amplification and detection platform, Applied Biosystems® 7500 Real-Time PCR System (Software v2.3 or higher).

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).

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 (1.5 mL or 2.0 mL tubes depending on the mastermix volume)

Assay Procedure

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 and FTD IC (by quick vortexing) and spin down briefly.
3. Prepare samples for extraction procedure (if applicable, thaw 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 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 incubation period, prepare beads as described in the NucliSENS® easyMAG® manual.
7. Once incubation finishes, 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 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 Biosystems® 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.

Table 3: Volume of Reagents Required for 1, 9 and 32 Reactions

Number of Reactions	1	9	32
2x RT PCR Buffer	12.5 µL	112.5 µL	400 µL
Primer/Probe Mix	1.5 µL	13.5 µL	48 µL
25x RT PCR Enzyme	1 µL	9 µL	32 µL
Total	**15 µL**	**135 µL**	**480 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 (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:

   - 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.

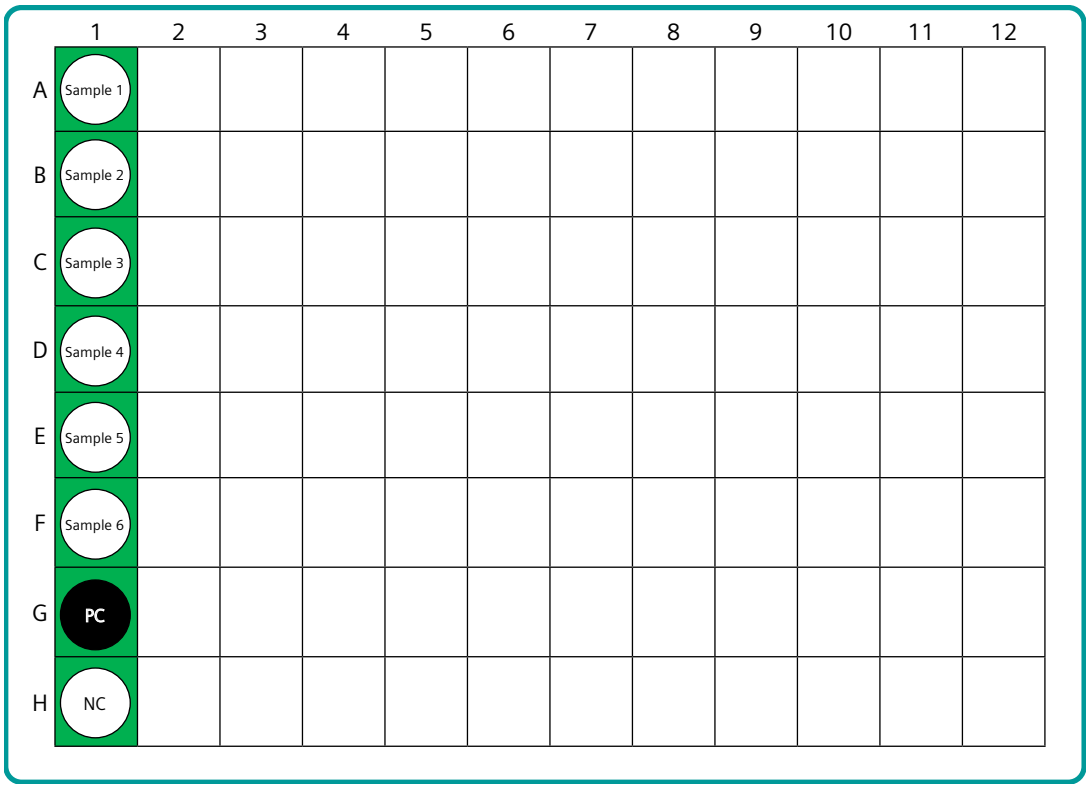
   a. Pipette the required amount of 25x RT-PCR Enzyme in each of the tubes containing SCoV2 PP Mix and 2x RT-PCR Buffer (see Table 3).
   b. Vortex master mix briefly and spin it down.
   c. Use master mix immediately and do not store after use.

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

NOTE: Each master mix on the plate must have a corresponding FTD PC and FTD NC to perform analysis.

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) • 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 F1.
3. Add 10 µL of the FTD PC into well G1.
4. Add 10 µL of the extracted FTD NC into well H1.
5. Seal plate with appropriate adhesive film.
6. Gently vortex plate, then centrifuge briefly.
7. 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.

Criteria for a Valid Run

The run is considered valid and patient results are reported 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 and FTD NC (or each extracted material) must show a positive amplification trace for the FTD IC. The FTD IC must fall below a Ct of 33.

Results

Interpretation of Results

The results of interpretation of clinical samples and controls are shown in Table 5:

Table 5: FTD SARS-CoV-2 Results

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	Sample is invalid. Retest the sample.
	Negative	Not detected	Invalid	Sample is invalid. Retest the sample.
	Positive	Ct < 33	Valid	SARS-CoV-2 detected.
	Positive	Ct > 33	Invalid	Sample is invalid. Retest the sample.
	Positive	Not detected	Invalid	Sample is invalid. Retest the sample.
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 one of the pathogens targeted by the kit. An absence of an exponential trace indicates an absence or undetectable load of nucleic acid.

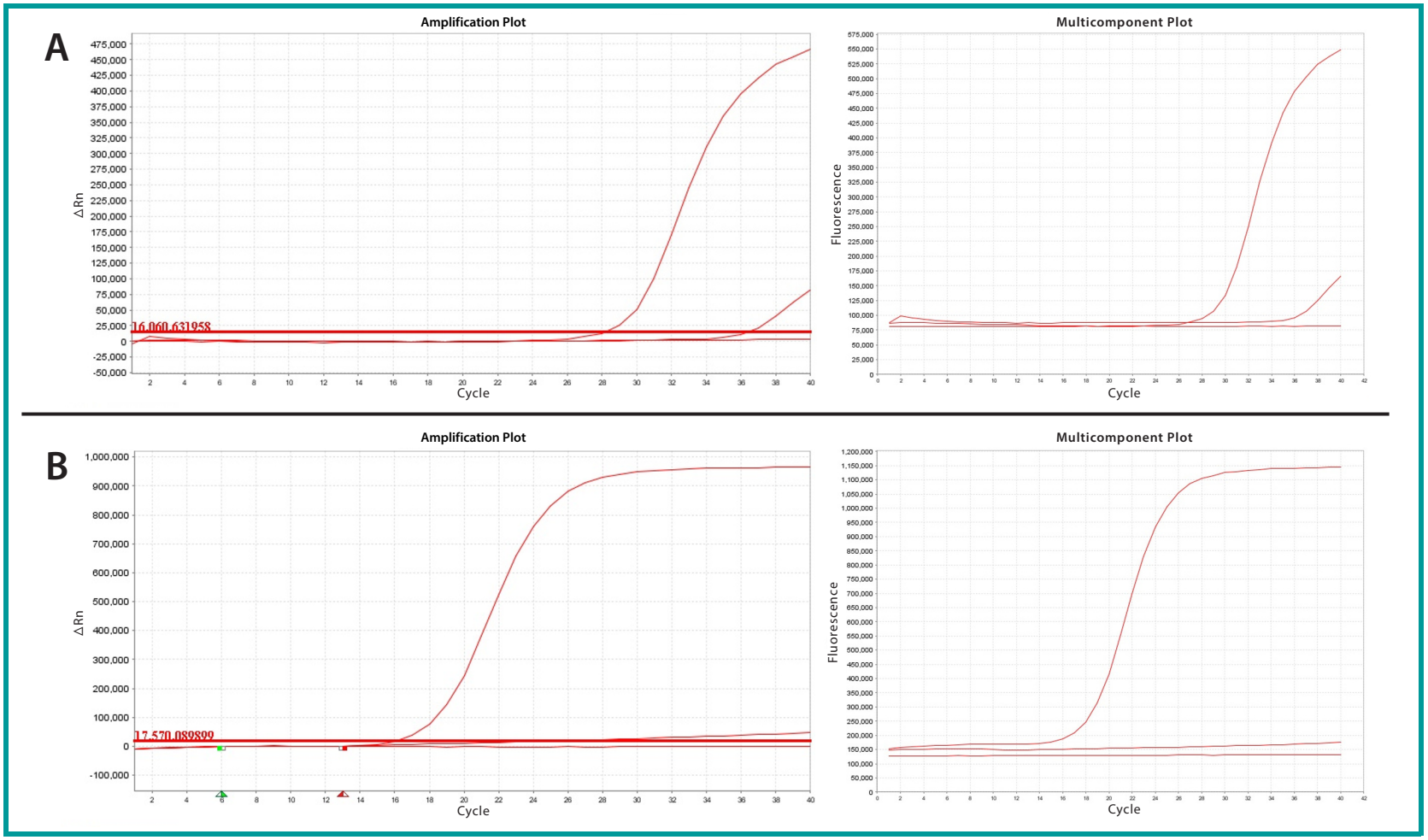
The FTD IC must be positive in all extracted materials (samples and FTD NC).

