Fast Track DIAGNOSTICS
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IVD CE

FTD™ Respiratory pathogens 21

Current Revision and Date	11414180_en Rev. C, 2021-07			
Product Name	FTD Respiratory pathogens 21 (FTD-2.1-32)	REF	10921702	Σ 32
	FTD Respiratory pathogens 21 (FTD-2.1-64)	REF	10921703	Σ 64
Specimen Type	Nasopharyngeal swabs			
Processed Sample Volume	200 µL required			

FTD Respiratory pathogens 21 was validated with the Thermo Fisher Scientific Applied Biosystems® 7500 Real-Time PCR System and the NucliSENS® easyMAG® (bioMérieux).

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Intended Use

FTD Respiratory pathogens 21 is a qualitative *in vitro* nucleic acid amplification test for the detection and differentiation of specific viral and bacterial nucleic acids in nasopharyngeal swab specimens of human origin.

The test is intended as an aid in the diagnosis of respiratory infections caused by influenza A virus (IAV), influenza A virus H1N1 swine-lineage (IAV [H1N1] swl), influenza B virus (IBV), human rhinovirus (HRV), human coronaviruses (HCoV) 229E, NL63, HKU1, and OC43, human parainfluenza viruses (HPIV) 1 through 4, human metapneumoviruses (HMPV) A and B, human bocavirus (HBoV), *Mycoplasma pneumoniae* (*M. pneumoniae*), human respiratory syncytial viruses (HRSV) A and B, human parechovirus (HPeV), enterovirus (EV), and human adenovirus (HAdV).

For *in vitro* diagnostic use.

Summary and Explanation

Acute respiratory pathogen infections are common causes of acute local and systemic disease with substantial morbidity and mortality in pediatric, adult and immunocompromised patients. Respiratory symptoms may include nasal discharge, congestion, fever, coughing, wheezing, and headache. Globally, pathogen transmission occurs mainly through an infected person, spreading it to other people from the droplets made when they cough, sneeze, or talk. Early and accurate diagnosis of respiratory pathogens diminishes complications, and unnecessary laboratory testing and enables the physician in determining appropriate immediate patient treatment.[1,2]

Simultaneous detection of several respiratory pathogens became feasible with the introduction of molecular multiplex assays as compared to traditional singleplex tests.[3] In this diagnostic context, FTD Respiratory pathogens 21 was designed for simultaneous detection of viral and bacterial nucleic acids from nasopharyngeal swabs of human origin as an aid in the evaluation of respiratory infections.

Pathogens

Influenza viruses are encapsulated single-stranded negative-sense ribonucleic acid (RNA) viruses belonging to the *Orthomyxoviridae* family.[4] Influenza A and B viruses are major causes of seasonal epidemics of respiratory infections in children and adults with rapid onset of fever.

Influenza A viruses (IAV) are further subtyped by their surface glycoproteins, the hemagglutinin (HA) and the neuraminidase (NA).[4] Genetically distinct 18 HA and 11 NA subtypes have been identified in circulating influenza A viruses. However, only two IAV subtypes (*i.e.*, H1N1 and H3N2) are currently in general circulation among humans.[5] The most frequently identified subtypes of avian influenza that have caused human infections are H5, H7, and H9 viruses.[5]

FTD Respiratory pathogens 21 contains two assays for the detection of IAV: a general assay that detects all subtypes of IAV and a secondary assay specifically targeting the NA gene of the 2009-pandemic swine-lineage **Influenza A virus subtype H1N1(IAV [H1N1] swl)** (see *Performance Characteristics – Inclusivity* section on page 33).

Influenza B viruses (IBV) are not divided into subtypes, but are further classified into lineages and strains. Currently circulating influenza B viruses belong to one of two lineages: B/Yamagata and B/Victoria.[5]

FTD Respiratory pathogens 21 detects both lineages of IBV (see *Performance Characteristics – Inclusivity* section on page 33).

Human rhinoviruses (HRV) are the most frequent viral infective agents in humans and the predominant cause of the common cold. Human rhinoviruses are prevalent throughout the entire year.[6] Human rhinoviruses are single-stranded, positive-sense RNA viruses belonging to the genus *Enterovirus* of the *Picornaviridae* family. These are classified into three genetically distinct species (A, B, and C) with more than 150 serotypes in total.[6]

Recently, these viruses have been increasingly reported to be associated with asthma and Chronic Obstructive Pulmonary Disease (COPD) exacerbations.[7,8]

FTD Respiratory pathogens 21 detects all three species of the HRV (see *Performance Characteristics – Inclusivity* section on page 33).

Human coronaviruses (HCoV) are a common cause of infections of the respiratory tract, including bronchiolitis and pneumonia. Human coronaviruses are large, enveloped, single-stranded, positive-sense RNA viruses belonging to the *Coronaviridae* family.[9] Coronaviruses are frequently co-detected with other respiratory viruses, particularly with human respiratory syncytial virus (HRSV).[10] Whether coronaviruses contribute to disease severity in such coinfections is currently unclear.[10]

FTD Respiratory pathogens 21 detects all four species of coronaviruses: NL63, 229E, OC43 and HKU1 (see *Performance Characteristics – Inclusivity* section on page 33).

Human parainfluenza viruses (HPIV) are common community-acquired respiratory pathogens that are associated with every kind of upper and lower respiratory tract illness. These pathogens are single-stranded, negative-sense RNA viruses of the *Paramyxoviridae* family. Human parainfluenza viruses are divided into two genera, *Respirovirus* and *Rubulavirus*, and four species: HPIV-1, HPIV-3 and HPIV-2, HPIV-4, respectively.[11]

FTD Respiratory pathogens 21 detects all four species (see *Performance Characteristics – Inclusivity* section on page 33).

Human metapneumoviruses (HMPV) are a leading cause of acute respiratory infections, particularly in children, immunocompromised patients, and the elderly.[12] Human metapneumoviruses are non-segmented, single-stranded, negative-sense RNA viruses belonging to the genus *Metapneumovirus* of the *Pneumoviridae* family.[13] Two HMPV genotypes, A and B, exist with similar severity of illness.[14]

FTD Respiratory pathogens 21 detects both genotypes (see *Performance Characteristics – Inclusivity* section on page 33).

Human bocaviruses (HBoV) are small, single-stranded deoxyribonucleic acid (DNA) viruses linked with acute respiratory and gastrointestinal infections. Four subtypes are known to cause infections in humans, but only subtype one causes respiratory tract infections. HBoV infection causes mild-to-severe, primarily lower respiratory tract infections in children.[15] Additionally, HBoV infections are frequently accompanied by at least another viral and/or bacterial respiratory and/or gastroenteric pathogen infection.[16]

FTD Respiratory pathogens 21 detects HBoV subtype 1 (see *Performance Characteristics – Inclusivity* section on page 33).