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 set up the baseline correctly.

Limitations

For *in vitro* diagnostic use.

- The sample storage and shipment instructions provided are recommendations. Verification and validation data for specimen collection and handling (transport and storage) are not available.
- 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.
- Remel M4RT® transport medium is not recommended for use with FTD SARS-CoV-2.
- Detection of pathogens may be affected by the presence of inhibitors other than those specified in the *Performance Characteristics* section.

Performance Characteristics

Performance characteristics show the analytical and clinical performance data of FTD SARS-CoV-2. The analytical performance (analytical sensitivity, analytical specificity, inclusivity, and precision) was evaluated using the NucliSENS® easyMAG® (bioMérieux) extraction system and the Applied Biosystems® 7500 (Thermo Fisher Scientific) real-time thermocycler.

Analytical Sensitivity

The analytical sensitivity is the ability of the assay to consistently detect a given target sequence in the tested biological specimens. The lowest concentration detectable in greater than or equal to 95% of tested specimens is then defined as the "Limit of Detection" (LoD).[8]

The LoD of FTD SARS-CoV-2 was determined empirically by testing serial dilutions of quantified extracted RNA of SARS-CoV-2 (BetaCoV/Germany/BavPat1/2020 p.1).

Using Probit (PROBability unITS) Regression Analysis, the LoD for SARS-CoV-2 was evaluated to be 1155 copies per milliliter (see Table 6).

Table 6: Analytical Sensitivity of FTD SARS-CoV-2

Pathogen	Units	LoD from Probit Analysis	95% Confidence Interval
SARS-CoV-2	cop/mL	1155	854–1508

Legend: cop/mL = copies per milliliter, LoD = Limit of detection, Probit = PROBability unITS Regression Analysis

The LoD previously calculated with Probit analysis was then confirmed using another primer and probe mix (PP mix) lot. This LoD confirmation test was performed by testing 24 replicates at a concentration equivalent to the upper confidence limit previously established. This LoD confirmatory study was performed by two operators, using two different instruments and one PP mix lot, across 2 days.

It can be concluded that the 95% LoD of FTD SARS-CoV-2 is 1155 cop/mL, which corresponds to 11.5 cop/reaction.

Analytical Specificity

The analytical specificity of a PCR multiplex assay is the ability of a measurement to measure solely the measurand.[8] The analytical specificity is referred to as:

- Cross-reactivity: The ability of the test to specifically detect the intended pathogens (including relevant subtypes when specified), but no other organism in biological samples.
- Specificity: The assurance that the test will not report false positive results when testing negative samples.

FTD SARS-CoV-2 analytical specificity was validated using an *in silico* analysis as well as *in vitro* testing as detailed below.

Cross-Reactivity

In Silico Analysis

The specificity of FTD SARS-CoV-2 was determined using *in silico* analysis, applying the Basic Local Alignment Search Tool (BLAST). Regions of similarity were searched for all primers and probes of this assay analyzing the complete National Center for Biotechnology Information (NCBI) Nucleotide collection database (non-redundant protein [nr]/non-redundant nucleotide [nt]), excluding sequences belonging to the targeted organism. A sequence similarity of at least 90% with the respective assay (primer/probe) sequences was used as criteria for mapping to check for potential matches producing amplicons. A maximum of four mismatches between oligo and target sequence was allowed when mapping the primers and probes.

The results of the *in silico* analysis are presented in Table 7. Data showed a potential cross-reactivity with one sequence from Bat coronavirus isolate RaTG13 (Accession number MN996532.1) and Pangolin coronavirus isolate MP789 (Accession number MT084071.1). Both viruses belong to the SARS-related coronaviruses. Pairwise sequence alignment between Bat CoV RaTG13 and SARS-CoV-2 (Accession number NC_045512) revealed a sequence identity of 96.1%. There is evidence that RaTG13 is the closest relative of SARS-CoV-2 and they form a distinct lineage from other SARS-related coronaviruses.[9] Pairwise sequence alignment between Pangolin CoV MP789 and SARS-CoV-2 (Accession number NC_045512) revealed a sequence identity of 78.7%. The group that identified the pangolin isolate showed that the pangolin coronavirus (pangolin-CoV-2020) is genetically associated with both SARS-CoV-2 and a group of bat coronaviruses.

However, phylogenetic analyses did not support that the SARS-CoV-2 arose directly from the pangolin-CoV-2020.[10] No other cross-reactivity was found with FTD SARS-CoV-2.

Table 7: Cross-Reactivity of FTD SARS-CoV-2

Pathogen	Potential Cross-Reactivity
SARS-CoV-2	Bat coronavirus isolate RaTG13 (MN996532.1)
	Pangolin coronavirus isolate MP789 (MT084071.1)

In Vitro Analysis

FTD SARS-CoV-2 was tested *in vitro* for potential cross-reaction with common human flora organisms and the pathogenic organisms causing respiratory infections. Cross-reactivity testing was performed using several pools of contrived samples, either pooled or tested separately, and extracted RNA/DNA. Each pool was spiked with a maximum of five pathogens or samples. The pools and samples were extracted in triplicate using the NucliSENS® easyMAG® and evaluated on the Applied Biosystems® 7500.

The list of the pathogens tested is displayed in Table 8 along with the culture information, tested concentration and the results of the analysis. No unspecific signal was detected.

Table 8: Cross-Reactivity Panel Tested with FTD SARS-CoV-2

Target	Provider	Catalog Number	Tested Concentration	Units	Result
Human Adenovirus 1 (Adenoid 71)	American Type Culture Collection (ATCC®)	VR-1	1.13E+06	$TCID_{50}$/mL	No cross-reactivity
Human parainfluenza virus 2, Greer		VR-92	1.13E+06	$TCID_{50}$/mL	No cross-reactivity
Human enterovirus, human echovirus 1; Strain: Farouk		VR-1808	2.00E+06	$TCID_{50}$/mL	No cross-reactivity
Human rhinovirus 1A; Strain: 2060		VR-1559	1.13E+07	$TCID_{50}$/mL	No cross-reactivity
Streptococcus pneumoniae	DSMZ	DSM 20566	1.24E+08	cop/mL	No cross-reactivity
Human metapneumovirus (hMPV) A, Type: A1; Strain: IA10-2003	ZeptoMetrix	0810156CFHI	6.82E+05	$TCID_{50}$/mL	No cross-reactivity
Human parainfluenza virus 3, C 243	ATCC®	VR-93	1.13E+06	$TCID_{50}$/mL	No cross-reactivity
Human parainfluenza virus 4, M-25		VR-1378	2.00E+05	$TCID_{50}$/mL	No cross-reactivity
Human metapneumovirus (hMPV) B, Type: B1; Strain: Peru2-2002	ZeptoMetrix	0810156CFHI	1.56E+05	$TCID_{50}$/mL	No cross-reactivity
Human parainfluenza virus 1, C35	ATCC®	VR-94	2.00E+05	$TCID_{50}$/mL	No cross-reactivity
Human coronavirus OC43		VR-1558	1.41E+04	$TCID_{50}$/mL	No cross-reactivity
Human coronavirus NL63	ZeptoMetrix	0810228CFHI	8.39E+03	$TCID_{50}$/mL	No cross-reactivity
Influenza B, Florida/04/06		0810255CFHI	Unknown (Ct: 20.3)[a]	—	No cross-reactivity
Chlamydophila pneumoniae, Strain: TW-183	ATCC®	VR-1435	2.01E+04	$TCID_{50}$/mL	No cross-reactivity
Haemophilus influenzae		9006	5.71E+02	CFU/mL	No cross-reactivity
Legionella pneumophila		33152	1.00E+03	CFU/mL	No cross-reactivity
Bordetella pertussis, H898		BAA-2702	1.43E+03	CFU/mL	No cross-reactivity
Mycoplasma pneumoniae	DSMZ	DSM 23978	Unknown (Ct: 21)[a]	—	No cross-reactivity

Table 8: Cross-Reactivity Panel Tested with FTD SARS-CoV-2 (Continued)

Target	Provider	Catalog Number	Tested Concentration	Units	Result
Respiratory syncytial virus (RSV A), A2	ATCC®	VR-1540	3.14E+06	PFU/mL	No cross-reactivity
Respiratory syncytial virus (RSV B), Strain: CH93(18)-18	ZeptoMetrix	0810040CFHI	3.26E+05	$TCID_{50}$/mL (prior to heat-inactivation)	No cross-reactivity
SARS-Coronavirus, HKU39849[b]	European Virus Archive - GLOBAL (EVAg)	011N-03868	Unknown (Ct: 18)[c]	—	No cross-reactivity
MERS-Coronavirus[b]		011N-03868	Unknown (Ct: 29)[c]	—	No cross-reactivity
Mycobacterium tuberculosis[d]	Vircell	MBC034	1.00E+06	cop/mL	No cross-reactivity
Human coronavirus 229E	EVAg	011N-03868	Unknown (Ct: 30.3)[a]	—	No cross-reactivity
Human coronavirus HKU1	Discovery Life Sciences (DLS)	DLS0085250	Unknown (Ct: 14.9)[a]	—	No cross-reactivity
Influenza A[b]	Vircell	MBC028	1.00E+06	cop/mL	No cross-reactivity
Streptococcus salivarius	DSMZ	DSM 20560	5.91E+07	cop/mL	No cross-reactivity
Staphylococcus epidermidis		DSM 20044	8.05E+06	cop/mL	No cross-reactivity
Legionella sainthelensi		DSM 25322	2.29E+08	cop/mL	No cross-reactivity
Legionella spiritensis		DSM 19324	4.08E+08	cop/mL	No cross-reactivity
Streptococcus pyogenes		DSM 20565	6.82E+08	cop/mL	No cross-reactivity
Pneumocystis carinii (Pneumocystis jirovecii)	ATCC®	PRA-159	1.64E+08	cop/mL	No cross-reactivity
Candida albicans		ATCC-14053	2.99E+06	CFU/mL	No cross-reactivity
Pseudomonas aeruginosa		ATCC-10145	3.10E+07	CFU/mL	No cross-reactivity

[a] Ct value determined with FTD Respiratory pathogens 21 (CE-IVD).
[b] Extracted RNA.
[c] Ct value given by supplier.
[d] Extracted DNA.

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

Negative Material

Negative material (extracted negative controls, extracted negative clinical samples or non-template controls) has been tested *in vitro* in order to evaluate the occurrence of potential non-specific amplification when using FTD SARS-CoV-2. The derived analytical specificity is displayed in Table 9. Overall, an analytical specificity of 100% was reached.

Table 9: Analytical Specificity of FTD SARS-CoV-2

Pathogen	Tested Sample	Positive Results	Total Reactions	Analytical Specificity %	Confidence Interval %
SARS-CoV-2	Negative clinical sample	0	50	100	92.89–100.00
	Negative control	0	35	100	90.00–100.00
	Non-template control	0	34	100	89.72–100.00
	Total	**0**	**119**	**100**	**96.95–100.00**

Inclusivity

Inclusivity (or analytical reactivity) 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 then 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. A maximum of 4 mismatches between oligo (primer and probe) and the target sequence were allowed when mapping the primers and probes, as generally an imperfect match still produces pairing and amplification.

- The sequence alignment showed that the SARS-CoV-2 N gene assay detected 100% of the sequences from the GenBank database. 99.21% were detected with no mismatch and 0.79% of the sequences were detected with 1 mismatch.
- The sequence alignment showed that the SARS-CoV-2 N gene assay detected 100% of the analyzed sequences from the GISAID database. 99.21% were detected with no mismatch and 0.79% of the sequences were detected with 1 mismatch.
- The sequence alignment showed that the SARS-CoV-2 ORF1ab assay detected 100% of the analyzed sequences from the GenBank database. 99.49% of the sequences were detected with no mismatch and 0.51% of the sequences were detected with 1 mismatch.
- The sequence alignment showed that the SARS-CoV-2 ORF1ab assay detected 100% of the analyzed sequences from the GISAID database. 99.49% of the sequences were detected with no mismatch and 0.51% of the sequences were detected with 1 mismatch.

Due to the dual-target approach, only 9 of all the sequences that present 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. The inclusivity analysis results are summarized in Table 10.