Mycoplasma pneumoniae (M. pneumoniae) is an important bacterial pathogen responsible for respiratory tract infections in both children as well as adults that can range in severity from mild to life-threatening.[17] The bacterium belonging to the *Mycoplasmataceae* family lacks a peptidoglycan cell wall which makes it intrinsically resistant to antimicrobials that target the cell wall and are undetectable by gram staining.[18] *M. pneumoniae* is broadly divided into two genetic groups, subtype 1 and subtype 2.[18]

FTD Respiratory pathogens 21 detects both subtypes (see *Performance Characteristics – Inclusivity* section on page 33).

Human respiratory syncytial viruses (HRSV) are an important cause of severe lower respiratory tract infections in children, immunocompromised patients, and the elderly.[19] Human respiratory syncytial viruses are enveloped, non-segmented, single-stranded, negative-sense RNA viruses belonging to the genus *Orthopneumovirus* of the *Pneumoviridae* family.[20] Two subtypes of HRSV have been described (A and B).[21]

FTD Respiratory pathogens 21 detects both subtypes (see *Performance Characteristics – Inclusivity* section on page 33).

Human parechoviruses (HPeV) are single-stranded, positive-sense RNA viruses belonging to species Parechovirus A of the *Picornaviridae* family. Parechovirus A consists of 19 distinct genotypes (HPeV-1 to 19)[22,23] with HPeV-1 and HPeV-3 being the most frequently detected HPeV genotypes worldwide.[24] Human parechovirus infections are often asymptomatic but are also associated with a wide variety of clinical presentations ranging from mild gastrointestinal and respiratory infections to severe disease like sepsis, and meningitis, particularly in neonates.[25] While HPeV-1 is usually associated with gastrointestinal and respiratory symptoms, HPeV-3 infection has been related to sepsis-like illness and meningitis

in young children. HPeV-3 is one of the most important emerging pathogens for pediatricians to pay attention to because of the severity of disease.[26]

FTD Respiratory pathogens 21 detects HPeV subtypes 1–8, 10, 14 and 16–18 (see *Performance Characteristics – Inclusivity* section on page 33).

Enteroviruses (EV) are known to cause self-limiting febrile illnesses in infants and young children, but can also cause severe outbreaks of neurological and respiratory infections. Enteroviruses are now considered the most common cause of meningitis, myelitis, and paralysis in patients.[27] Enteroviruses are members of the *Enterovirus* genus of the *Picornaviridae* family, and contain a single-stranded, positive-sense RNA genome.[27,28] Enteroviruses which infect humans are classified into four species (A through D) that are based upon genetic divergence. The most well-known enteroviruses, include polioviruses, coxsackieviruses, echoviruses, and other enteroviruses.[28] Transmission of enteroviruses occur through the fecal-oral or respiratory route, with incubation periods lasting 3 to 21 days before symptoms appear.

FTD Respiratory pathogens 21 detects EV species A through D (see *Performance Characteristics – Inclusivity* section on page 33).

Human adenoviruses (HAdV) are non-enveloped, double-stranded DNA viruses of the genus *Mastadenovirus* in the *Adenoviridae* family. Human adenoviruses typically causes mild infections involving the upper or lower respiratory tract, gastrointestinal tract, or conjunctiva. HAdV infection may occur in healthy children and adults, but infections are more commonly seen in young children due to their lack of humoral immunity.[29] The disease in immunocompromised patients causes prolonged and more severe infections with increased mortality, compared to those who are immunocompetent.[30] Clinical manifestations in immune deficient patients depend upon underlying disease, affected organ system, patient age, and virus serotype, and include: pneumonia, hepatitis, hemorrhagic cystitis, colitis, pancreatitis, and meningoencephalitis.[31]

Human adenoviruses are known to have seven different species of the virus, HAdV A through G, and also includes over 50 serotypes and 70 distinct genotypes.[32]

FTD Respiratory pathogens 21 detects all seven species of HAdV (see *Performance Characteristics – Inclusivity* section on page 33).

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 type 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 the 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, and thereby allowing the emission of fluorescence.

The level of fluorescence increases by 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 Respiratory pathogens 21 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 siemens-healthineers.com. Strict adherence to the following warnings and precautions are required when running FTD Respiratory pathogens 21.

WARNING

The 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 general industry practice for respiratory specimens handling and storage that will help ensure accurate test results.

This test is for use with extracted nucleic acids from nasopharyngeal swabs of human origin.

Respiratory pathogen detection depends on the collection of high-quality specimens, their rapid transport to the laboratory, and 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 laboratory's procedure. To protect the viral or bacterial DNA/RNA from degradation, correct specimen handling is very important (as recommended by CDC[33]).

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 of samples is required for sample storage.

Collecting the Specimen

Collect all specimens according to the standard technique from the laboratory or clinician.

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[33]).

Storing and Transporting the Specimen

Nasopharyngeal swabs should be refrigerated at temperatures of 2°C to 8°C for a maximum of 48 hours. Transport on wet ice or refrigerant gel packs is recommended. Keep specimens frozen at or below -70°C for long-term storage (as recommended by CDC[33]).

NOTES:

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

Procedure

Materials Provided

Table 1 details the components for FTD Respiratory pathogens 21.

Table 1: FTD Respiratory pathogens 21 Components

Reagent	Composition	Description / Quantity	Storage
FluRhino PP Mix	Synthetic oligonucleotides, buffer	PP mix for IAV, IBV, IAV (H1N1) swl and HRV 32 reactions: 1 x 48 µL 64 reactions: 2 x 48 µL	-30°C to -10°C
COR PP Mix		PP mix for HCoV NL63, HCoV OC43, HCoV 229E and HCoV HKU1 32 reactions: 1 x 48 µL 64 reactions: 2 x 48 µL	
ParaEAV PP Mix		PP mix for HPIV-2, HPIV-3, HPIV-4 and IC 32 reactions: 1 x 48 µL 64 reactions: 2 x 48 µL	
BoMpPf1 PP Mix		PP mix for HPIV-1, HMPV A and B, HBoV and *M. pneumoniae* 32 reactions: 1 x 48 µL 64 reactions: 2 x 48 µL	
RsEPA PP Mix		PP mix for HRSV A and B, HAdV, EV and HPeV 32 reactions: 1 x 48 µL 64 reactions: 2 x 48 µL	

Table 1: FTD Respiratory pathogens 21 Components (Continued)

Reagent	Composition	Description / Quantity	Storage
Resp21 PC	Double-stranded circular DNA molecules, buffer, stabilizing agents	Plasmid pool for IAV, IBV, IAV (H1N1) swl, HRV, HCoV NL63, HCoV 229E, HCoV OC43, HCoV HKU1, HPIV-1 through HPIV-4, HMPV, HBoV, *M. pneumoniae*, HRSV, HAdV, EV and HPeV 32 reactions: 1 x 750 µL 64 reactions: 2 x 750 µL	-30°C to -10°C
Negative Ctrl	Nuclease-free water	— 32 reactions: 1 x 2000 µL 64 reactions: 1 x 4000 µL	
Internal Ctrl	Double-stranded circular DNA molecules, buffer, <5.0% guanidine hydrochloride, <0.1% maleic acid	— 32 reactions: 1 x 128 µL 64 reactions: 2 x 128 µL	
25x RT-PCR Enz.	Enzymes, buffer, glycerol, Ribonuclease (RNase) inhibitors, stabilizing agents	25x RT-PCR Enzyme mix 32 reactions: 1 x 160 µL 64 reactions: 2 x 160 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: 2 x 1000 µL 64 reactions: 4 x 1000 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 or 64 reactions. Each kit includes IC, NC and PC components.

REF	Contents	Number of Reactions
10921702 (FTD-2.1-32)	FTD Respiratory pathogens 21	32
10921703 (FTD-2.1-64)	FTD Respiratory pathogens 21	64

Materials Required but Not Provided

The kit has been validated on Applied Biosystems® 7500 using NucliSENS® easyMAG® as an extraction method.

The following 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
N/A	Nuclease-free water
280135	NucliSENS® easyMAG®, Disposables

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) and internal control (IC).
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 recommended volumes.

Table 2: Recommended 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, 9, 32 and 64 Reactions

Number of Reactions		1	9	32	64
	2x RT PCR Buffer	12.5 µL	112.5 µL	400 µL	800 µL
	Primer/Probe Mix	1.5 µL	13.5 µL	48 µL	96 µL
	25x RT PCR Enzyme	1 µL	9 µL	32 µL	64 µL
	Total	**15 µL**	**135 µL**	**480 µL**	**960 µL**

2. Prepare a separate 1.5 mL tube per primer/probe mix and label accordingly. Pipette required amount of 2x RT-PCR buffer based on the number of reactions (see Table 3).
3. Pipette the required amount of FluRhino PP Mix, COR PP Mix, ParaEAV PP Mix, BoMpPf1 PP Mix and RsEPA 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 FluRhino PP Mix, COR PP Mix, ParaEAV PP Mix, BoMpPf1 PP Mix, and RsEPA PP Mix and 2x RT-PCR buffer (see Table 3).

   b. Vortex master mix briefly and spin it down.

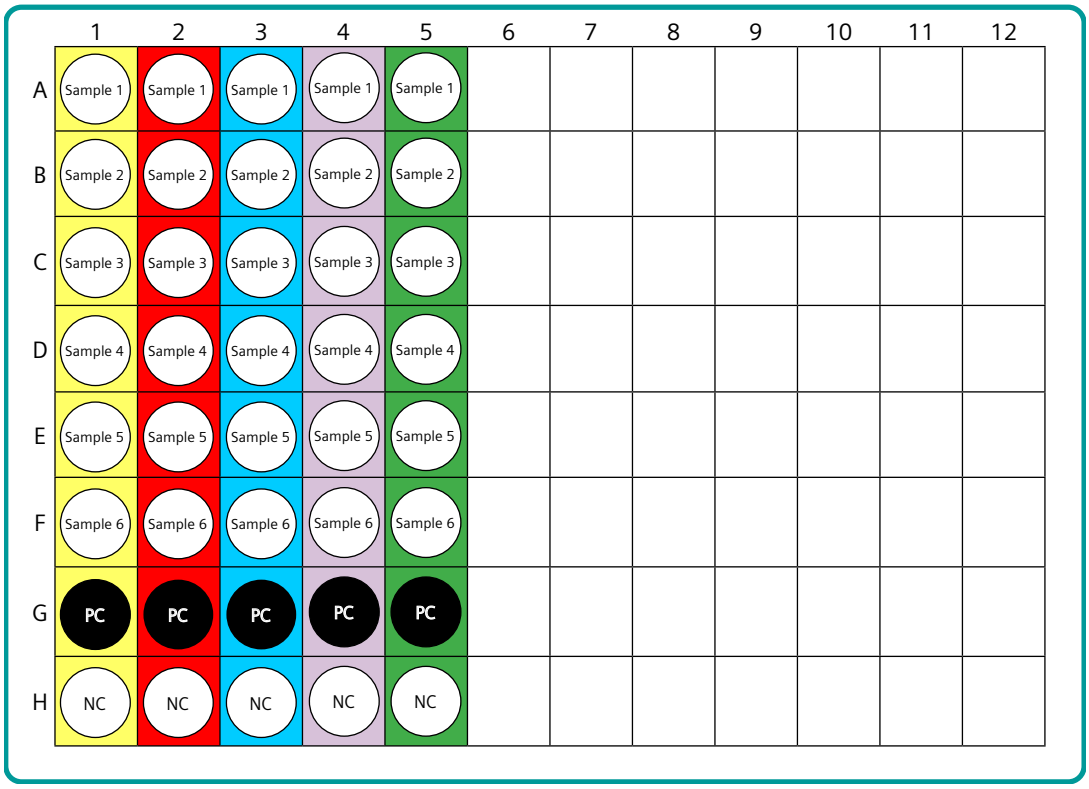
   c. Use master mix immediately and do not store after use.

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: Yellow = FluRhino master mix (A1–H1) • Red = COR master mix (A2–H2) • Blue = ParaEAV master mix (A3–H3) • Purple = BoMpPf1 master mix (A4–H4) • Green = RsEPA master mix (A5–H5) • Positive Control (G1–G5) • Negative Control (H1–H5)

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

1. Pipette the 15 µL of each mix into the wells identified below:
   a. FluRhino master mix into wells A1 to H1.
   b. COR master mix into wells A2 to H2.
   c. ParaEAV master mix into wells A3 to H3.
   d. BoMpPf1 master mix into wells A4 to H4.
   e. RsEPA master mix into wells A5 to H5.
2. Add 10 µL of the extracted samples to wells A1 to F1, A2 to F2, A3 to F3, A4 to F4 and A5 to F5.
3. Add 10 µL of the PC to wells G1 to G5.
4. Add 10 µL of the extracted NC to wells H1 to H5.

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

PP Mix and Thermocycler Detection Settings							
	Master Mix					Dye	Detection Wavelength (nm)[a]
	FluRhino	COR	ParaEAV	BoMpPf1	RsEPA		
Pathogen	IAV	HCoV 229E	HPIV-3	HPIV-1	HRSV A and B	green	520
	HRV	HCoV NL63	HPIV-2	HMPV A and B	HPeV	yellow	550
	IBV	HCoV HKU1	HPIV-4	HBoV	EV	orange	610
	IAV (H1N1) swl	HCoV OC43	IC (EAV)	*M. pneumoniae*	HAdV	red	670

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

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

Completion of Run

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

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 Respiratory pathogens 21.