Table 10: Inclusivity of FTD SARS-CoV-2

Assay	Database	Complete Genomes Tested	Complete Genomes Detected	Detection Rate (%)
SARS-CoV-2 (N gene)	GISAID	78945	78945	100
	GenBank	7354	7354	100
SARS-CoV-2 (ORF1ab)	GISAID	79273	79273	100
	GenBank	7711	7711	100

Precision

Precision refers to how well a given measurement can be reproduced when a test is applied repeatedly to multiple aliquots of a single homogeneous sample. FTD SARS-CoV-2 precision was assessed by repeatability and reproducibility studies with test material at a concentration at upper LoD (see Table 11).

Repeatability evaluates measurements carried out under the same conditions (intra-assay variation), whereas reproducibility evaluates results of measurements under changed conditions (*i.e.*, time, operator and cycler). The precision of each study was expressed based on statistical measurements of imprecision (standard deviation and coefficient of variation).

Commercially available quantified RNA of SARS-CoV-2 was tested at a concentration at upper LoD with FTD SARS-CoV-2 on the Applied Biosystems® 7500. Results were collected over multiple runs and days. Runs were executed by different operators on different cyclers using one PP mix lot.

Table 11 presents the results of the precision study. The data demonstrated a repeatability imprecision of 2.10% and a reproducibility imprecision of 2.49%.

Table 11: Precision Study for FTD SARS-CoV-2

Target and Sample	N	Repeatability SD	Reproducibility SD	Reaptability CV (%)	Reproducibility CV (%)
SARS-CoV-2	23	0.80 (0.61–1.15)	0.95 (0.64–1.79)	2.10 (1.61–3.03)	2.49 (1.69–4.71)

Legend: N = Total sample size, SD = Standard deviation, CV = Coefficient of variation

Interfering Substances

An interference study was conducted to evaluate the susceptibility of FTD SARS-CoV-2 to provide erroneous results in presence of potential interfering substances in the clinical sample. Artificial matrix was spiked with an interfering substance, the solvent or left untreated. Each sample was then extracted in triplicates using NucliSENS® easyMAG®. The eluate was spiked with SARS-CoV-2 RNA (BetaCoV/Germany/BavPat1/2020 p.1) at 3x LoD concentration. RT-PCR with one lot of FTD SARS-CoV-2 was performed on the Applied Biosystems® 7500. The list of the tested substances and their interfering power are documented in Table 12. The data showed that none of the tested substances interfered with the PCR results.

Table 12: Potential Interfering Substance Evaluated

Substance	Provider	Tested Concentration	Results
Whole blood	Biomex	10% (v/v)	No interference
Mucin (porcine)	Sigma	60 µg/mL	No interference
Salbutamol		1.7 µmol/L	No interference
Nasal spray (Xylometazoline)	Ratiopharm	10% (v/v)	No interference
Nasal spray (Salts)	Emsan	10% (v/v)	No interference
Guaifenesin	Sigma	15.2 mmol/L	No interference
Acetylcystein	USP	920 µmol/L	No interference
Nicotine	Sigma	6.2 µmol/L	No interference
Benzocaine		0.63 mg/mL	No interference
Oseltamivir		1.5 mg/mL	No interference

Legend: v/v = volume to volume, µg/mL = micrograms per milliliter, µmol/L = micromoles per liter, mmol/L = millimoles per liter, mg/mL = milligrams per milliliter

Clinical Performance

The clinical performance of FTD SARS-CoV-2 was established using prospectively collected nasopharyngeal and oropharyngeal swabs. A total of 101 specimens were collected from symptomatic patients with suspicion of COVID-19. The clinical performance study was conducted in a diagnostic laboratory in Luxembourg and was evaluated by comparing FTD SARS-CoV-2 results using the NucliSENS® easyMAG® extraction method and the Applied Biosystems® 7500 Real-Time PCR System, with the results obtained from a CE-IVD nucleic acid amplification test (NAAT) comparator kit.

Results are displayed in Table 13.

Table 13: Diagnostic Sensitivity and Specificity Obtained by FTD SARS-CoV-2

Pathogen	Specimen Type	Diagnostic Sensitivity		95% Confidence Interval	Diagnostic Specificity		95% Confidence Interval
		Percentage	Total Number		Percentage	Total Number	
SARS-CoV-2	NPS	100%	12/12	(73.54–100)	100%	3/3	(29.24–100)
	OPS	100%	31/31	(88.78–100)	100%	55/55	(93.51–100)
Overall		**100%**	**43/43**	**(91.78–100)**	**100%**	**58/58**	**(93.84–100)**

Legend: NPS = nasopharyngeal swab, OPS = oropharyngeal swab

The results showed an overall diagnostic sensitivity of 100% (95% Confidence Interval: 91.78–100) and an overall diagnostic specificity of 100% (95% Confidence Interval: 93.84–100) for the detection of SARS-CoV-2 in both matrices using FTD SARS-CoV-2.

Troubleshooting

Table 14 describes a non-exhaustive list of control errors that a user may observe with FTD SARS-CoV-2 and suggested corrective actions.

Table 14: Control Errors

Observation	Possible Cause	Corrective Action
FTD PC 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 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. • If the problem persists, consider the presence of interfering material in the samples.
	Amplification of FTD IC was inhibited or the extraction of the FTD IC was inadequate.	
	High viral load may cause competitive signals with the FTD IC causing the sample(s) to become invalid.	Consider diluting sample(s) and repeat the analysis.
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.

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.

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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
	*In vitro* diagnostic medical device		Contains sufficient for <*n*> tests
	Catalog number		Batch code
	Manufacturer		Use-by date
	Date of manufacture		Keep away from sunlight
	CE Mark	**YYYY-MM-DD**	Date format (Year-Month-Day)
	CE Mark with identification number of notified body	**YYYY-MM**	Date format (Year-Month)
	Consult instructions for use		Store upright
	Caution/Warning		Irritant
	Temperature limit		Made in Luxembourg

Legal Information

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2.2 Product code 11416284 IFU

Fast Track DIAGNOSTICS

A Siemens Healthineers Company

IVD CE

FTD™ SARS-CoV-2

Current Revision and Date	11416283_en Rev. A, 2020-05		
Product Name	FTD SARS-CoV-2 (FTD-114-96)	REF 11416284	Σ 96
Specimen Types	Nasopharyngeal and Oropharyngeal swabs		
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).

Table of Contents

Intended Use

FTD SARS-CoV-2 is a qualitative *in vitro* nucleic acid amplification test for the detection of severe respiratory syndrome coronavirus 2 (SARS-CoV-2) nucleic acids in nasopharyngeal and oropharyngeal swabs of patients with signs and symptoms of SARS-CoV-2 infection in conjunction with clinical and epidemiological risk factors, who are suspected of Coronavirus Disease 2019 (COVID-19).

The test is intended as an aid in the diagnosis of infections caused by the new human coronavirus SARS-CoV-2.

For *in vitro* diagnostic use.

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 severity of clinical signs requires that approximately 20% of patients remain in hospital and 5% require admission to intensive care. The most serious forms are observed mainly in people who are vulnerable because of their age (over 70) or associated diseases. However, the infection can also be asymptomatic or paucisymptomatic (*i.e.*, causing little or no clinical manifestations) in 30% to 60% of infected subjects. The duration of incubation is on average 5 days, with extremes of 2 to 12 days.[1]

The serial interval (*i.e.*, time between one person developing symptoms of a disease and a second person becoming infected and developing symptoms) is approximately 4 days. Thus, the serial interval of COVID-19 is shorter than its median incubation period. This suggests that a substantial proportion of secondary transmission may occur prior to illness onset.[2,3] This “silent transmission” makes this pandemic hard to contain and more likely to spread very quickly.