Table 5: FTD Respiratory pathogens 21 - Possible Results

PP Mix	Pathogen	Signal in Green Channel	Signal in Yellow Channel	Signal in Orange Channel	Signal in Red Channel
FluRhino	IAV	POS	—	—	—
	HRV	—	POS	—	—
	IBV	—	—	POS	—
	IAV (H1N1) swl	POS	—	—	POS
COR	HCoV 229E	POS	—	—	—
	HCoV NL63	—	POS	—	—
	HCoV HKU1	—	—	POS	—
	HCoV OC43	—	—	—	POS

[1] Specimens with a Ct above 35.

Table 5: FTD Respiratory pathogens 21 - Possible Results (Continued)

PP Mix	Pathogen	Signal in Green Channel	Signal in Yellow Channel	Signal in Orange Channel	Signal in Red Channel
ParaEAV	HPIV-3	POS	—	—	—
	HPIV-2	—	POS	—	—
	HPIV-4	—	—	POS	—
	IC (EAV)	—	—	—	POS
BoMpPf1	HPIV-1	POS	—	—	—
	HMPV A and B	—	POS	—	—
	HBoV	—	—	POS	—
	M. pneumoniae	—	—	—	POS
RsEPA	HRSV A and B	POS	—	—	—
	HPeV	—	POS	—	—
	EV	—	—	POS	—
	HAdV	—	—	—	POS

Legend: POS = Positive, Empty = Negative

ATTENTION:

According to results collected from FluRhino master mix:

- If only IAV is positive, patient is IAV positive.
- If IAV and IAV (H1N1) swl are positive, patient is IAV (H1N1) swl positive.

According to results collected from both FluRhino master mix and RsEPA master mix:

- If only HRV (FluRhino master mix) is positive, patient is HRV positive.
- If only EV (RsEPA master mix) is positive, patient is EV positive.
- If HRV and EV (FluRhino master mix and RsEPA master mix) are positive, patient has either a co-infection caused by both HRV and EV or a single EV infection[1],[2].

The results will be reported as 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.

[1] HRV non-specific amplification was observed in 2.27%. Refer to the caution information on the next page for details.

[2] The *In silico* analysis reported some EV species can be amplified by the HRV assay. Refer to *Performance Characteristics* for *Cross-Reactivity* under the *In Silico Analysis* section for more information.

For example, if a patient sample analyzed with the FluRhino master mix (see Table 5) displays an exponential fluorescence trace in the:

- Green channel – this sample contains a detectable load of IAV RNA and no/undetectable load of HRV, IBV, and IAV (H1N1) swl nucleic acid.
- Orange channel – this sample contains a detectable load of IBV RNA and no/undetectable load of IAV, HRV, and IAV (H1N1) swl nucleic acid.

It is also possible to have several exponential fluorescence traces in the same samples; according to the color of the signal, it will indicate the presence of nucleic acid from IAV, HRV, IBV, and/or IAV (H1N1) swl.

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

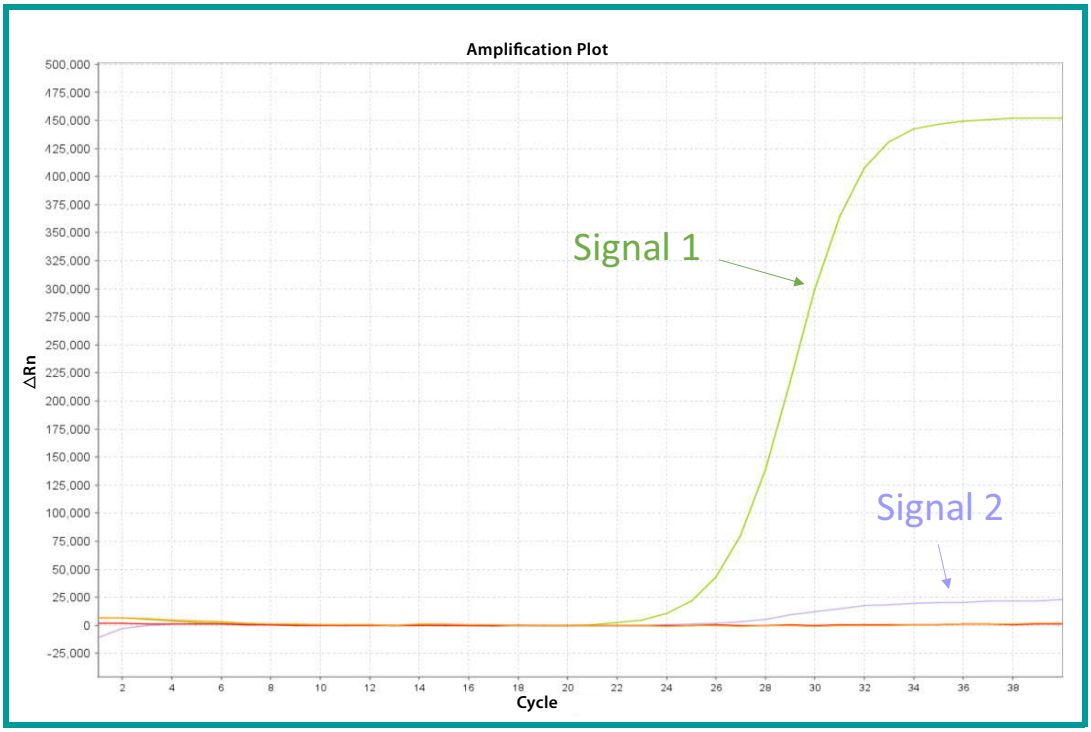
WARNING
IMPORTANT INFORMATION REGARDING FALSE POSITIVE RESULTS

In order to avoid a report of false positive results, pay attention to the following section:

1. **Crosstalk**

   It was noticed that in some cases a strong positive signal in one channel can lead to the appearance of a weaker non-specific signal in another channel (crosstalk). An illustration of this situation is provided in Figure 2: A high-positive signal in the green channel (signal 1, in green) can lead to a weaker signal in the yellow channel (signal 2, in purple). The fluorescence intensity and Ct value of signal 2 will always be lower than those of signal 1.

Figure 2: Illustration of Crosstalk



In case of a suspected crosstalk:

a. Check Table 6 as to whether your results belong to observed crosstalk using FTD Respiratory pathogens 21. If yes, follow steps b. to d.

b. The stronger signal should be considered as a real positive.

c. The weaker signal should be considered as inconclusive as a co-infection cannot be formally excluded.

d. An alternative test is required to confirm the status of the sample for the pathogen associated with the weaker signal.

Table 6 details potential crosstalk for the different PP mixes of FTD Respiratory pathogens 21.

Table 6: Potential Crosstalk Observed using FTD Respiratory pathogens 21

PP Mix	Green	Yellow	Orange	Red
FluRhino	**True positive (IAV)**	Potential crosstalk (HRV FP)		
COR				

Table 6: Potential Crosstalk Observed using FTD Respiratory pathogens 21 (Continued)

PP Mix	Green	Yellow	Orange	Red
ParaEAV		**True positive (HPIV-2)**	Potential crosstalk (HPIV-4 FP)	
BoMpPf1	**True positive (HPIV-1)**	Potential crosstalk (HMPV A and B FP)		
RsEPA	**True positive (HRSV A and B)**	Potential crosstalk (HPeV FP)		

Legend: FP = False Positive, Green text = strong positive signal to report as positive, Red text = potential crosstalk signal to disregard

2. **Non-Specific Low Positive Signals**

In house data revealed that some non-specific low positive signals may be randomly generated when testing samples. The non-specific signals are positive amplification signals with high cycle threshold (Ct) values (greater than 35). Table 7 displays the impacted pathogens and the occurrence rate of those signals (see *Performance Characteristics – Negative Material* section on page 32) together with the minimal Ct value observed internally as a reference. These Ct values are valid for use of this product in combination with the easyMAG® (bioMérieux) and Applied Biosystems®7500 (Thermo Fisher Scientific) instruments.

In case you observe a low positive result with a Ct value greater than 35:

a. Check Table 7 as to whether your results belong to the listed pathogens. If yes, follow steps b. to c.

b. Consider this result as inconclusive.

c. Retest the sample with an alternative method.

Table 7: Occurrence Rate and Cut-Off Ct Value for Non-Specific Low Positive Signals

PP Mix	Pathogen	Channel	Occurrence Rate	Minimum Ct
FluRhino	HRV	yellow	2.27%	36.6
	IAV (H1N1) swl	red	0.57%	38.2
COR	HCoV 229E	green	0.39%	37.6
	HCoV NL63	yellow	0.39%	38.5
	HCoV HKU1	orange	0.39%	39.8

Table 7: Occurrence Rate and Cut-Off Ct Value for Non-Specific Low Positive Signals (Continued)

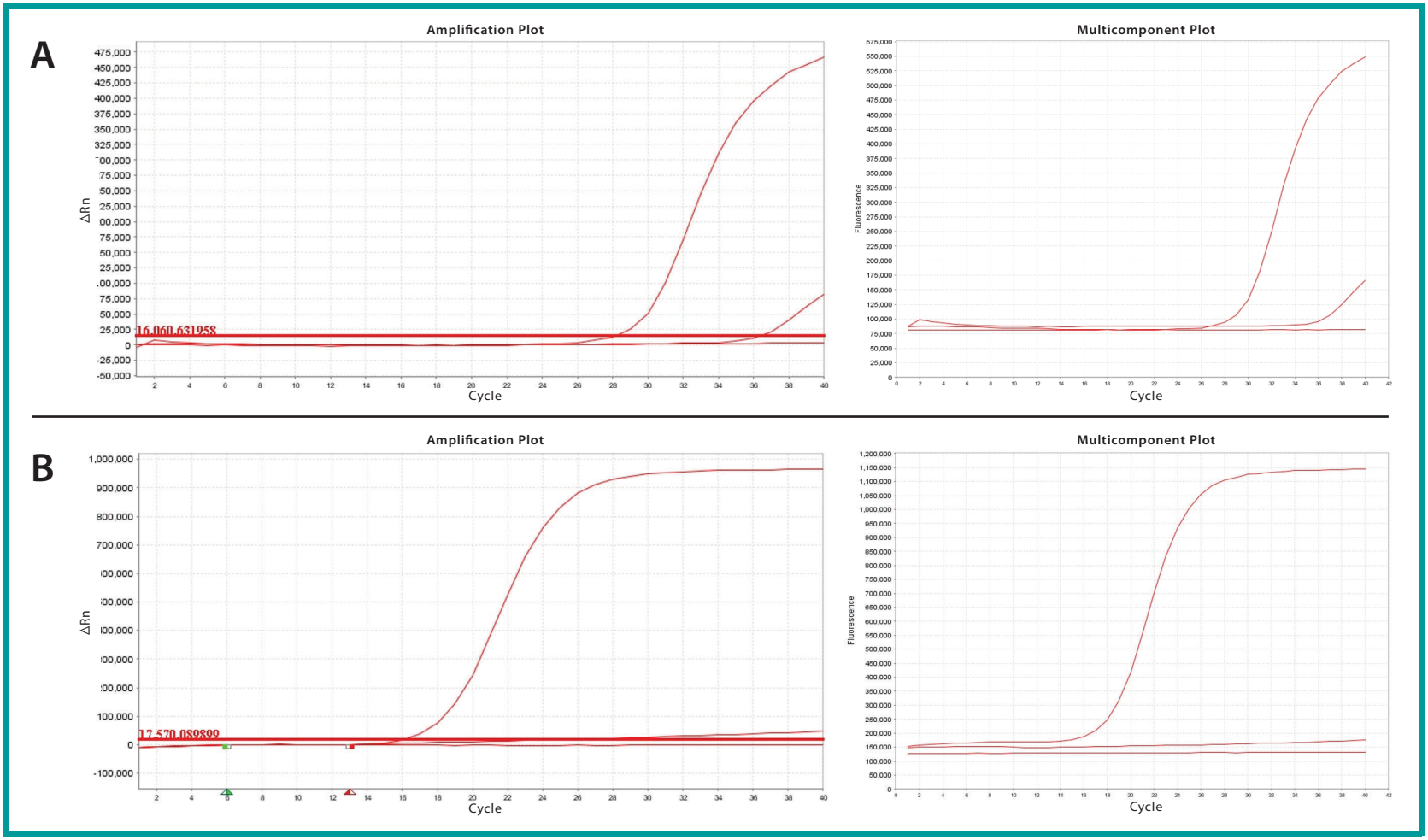
PP Mix	Pathogen	Channel	Occurrence Rate	Minimum Ct
BoMpPf1	HMPV A and B	yellow	0.78%	38.8
RsEPA	HRSV A and B	green	0.45%	39.0
	EV	orange	2.27%	37.6
	HAdV	red	4.76%	36.1

Legend: Ct = Cycle threshold value observed internally

3. 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 3 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 3: 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.
- The performance of this test has not been verified or validated in immunocompromised individuals or in patients without signs and symptoms of respiratory infection.
- The performance of this test has not been verified or validated in patients who received influenza vaccine.
- Other parameters can lead to false positive, negative or invalid results related to patient conditions (use of antiviral/bacterial therapy, patient age, patient history of respiratory infections, presence of symptoms and the stage of infection).
- Low levels of viruses/bacteria below the limit of detection might be detected. However, results may not be reproducible.
- This test is not a diagnostic test to confirm the presence of the pathogen and shall not be the only element 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.
- This test is not intended to replace any medical examination by a professional and results shall be interpreted in conjunction with other laboratory and clinical findings (*e.g.*, clinical history, epidemiological data or other data) available to the clinician examining the patient.
- Though infrequent, mutations within the highly conserved regions of the targets for viruses/bacteria detected by the kit may occur. As a consequence, primer and probe combinations may fail to detect the presence of this virus/bacterium.
- 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 the FTD Respiratory pathogens 21.