FTD SARS-CoV-2 is an aid in the identification of COVID-19 disease by the detection of SARS-CoV-2 ribonucleic acid (RNA) extracted from nasopharyngeal and oropharyngeal swabs of patients suspected of COVID-19 infection.

Pathogens

New human coronavirus SARS-CoV-2

Coronaviruses belong to a large family of encapsulated viruses with a positive-sense RNA genome, which can cause illness in animals and humans. The genera Alphacoronavirus (AlphaCoV) and Betacoronavirus (BetaCoV) contain seven known human coronaviruses that can cause mild respiratory tract infections, but also more severe infections like severe acute respiratory syndrome (SARS), Middle East respiratory syndrome (MERS) and COVID-19.

SARS-CoV-2, the causative agent of COVID-19, belongs to the genus Betacoronavirus and was firstly identified in humans in January 2020. The transmission route is still under investigation. Although bats are likely reservoir hosts for SARS-CoV-2, there is still ongoing research investigating if pangolins (*Manis javanica*) could be considered as possible intermediate hosts for this novel human virus.[4] Human-to-human transmission occurs via droplets or direct contact.[5]

FTD SARS-CoV-2 detects two different genomic regions specific for SARS-CoV-2 (see *Performance Characteristics – Inclusivity* section on page 23).

Principles of the Procedure

Method

This test is a real-time polymerase chain reaction (RT-PCR)-based process for detection of pathogens in human samples.

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 the pathogen(s) is added to a master mix to enable the RT-PCR reaction. 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 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 also processed as a sample (extraction and RT-PCR). It confirms the absence of contamination.

The FTD SARS-CoV-2 kit also 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.

Reagents

Warnings and Precautions

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 nasopharyngeal and oropharyngeal swabs 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 due to the sensitivity of several pathogens to external influences. 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[6]).

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.

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

Nasopharyngeal Swabs

For nasopharyngeal swab, insert the swab through the nares parallel to the palate until resistance is encountered or the distance is equivalent to that from the ear to the nostril of the patient indicating contact with the nasopharynx. Once inserted, gently turn the swab to obtain infected cells and place into the appropriate container (as recommended by CDC[6]).

Oropharyngeal Swabs

For oropharyngeal swabs, insert the swab into the posterior pharynx and tonsillar areas. Rub the swab gently over both tonsillar pillars and posterior oropharynx and avoid touching the tongue, teeth, and gums. Place then into the appropriate container (as recommended by CDC[6]).

Storing and Transporting the Specimen

Nasopharyngeal and oropharyngeal swabs should be transported in a viral transport medium or similar. Specimens which can be delivered promptly to the laboratory can be refrigerated and shipped at temperatures of 2–8°C (according to manufacturer instructions). 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[7]).

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 circular 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! While 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
11416284 (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 manufacturer (bioMérieux) 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 and IC (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 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 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.

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	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 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.

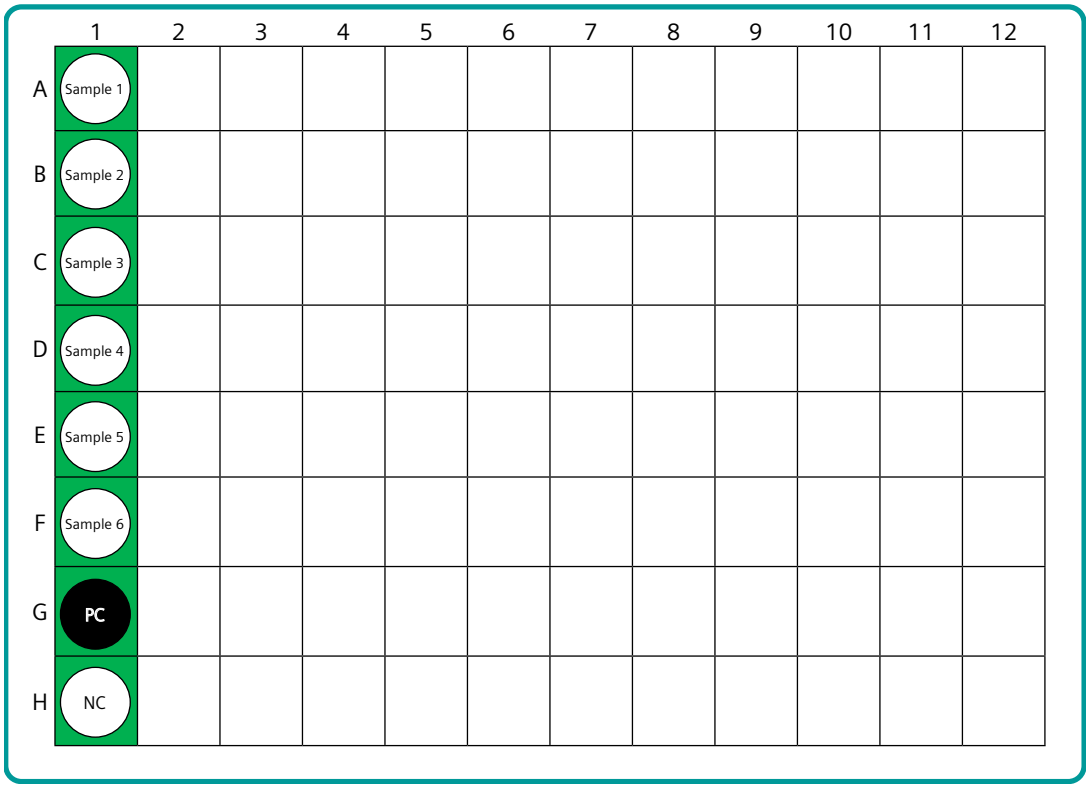
   a. Pipette the required amount of 25x RT-PCR enzyme in each of the tubes containing SCoV2 PP Mix and 2x RT-PCR buffer (see Table 3).
   b. Vortex master mix briefly and spin it down.
   c. Use master mix immediately and do not store after use.

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

NOTE: Each master mix on the plate must have a corresponding PC and NC to perform analysis.

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) • 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 F1.
3. Add 10 µL of the PC into well G1.
4. Add 10 µL of the extracted NC into well H1.
5. Seal plate with appropriate adhesive film.
6. Gently vortex plate, then centrifuge briefly.
7. 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.

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. If there is a potential contamination (appearance of a curve in the NC or a cluster of curves in specimens at high Ct[1]), results obtained are not interpretable and the whole run (including extraction) must be repeated.

2. All PC must show a positive (*i.e.*, exponential) amplification trace. The PC must fall below a Ct of 33.

3. All samples and NC (or each extracted material) must show a positive amplification trace for the IC. The IC must fall below a Ct of 33.