Analytical Sensitivity

The analytical sensitivity is the ability of the kit 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 “limit of detection” (LoD).[34]

The LoD of FTD Respiratory pathogens 21 was determined empirically by testing serial dilutions of quantified material. An exhaustive list of the products used for the analytical sensitivity study is documented in Table 8.

Table 8: Products Used for the LoD Determination of FTD Respiratory pathogens 21

Pathogens	Product	Supplier
IAV	AMPLIRUN® INFLUENZA A H1 RNA CONTROL	Vircell S.L.
HRV	AMPLIRUN® RHINOVIRUS RNA CONTROL	
IBV	AMPLIRUN® INFLUENZA B RNA CONTROL	
IAV (H1N1) swl	AMPLIRUN® NOVEL INFLUENZA A H1N1 RNA CONTROL	
HCoV 229E	Synthetic RNA	Bio-synthesis Inc.
HCoV NL63		
HCoV HKU1		
HCoV OC43		
HPIV-3	AMPLIRUN® PARAINFLUENZA 3 RNA CONTROL	Vircell S.L.
HPIV-2	AMPLIRUN® PARAINFLUENZA 2 RNA CONTROL	
HPIV-4	AMPLIRUN® PARAINFLUENZA 4 RNA CONTROL	
HPIV-1	AMPLIRUN® PARAINFLUENZA 1 RNA CONTROL	
HMPV	Synthetic RNA	Bio-synthesis Inc.
HBoV	Synthetic DNA	Eurofins

Table 8: Products Used for the LoD Determination of FTD Respiratory pathogens 21 (Continued)

Pathogens	Product	Supplier
M. pneumoniae	AMPLIRUN® MYCOPLASMA PNEUMONIAE DNA CONTROL	Vircell S.L.
HRSV A	AMPLIRUN® RESPIRATORY SYNCYTIAL VIRUS (subtype A) RNA CONTROL	
HRSV B	AMPLIRUN® RESPIRATORY SYNCYTIAL VIRUS (subtype B) RNA CONTROL	
HPeV	AMPLIRUN® PARECHOVIRUS 1 RNA CONTROL	
EV	AMPLIRUN® ENTEROVIRUS 68 RNA CONTROL	
HAdV	AMPLIRUN® ADENOVIRUS DNA CONTROL	

For determination of the pathogen specific analytical sensitivity, dilution series were performed with a minimum of 24 replicates per pathogen and dilution step. This analysis included the use of at least three different product lots, operators and instruments. Using Probit (PROBability unITS) Regression, the LoD for the respective pathogens were evaluated (see Table 9).

Table 9: Analytical Sensitivity of FTD Respiratory pathogens 21

PP Mix	Pathogen	Units	LoD from Probit Analysis	95% Confidence Interval
FluRhino	IAV	gen.cop/mL	2.8×10^4	$1.8 \times 10^4 – 4.0 \times 10^4$
	HRV	gen.cop/mL	7.6×10^4	$5.4 \times 10^4 – 9.2 \times 10^4$
	IBV	gen.cop/mL	9.8×10^2	$6.5 \times 10^2 – 1.3 \times 10^3$
	IAV (H1N1) swl (green)*	gen.cop/mL	1.9×10^4	$1.1 \times 10^4 – 2.6 \times 10^4$
	IAV (H1N1) swl (red)*	gen.cop/mL	5.9×10^4	$3.1 \times 10^4 – 8.6 \times 10^4$
COR	HCoV 229E	cop/mL	1.6×10^4	$1.3 \times 10^4 – 2.0 \times 10^4$
	HCoV NL63	cop/mL	1.9×10^4	$1.4 \times 10^4 – 2.4 \times 10^4$
	HCoV HKU1	cop/mL	4.9×10^4	$3.0 \times 10^4 – 6.8 \times 10^4$
	HCoV OC43	cop/mL	2.9×10^4	$2.1 \times 10^4 – 3.6 \times 10^4$
ParaEAV	HPIV-3	gen.cop/mL	2.7×10^4	$1.5 \times 10^4 – 3.8 \times 10^4$
	HPIV-2	gen.cop/mL	5.6×10^4	$3.2 \times 10^4 – 8.5 \times 10^4$
	HPIV-4	gen.cop/mL	9.9×10^4	$5.6 \times 10^3 – 4.0 \times 10^5$

Table 9: Analytical Sensitivity of FTD Respiratory pathogens 21 (Continued)

PP Mix	Pathogen	Units	LoD from Probit Analysis	95% Confidence Interval
BoMpPf1	HPIV-1	gen.cop/mL	5.8×10^3	$4.6 \times 10^3 – 7.1 \times 10^3$
	HMPV	cop/mL	1.4×10^4	$1.1 \times 10^4 – 1.8 \times 10^4$
	HBoV	cop/mL	1.6×10^4	$1.2 \times 10^4 – 2.1 \times 10^4$
	M. pneumoniae	gen.cop/mL	3.7×10^3	$2.9 \times 10^3 – 4.8 \times 10^3$
RsEPA	HRSV A	gen.cop/mL	1.0×10^3	$6.9 \times 10^2 – 1.4 \times 10^3$
	HRSV B	gen.cop/mL	1.2×10^4	$8.0 \times 10^3 – 1.5 \times 10^4$
	HPeV	gen.cop/mL	4.1×10^4	$3.2 \times 10^4 – 5.1 \times 10^4$
	EV	gen.cop/mL	1.4×10^5	$1.0 \times 10^5 – 1.9 \times 10^5$
	HAdV	gen.cop/mL	1.5×10^5	$1.2 \times 10^5 – 1.9 \times 10^5$

Legend: PP = primer/probe, gen.cop/mL = genome copies per milliliter, cop/mL = copies per milliliter, * = IAV (H1N1) swl is targeted by two assays: one targeting all IAV (green) and one specific for the IAV (H1N1) swl (red). The LoD of IAV (H1N1) swl detected by the IAV assay is lower than the LoD of IAV (H1N1) swl detected by the IAV (H1N1) swl specific assay

Results of the Probit analysis were further confirmed by testing 24 replicates at a concentration equivalent to the upper confidence limit provided in Table 9. This LoD confirmation study was performed using different instruments, operators and reagent lots. All pathogens demonstrated a detection rate superior to 95%.

Analytical Specificity

The analytical specificity of a PCR multiplex assay is the ability of a measurement to measure solely the measurand.[34] The analytical specificity is referred to:

- 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 Respiratory pathogens 21 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 Respiratory pathogens 21 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 in the complete 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 10. Data does not show any cross-reactivity for FTD Respiratory pathogens 21, except for HRV.

Table 10: Cross-Reactivity of FTD Respiratory pathogens 21

Pathogens	Potential Cross-Reactivity
enterovirus	No potential cross-reactivity
human adenovirus	No potential cross-reactivity
human bocavirus	No potential cross-reactivity
human coronavirus 229E	No potential cross-reactivity
human coronavirus HKU1	No potential cross-reactivity
human coronavirus NL63	No potential cross-reactivity
human coronavirus OC43	No potential cross-reactivity
human metapneumoviruses	No potential cross-reactivity
human parainfluenza 1 virus	No potential cross-reactivity
human parainfluenza 2 virus	No potential cross-reactivity
human parainfluenza 3 virus	No potential cross-reactivity
human parainfluenza 4 virus	No potential cross-reactivity
human parechovirus	No potential cross-reactivity
human respiratory syncytial viruses	No potential cross-reactivity
human rhinovirus	Potential cross-reactivity with other enterovirus species[a]
influenza A virus	No potential cross-reactivity
influenza A virus H1N1 swl	No potential cross-reactivity
influenza B virus	No potential cross-reactivity
mycoplasma pneumoniae	No potential cross-reactivity

[a] Human rhinovirus belongs to the enterovirus genus.

In Vitro Analysis

FTD Respiratory pathogens 21 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. Each pool was spiked with a maximum of five pathogens. The pools were extracted using NucliSENS® easyMAG® and evaluated in triplicate on the Applied Biosystems® 7500 (Thermo Fisher Scientific).

The list of the pathogens tested is displayed in Table 11 along with the culture information and the results of the analysis. No unspecific signal was detected.

Table 11: Cross-Specificity Panel Tested with FTD Respiratory pathogens 21

Target	Provider and Catalog Number	Stock Concentration	Units	Results
Bacteroides fragilis	ATCC-25285	9.11E+06	CFU/vial	Not detected
BK virus	ATCC-VR-837	5.00E+03	TCID50/mL	Not detected
Campylobacter jejuni; subsp. Jejuni NCTC11168	ATCC-700819	1.00E+04	CFU/vial	Not detected
Candida albicans	ATCC-14053	2.09E+08	CFU/vial	Not detected
Candida parapsilosis	ATCC-22019	1.80E+08	CFU/vial	Not detected
Candida tropicalis	ATCC-750	3.50E+07	CFU/vial	Not detected
Chlamydia pneumoniae; J-21	ATCC-VR-1435	1.0E+4.75	TCID50/ 0.2mL	Not detected
Chlamydia trachomatis; Serovar B, Strain Har-36	ATCC-VR-573	>5 10E+03	TCID50/mL	Not detected
Clostridium perfringens; Strain S 107	ATCC-13124	1.60E+06	CFU/vial	Not detected
Cytomegalovirus	ATCC-VR-1788	2.34E+05	TCID50/mL	Not detected
Enterobacter aerogenes	ATCC-13048	1.84E+07	CFU/vial	Not detected
Enterobacter cloacae	ATCC-13047	1.00E+06	CFU/vial	Not detected
Enterococcus faecalis	ATCC-19433	1.70E+09	CFU/vial	Not detected
Enterococcus faecium; Strain NCTC 7171	ATCC-19434	3.30E+09	CFU/vial	Not detected
Epstein Barr Virus; Strain: B95-8	ATCC-VR-1492	5.00E+03	TCID50/mL	Not detected
Helicobacter pylori	ATCC-43504	1.00E+04	CFU/vial	Not detected
Herpes Simplex Virus 1; MacIntyre	ATCC-VR-539	2.80E+07	TCID50/mL	Not detected
Herpes Simplex Virus 2; MS	ATCC-VR-540	1.58E+07	TCID50/mL	Not detected
Klebsiella oxytoca	ATCC-13182	1.00E+04	CFU/vial	Not detected
Lactobacillus crispatus; Strain VPI 3199	ATCC-33820	>1.00E+04	CFU/vial	Not detected
Lactobacillus gasseri; DSM 20243 [63AM]	ATCC-33323	>1.00E+04	CFU/vial	Not detected
Lactobacillus iners; Strain AB107	ATCC-55195	>1.00E+04	CFU/vial	Not detected

Table 11: Cross-Specificity Panel Tested with FTD Respiratory pathogens 21 (Continued)

Target	Provider and Catalog Number	Stock Concentration	Units	Results
Lactobacillus jensenii	ATCC-25258	1.00E+04	CFU/vial	Not detected
Legionella pneumoniae	ATCC-33152	1.00E+04	CFU/vial	Not detected
Leishmania donovani Khartoum	ATCC-30030	1.54E+07	cells/mL	Not detected
Leptospira alexanderi	ATCC-700520	Not Available	Not Available	Not detected
Listeria monocytogenes	ATCC-15313	1.00E+04	CFU/vial	Not detected
Measles Virus (Rubeola) Schwartz, Genotype A	PHE-0809212v	3.20E+02	pfu/mL	Not detected
Mumps virus	ATCC-VR-106	1.60E+05	TCID50/mL	Not detected
Mycoplasma genitalium G37	ATCC-33530	1.00E+11	gen.cop/mL	Not detected
Mycoplasma hominis	ATCC-33131	4.80E+08	CFU/vial	Not detected
Neisseria elongata; subsp. Glycolytica 6171/75	ATCC-29315	1.00E+04	CFU/vial	Not detected
Neisseria gonorrhoeae; FA1019	ATCC-700825	1.00E+04	CFU/vial	Not detected
Neisseria lactamica	ATCC-23970	1.00E+03	CFU/vial	Not detected
Neisseria meningitis; M1027	ATCC-53417	1.95E+09	gen.cop/µL	Not detected
Neisseria mucosa	ATCC-19696	1.00E+04	CFU/vial	Not detected
Neisseria subflava; CDN-17 [NRL 30, 017, h231]	ATCC-49275	1.00E+04	CFU/vial	Not detected
Pseudomonas aeruginosa	ATCC-10145	2.17E+09	CFU/vial	Not detected
Serratia marcescens	ATCC-13880	8.90E+06	CFU/vial	Not detected
Streptococcus agalactiae	ATCC-13813	3.40E+08	cells/mL	Not detected
Treponema pallidum	ATCC-BAA-2642SD	>1.00E+05	gen.cop/µL	Not detected
Ureaplasma parvum	ATCC-27815	1.00E+08	CFU/vial	Not detected
Ureaplasma urealyticum	ATCC-27618	1.00E+09	CFU/vial	Not detected
Varicella-zoster virus; Ellen	ATCC-VR-1367	9.20E+03	TCID50/mL	Not detected

Legend: CFU = colony-forming unit, gen.cop/mL = genome copies per milliliter, gen.cop/µL = genome copies per microliter, TCID50 = Median Tissue Culture Infection Dose, pfu = plaque-forming unit

Negative Material

Negative material (extracted negative controls, extracted negative clinical samples or nuclease-free water) has been tested *in vitro* in order to evaluate the occurrence of potential non-specific amplification when using FTD Respiratory pathogens 21. The derived analytical specificity of each pathogen is displayed in Table 12. Overall, an analytical specificity of 99.3% was reached.