Results

Interpretation of Results

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

Table 5: FTD SARS-CoV-2 – Possible Results

PP Mix	Pathogen	Signal in Green Channel	Signal in Yellow Channel	Signal in Orange Channel	Signal in Red Channel
SCoV2	SARS-CoV-2	POS	—	—	—
	—	—	—	—	—
	—	—	—	—	—
	IC (EAV)	—	—	—	POS

Legend: POS = Positive, Empty = Negative

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

[1] Specimens with a Ct above 35.

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 one of the pathogens targeted by the kit. An absence of an exponential trace indicates an absence or undetectable load of nucleic acid.

For example, if a patient sample analyzed with the SCoV2 master mix (see Table 5) displays an exponential fluorescence trace in the Green channel – this sample contains a detectable load of SARS-CoV-2 RNA.

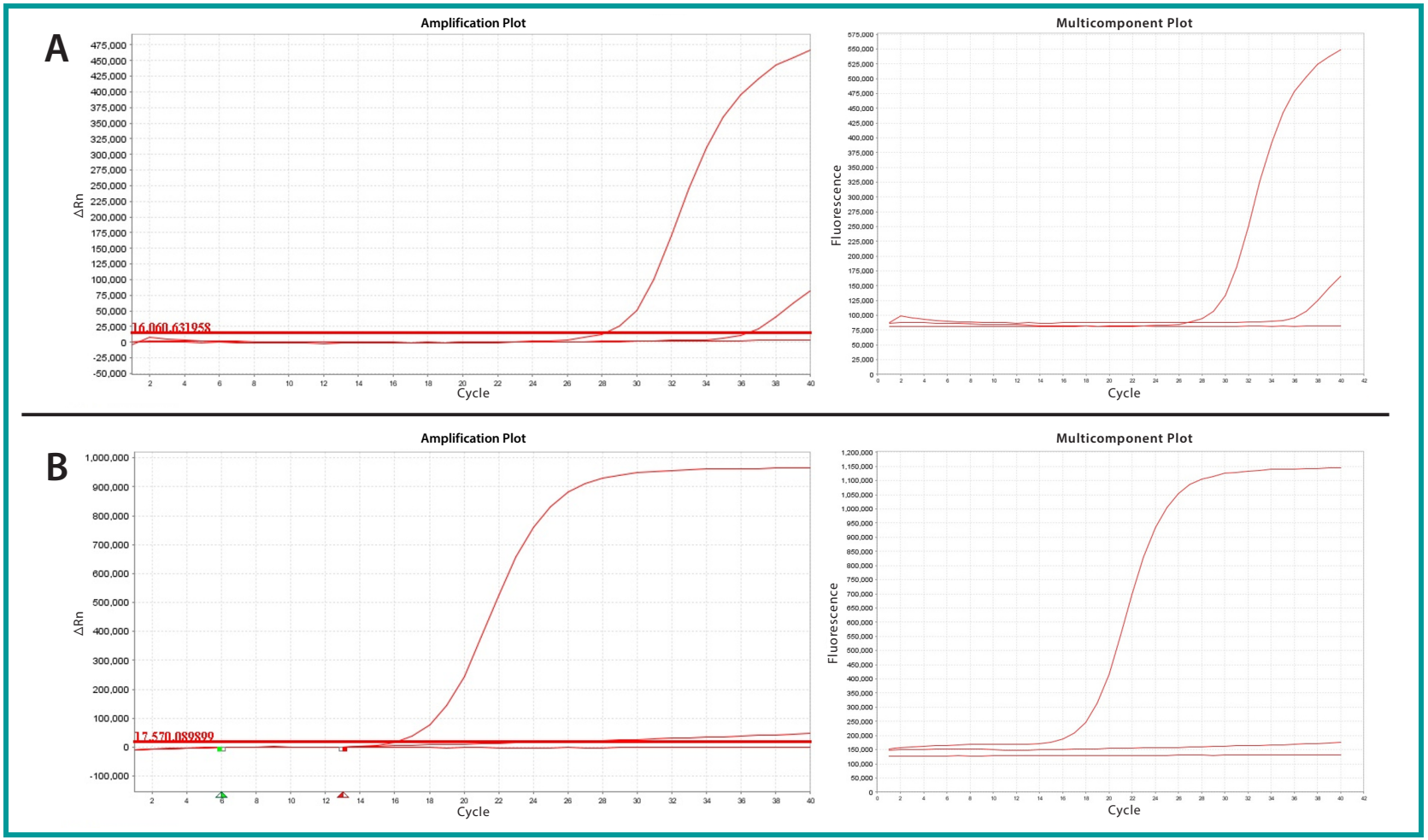
The IC must be positive (red channel) for each extracted material (samples and NC).

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

For *in vitro* diagnostic use.

- The sample storage and shipment instructions provided are recommendations. Verification and validation data for specimen collection and handling (transport and storage) are not available.
- 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.
- Remel M4RT® transport medium is not recommended for use with FTD SARS-CoV-2.
- 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.
- Detection of pathogens may be affected by the presence of inhibitors other than those specified in the *Performance Characteristics* section.

Performance Characteristics

Performance characteristics show the analytical and clinical performance data of FTD SARS-CoV-2. The analytical performance (analytical sensitivity, analytical specificity, inclusivity, and precision) was evaluated using the NucliSENS® easyMAG® (bioMérieux) extraction system and the Applied Biosystems® 7500 (ThermoFisher Scientific) real-time thermocycler.

Analytical Sensitivity

The analytical sensitivity is the ability of the assay to consistently detect a given target sequence in the tested biological specimens. The lowest concentration detectable in greater than or equal to 95% of tested specimens is then defined as the "Limit of Detection" (LoD).[8]

The LoD of FTD SARS-CoV-2 was determined empirically by testing serial dilutions of quantified extracted RNA of SARS-CoV-2 (BetaCoV/Germany/BavPat1/2020 p.1).

Using Probit (PROBability unITs) Regression Analysis, the LoD for SARS-CoV-2 was evaluated to be 1155 copies per milliliter (see Table 6).

Table 6: Analytical Sensitivity of FTD SARS-CoV-2

Pathogen	Units	LoD from Probit Analysis	95% Confidence Interval
SARS-CoV-2	cop/mL	1155	854–1508

Legend: cop/mL = copies per milliliter, LoD = Limit of detection, Probit = PROBability unITs Regression Analysis

The LoD previously calculated with Probit analysis was then confirmed using another primer and probe mix (PP mix) lot. This LoD confirmation test was performed by testing 24 replicates at a concentration equivalent to the upper confidence limit previously established. This LoD confirmatory study was performed by two operators, using two different instruments and one PP mix lot, across 2 days.

It can be concluded that the 95% LoD of FTD SARS-CoV-2 is 1155 cop/mL, which corresponds to 11.5 cop/reaction.

Analytical Specificity

The analytical specificity of a PCR multiplex assay is the ability of a measurement to measure solely the measurand.[8] The analytical specificity is referred to as:

- Cross-reactivity: The ability of the test to specifically detect the intended pathogens (including relevant subtypes when specified), but no other organism in biological samples.
- Specificity: The assurance that the test will not report false positive results when testing negative samples.

FTD SARS-CoV-2 analytical specificity was validated using an *in silico* analysis as well as *in vitro* testing as detailed below.

Cross-Reactivity

In Silico Analysis

The specificity of FTD SARS-CoV-2 was determined using *in silico* analysis, applying the Basic Local Alignment Search Tool (BLAST). Regions of similarity were searched for all primers and probes of this assay analyzing the complete National Center for Biotechnology Information (NCBI) Nucleotide collection database (non-redundant protein [nr]/non-redundant nucleotide [nt]), excluding sequences belonging to the targeted organism. A sequence similarity of at least 90% with the respective assay (primer/probe) sequences was used as criteria for mapping to check for potential matches producing amplicons. A maximum of four mismatches between oligo and target sequence was allowed when mapping the primers and probes.

The results of the *in silico* analysis are presented in Table 7. Data showed a potential cross-reactivity with one sequence from Bat coronavirus isolate RaTG13 (Accession number MN996532.1) and Pangolin coronavirus isolate MP789 (Accession number MT084071.1). Both viruses belong to the SARS-related coronaviruses. Pairwise sequence alignment between Bat CoV RaTG13 and SARS-CoV-2 (Accession number NC_045512) revealed a sequence identity of 96.1%. There is evidence that RaTG13 is the closest relative of SARS-CoV-2 and they form a distinct lineage from other SARS-related coronaviruses.[9] Pairwise sequence alignment between Pangolin CoV MP789 and SARS-CoV-2 (Accession number NC_045512) revealed a sequence identity of 78.7%. The group that identified the pangolin isolate showed that the pangolin coronavirus (pangolin-CoV-2020) is genetically associated with both SARS-CoV-2 and a group of bat coronaviruses.

However, phylogenetic analyses did not support that the SARS-CoV-2 arose directly from the pangolin-CoV-2020.[10] No other cross-reactivity was found with FTD SARS-CoV-2.

Table 7: Cross-Reactivity of FTD SARS-CoV-2

Pathogen	Potential Cross-Reactivity
SARS-CoV-2	Bat coronavirus isolate RaTG13 (MN996532.1)
	Pangolin coronavirus isolate MP789 (MT084071.1)

In Vitro Analysis

FTD SARS-CoV-2 was tested *in vitro* for potential cross-reaction with common human flora organisms and the pathogenic organisms causing respiratory infections. Cross-reactivity testing was performed using several pools of contrived samples and extracted RNA/DNA. Each pool was spiked with a maximum of five pathogens. The pools were extracted in triplicate using NucliSENS® easyMAG® and evaluated on the Applied Biosystems® 7500.

The list of the pathogens tested is displayed in Table 8 along with the culture information, tested concentration and the results of the analysis. No unspecific signal could be detected.

Table 8: Cross-Reactivity Panel Tested with FTD SARS-CoV-2

Target	Provider	Catalog Number	Tested Concentration	Units	Result
Human adenovirus (Ad. 71)	American Type Culture Collection (ATCC®)	VR-1	1.13E+06	TCID50/mL	No cross-reactivity
Human parainfluenza virus 2, Geer		VR-92	1.13E+06	TCID50/mL	No cross-reactivity
Human enterovirus, human echovirus 1; Strain: Farouk		VR-1808	2.00E+06	TCID50/mL	No cross-reactivity
Human rhinovirus 1A; Strain: 2060		VR-1559	1.13E+07	TCID50/mL	No cross-reactivity
Streptococcus pneumonia	DSMZ	20584-1018-011	1.24E+08	cop/mL	No cross-reactivity
Human metapneumovirus (hMPV) A, Type: A1; Strain: IA10-2003	Zeptometrix	0810161CFHI	6.82E+05	TCID50/mL	No cross-reactivity
Human parainfluenza virus 3, C 243	ATCC®	VR-93	1.13E+06	TCID50/mL	No cross-reactivity
Human parainfluenza virus 4, M-25		VR-1378	2.00E+05	TCID50/mL	No cross-reactivity
Human metapneumovirus (hMPV) B, Type: B1; Strain: Peru2-2002	Zeptometrix	0810156CFHI	1.56E+05	TCID50/mL	No cross-reactivity
Human parainfluenza virus 1, C35	ATCC®	VR-94	2.00E+05	TCID50/mL	No cross-reactivity
Human coronavirus OC43		VR-1558	1.41E+04	TCID50/mL	No cross-reactivity
Human coronavirus NL63	Zeptometrix	0810228CFHI	8.39E+03	TCID50/mL	No cross-reactivity
Influenza B, Florida/04/06		0810255CFHI	Unknown (Ct: 20.3)[a]	—	No cross-reactivity
Chlamydophila pneumoniae, Strain: TW-183	ATCC®	VR-2282	2.01E+04	cop/mL	No cross-reactivity
Haemophilus influenzae		9006	5.71E+02	CFU/mL	No cross-reactivity
Legionella pneumophila		33152	1.00E+03	CFU/mL	No cross-reactivity
Bordetella pertussis, H898		BAA-2702	1.43E+03	CFU/mL	No cross-reactivity
Mycoplasma pneumoniae	DSMZ	23978-1013-001	Unknown (Ct: 21)[a]	—	No cross-reactivity
Respiratory syncytial virus (RSV A), A2	ATCC®	VR-1540	3.14E+06	PFU/mL	No cross-reactivity
Respiratory syncytial virus (RSV B), Strain: CH93(18)-18	Zeptometrix	0810040CFHI	3.26E+05	TCID50/mL (prior to heat-inactivation)	No cross-reactivity

Table 8: Cross-Reactivity Panel Tested with FTD SARS-CoV-2 (Continued)

Target	Provider	Catalog Number	Tested Concentration	Units	Result
SARS-Coronavirus, HKU39849[b]	European Virus Archive - GLOBAL (EVAg)	SKU-011N-03868	Unknown (Ct: 18)[c]	—	No cross-reactivity
MERS-Coronavirus[b]		SKU-011N-03868	Unknown (Ct: 29)[c]	—	No cross-reactivity
Mycobacterium tuberculosis[d]	Vircell	MBC034	1.00E+06	cop/mL	No cross-reactivity
Human coronavirus 229E	EVAg	SKU-011N-03868	Unknown (Ct: 30.3)[a]	—	No cross-reactivity
Human coronavirus HKU1	DLS	85250	Unknown (Ct: 14.9)[a]	—	No cross-reactivity
Influenza A[b]	Vircell	MBC028	1.00E+06	cop/mL	No cross-reactivity

[a] Ct value determined with FTD Respiratory pathogens 21 (CE-IVD).
[b] Extracted RNA.
[c] Ct value given by supplier.
[d] Extracted DNA.

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

Negative Material

Negative material (extracted negative controls, extracted negative clinical samples or non-template controls) has been tested *in vitro* in order to evaluate the occurrence of potential non-specific amplification when using FTD SARS-CoV-2. The derived analytical specificity is displayed in Table 9. Overall, an analytical specificity of 100% was reached.

Table 9: Analytical Specificity of FTD SARS-CoV-2

Pathogen	Tested Sample	Positive Results	Total Reactions	Analytical Specificity %	Confidence Interval %
SARS-CoV-2	Negative clinical sample	0	50	100	92.89–100.00
	Negative control	0	39	100	90.00–100.00
	Non-template control	0	38	100	89.72–100.00
	Total	**0**	**127**	**100**	**96.95–100.00**

Inclusivity

Inclusivity (or analytical reactivity) 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 *in silico* analysis on all sequences available 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 1048 sequences (114 from GenBank and 934 from GISAID) were downloaded on 19 Mar 2020. SARS-CoV-2 primers and probes were then mapped to the sequences to check for potential matches producing amplicons. Incomplete sequences and sequences from animal were removed. This resulted in 901 sequences (96 from GenBank and 805 from GISAID) that were further analyzed (see Table 10). A maximum of four mismatches between oligo (primer and probe) and the target sequence was allowed when mapping the primers and probes, as most of the time an imperfect match still produces pairing and amplification.

The alignment showed that the SARS-CoV-2 N gene assay detected all sequences from the GenBank database with a maximum of one mismatch (three sequences concerned) and 100% of all sequences obtained from the GISAID database with a maximum of one mismatch (11 sequences concerned).

The SARS-CoV-2 ORF1ab assay was aligned against these same sequences. The results showed a 100% detection rate against the GenBank sequences with zero mismatch and 100% detection rate against the GISAID database with a maximum of one mismatch (eight sequences concerned).

For all of the 22 sequences, the mismatch is not located at a critical position that could impact detection. There is no overlap between the sequences showing mismatch with the assay target N gene or the one targeting ORF1ab. Due to our dual-target approach, at least one or both assays binds without any mismatch (100% homology) to the above mentioned 22 sequences.

The results of the inclusivity analysis are summarized in Table 10.

Table 10: Inclusivity of FTD SARS-CoV-2

Assays	Database	Complete Genomes Tested	Complete Genomes Detected	Detection Rate (%)
SARS-CoV-2 (N gene)	GenBank	96	96	100.0
	GISAID	805	805	100.0
SARS-CoV-2 (ORF1ab)	GenBank	96	96	100.0
	GISAID	805	805	100.0

Precision

Precision refers to how well a given measurement can be reproduced when a test is applied repeatedly to multiple aliquots of a single homogeneous sample. FTD SARS-CoV-2 precision was assessed by repeatability and reproducibility studies with test material at a concentration at upper LoD (see Table 11). Repeatability evaluates measurements carried out under the same conditions (intra-assay variation), whereas reproducibility evaluates results of measurements under changed conditions (*i.e.*, time, operator and cycler). The precision of each study was expressed based on statistical measurements of imprecision (standard deviation and coefficient of variation).

Commercially available quantified RNA of SARS-CoV-2 was tested at a concentration at upper LoD with FTD SARS-CoV-2 on the Applied Biosystems® 7500. Results were collected over multiple runs and days. Runs were executed by different operators on different cyclers using one PP mix lot.

Table 11 presents the results of the precision study. The data demonstrated a repeatability imprecision of 2.10% and a reproducibility imprecision of 2.49%.

Table 11: Precision Study for FTD SARS-CoV-2

Target and Sample	N	Repeatability SD	Reproducibility SD	Reaptability CV (%)	Reproducibility CV (%)
SARS-CoV-2	23	0.80 (0.61–1.15)	0.95 (0.64–1.79)	2.10 (1.61–3.03)	2.49 (1.69–4.71)

Legend: N = Total sample size, SD = Standard deviation, CV = Coefficient of variation

Interfering Substances

An interference study was conducted to evaluate the susceptibility of FTD SARS-CoV-2 to provide erroneous results in presence of potential interfering substances in the clinical sample. Artificial matrix was spiked with an interfering substance, the solvent or left untreated. Each sample was then extracted in triplicates using NucliSENS® easyMAG®. The eluate was spiked with SARS-CoV-2 RNA (BetaCoV/Germany/BavPat1/2020 p.1) at 3x LoD concentration. RT-PCR with one lot of FTD SARS-CoV-2 was performed on the Applied Biosystems® 7500. The list of the tested substances and their interfering power are documented in Table 12. The data showed that none of the tested substances interfered with the PCR results.

Table 12: Potential Interfering Substance Evaluated

Substance	Provider	Tested Concentration	Results
Whole blood	Biomex	10% (v/v)	No interference
Mucin (porcine)	Sigma	60 µg/mL	No interference
Salbutamol	Vaseline	1.7 µmol/L	No interference
Nasal spray (Xylo.)	Up&Up	10% (v/v)	No interference
Nasal spray (Salts)	Ratiopharm	10% (v/v)	No interference
Guaifenesin	Emsa	15.2 mmol/L	No interference
Acetylcystein	Sigma	920 µmol/L	No interference
Nicotine		6.2 µmol/L	No interference
Benzocaine		0.63 mg/mL	No interference
Oseltamivir		1.5 mg/mL	No interference

Legend: v/v = volume to volume, µg/mL = micrograms per milliliter, µmol/L = micromoles per liter, mmol/L = millimoles per liter, mg/mL = milligrams per milliliter, Xylo = Xylometazoline

Clinical Performance

The clinical performance of FTD SARS-CoV-2 was established using prospectively collected nasopharyngeal and oropharyngeal swabs. A total of 101 specimens were collected from symptomatic patients with suspicion of COVID-19. The clinical performance study was conducted in a diagnostic laboratory in Luxembourg and was evaluated by comparing FTD SARS-CoV-2 results using the NucliSENS® easyMAG® extraction method and the Applied Biosystems® 7500 Real-Time PCR System, with the results obtained from a CE-IVD nucleic acid amplification test (NAAT) competitor kit.

Results are displayed in Table 13.

Table 13: Diagnostic Sensitivity and Specificity Obtained by FTD SARS-CoV-2

Pathogen	Specimen Type	Diagnostic Sensitivity		95% Confidence Interval	Diagnostic Specificity		95% Confidence Interval
		Percentage	Total Number		Percentage	Total Number	
SARS-CoV-2	NPS	100%	12/12	(73.54–100)	100%	3/3	(29.24–100)
	OPS	100%	31/31	(88.78–100)	100%	55/55	(93.51–100)
Overall		**100%**	**43/43**	**(91.78–100)**	**100%**	**58/58**	**(93.84–100)**

Legend: NPS = nasopharyngeal swab, OPS = oropharyngeal swab

The results showed an overall diagnostic sensitivity of 100% (95% Confidence Interval: 91.78–100) and an overall diagnostic specificity of 100% (95% Confidence Interval: 93.84–100) for the detection of SARS-CoV-2 in both matrices using FTD SARS-CoV-2.

Troubleshooting

Table 14 describes a non-exhaustive list of control errors that a user may observe with FTD SARS-CoV-2 and suggested corrective actions.

Table 14: Control Errors

Observation	Possible Cause	Corrective Action
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.

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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
	*In vitro* diagnostic medical device		Contains sufficient for <*n*> tests
	Catalog number		Batch code
	Manufacturer		Use-by date
	Date of manufacture		Keep away from sunlight
	CE Mark	**YYYY-MM-DD**	Date format (Year-Month-Day)
	CE Mark with identification number of notified body	**YYYY-MM**	Date format (Year-Month)
	Consult instructions for use		Store upright
	Caution/Warning		Irritant
	Temperature limit		Made in Luxembourg

Legal Information

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