Table 12: Analytical Specificity of FTD Respiratory pathogens 21

PP Mix	Pathogens	Number of Positive Results	Total Reactions	Analytical Specificity %	Confidence Interval %
FluRhino	IAV	0	348	100	98.9–100
	HRV	8	352	97.7	95.6–99.0
	IBV	0	352	100	99.0–100
	IAV (H1N1) swl	2	350	99.4	98.0–99.9
COR	HCoV 229E	1	255	99.6	97.8–100
	HCoV NL63	1	257	99.6	97.8–100
	HCoV HKU1	1	257	99.6	97.9–100
	HCoV OC43	0	257	100	98.6–100
ParaEAV	HPIV-3	0	213	100	98.3–100
	HPIV-2	0	224	100	98.4–100
	HPIV-4	0	224	100	98.4–100
BoMpPf1	HPIV-1	0	257	100	98.6–100
	HBoV	0	253	100	98.6–100
	M. pneumoniae	0	257	100	98.6–100
	HMPV	2	257	99.2	97.2–99.9
RsEPA	HRSV A/B	2	441	99.5	98.4–99.9
	HPeV	0	440	100	99.2–100
	EV	10	440	97.7	95.9–98.9
	HAdV	21	441	95.2	92.8–97.0
				99.3	**Total***

*Total specificity is calculated as the average of the analytical specificity observed for each single pathogen.

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. Specific primers and probes were then mapped to the sequences 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, most of the time an imperfect match still produces pairing and amplification.

The results of the inclusivity analysis are summarized in Table 13.

Table 13: Inclusivity of FTD Respiratory pathogens 21

Pathogens	Subtypes Detected
enterovirus	Species A–D
human adenovirus	All seven species of HAdV (A–G)
human bocavirus	HBoV subtype 1
human coronavirus 229E	No subtypes defined
human coronavirus HKU1	
human coronavirus NL63	
human coronavirus OC43	
human metapneumoviruses	Genotypes A and B
human parainfluenza 1 virus	No subtypes defined
human parainfluenza 2 virus	
human parainfluenza 3 virus	
human parainfluenza 4 virus	
human parechovirus	Subtypes 1–8, 10, 14 and 16–18
human respiratory syncytial viruses	Subtypes A and B
human rhinovirus	Species HRV A, B and C
influenza A virus	Serotypes H1–H16
influenza A virus H1N1 swl	2009-pandemic swine-lineage influenza A virus subtype H1N1[a]
influenza B virus	Yamagata and Victoria lineages
mycoplasma pneumoniae	Subtypes 1 and 2

[a] Since 2016, two recurrent mismatches in the probe have become more and more frequent in sequences for H1N1p2009. The analyzed sequences from 2018 and 2019 found these two mismatches being present in 97.1% of the cases and it could lead to a reduced sensitivity.

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 Respiratory pathogens 21 precision was assessed by repeatability and reproducibility studies with test material at a concentration close to LoD (see Table 14) and high positive test material (see Table 15). Repeatability evaluates measurements carried out under the same conditions (intra-assay variation), whereas reproducibility evaluates results of measurements under changed conditions (inter-lot variation and inter-assay variation). The precision of each study was expressed based on statistical measurements of imprecision (standard deviation and coefficient of variation).

Repeatability studies (intra-assay variation) assess the precision of data collected between tests carried under the same conditions (*i.e.*, one reagent lot, one operator, one cycler). Commercial quantified samples for each pathogen were tested at a concentration close to the limit of detection with FTD Respiratory pathogens 21 on the Applied Biosystems® 7500 (Thermo Fisher Scientific).

At least 24 results were collected for each pathogen. Results were collected over multiple runs with N equaling eight associated with each run. The values in Table 14 only present repeatability (within-run) error and do not characterize a run to run error.

Table 14: Repeatability Study for FTD Respiratory pathogens 21

Pathogen	dF	SD	95% LL SD	95% UL SD	CV	95% LL CV	95% UL CV
IAV	28	0.69	0.55	0.94	2.0%	1.6%	2.7%
HRV	21	0.59	0.45	0.84	1.7%	1.3%	2.4%
IBV	28	0.73	0.58	0.99	2.1%	1.6%	2.8%
IAV (H1N1) swl	21	0.37	0.28	0.52	1.1%	0.8%	1.6%
HCoV 229E	35	0.68	0.55	0.89	1.9%	1.5%	2.5%
HCoV NL63	21	0.25	0.19	0.35	0.7%	0.6%	1.0%
HCoV HKU1	21	0.59	0.45	0.84	1.8%	1.4%	2.5%
HCoV OC43	21	0.56	0.43	0.80	1.6%	1.3%	2.3%
HPIV-3	35	0.43	0.35	0.56	1.3%	1.0%	1.7%
HPIV-2	21	0.27	0.21	0.38	0.8%	0.6%	1.1%
HPIV-4	28	0.56	0.44	0.75	1.5%	1.2%	2.1%
HPIV-1	35	0.56	0.46	0.74	1.6%	1.3%	2.1%
HumpA	35	0.45	0.36	0.58	1.2%	1.0%	1.6%
HBoV	28	0.46	0.37	0.63	1.4%	1.1%	1.9%
M. pneumoniae	28	0.56	0.44	0.75	1.6%	1.3%	2.2%
HRSV A	35	0.69	0.56	0.89	1.9%	1.5%	2.5%
HRSV B	21	0.38	0.29	0.54	1.1%	0.8%	1.6%

Table 14: Repeatability Study for FTD Respiratory pathogens 21 (Continued)

Pathogen	dF	SD	95% LL SD	95% UL SD	CV	95% LL CV	95% UL CV
HPeV	21	0.71	0.55	1.02	2.1%	1.6%	3.0%
EV	21	0.35	0.27	0.51	1.1%	0.8%	1.5%
HAdV	21	0.53	0.41	0.76	1.6%	1.2%	2.2%

Legend: dF = Degrees of Freedom (the effective sample size is calculated as one less than the sample size per run, multiplied by the number of runs for a given pathogen), SD = standard deviation, LL SD = Lower limit standard deviation, UL SD = Upper limit standard deviation, CV = Coefficient of variation, LL CV = Lower limit coefficient of variation, UL CV = Upper limit coefficient of variation

Data demonstrated a repeatability imprecision between 0.7% to 2.1% when testing sample close to the limit of detection.

Reproducibility studies assess the precision of the data collected between independent tests obtained under different conditions (*i.e.*, time, operator and/or product lots). Reproducibility study (inter-assay variation) was performed on panels of synthetic nucleic acids for each pathogen tested with two different lots of FTD Respiratory pathogens 21 on different Applied Biosystems® 7500 systems (Thermo Fisher Scientific) by different operators.

For each pathogen, two individual runs were performed. Each run included three replicates of the measurement and were performed by different operators, using different lots of reagents. The reproducibility values in Table 15 represent the variability of the combination of different lots, operators and instruments used in the study.

Additionally, values representing repeatability error between the results collected during this study are displayed in Table 15.

Table 15: Reproducibility Study for FTD Respiratory pathogens 21

Pathogen	n	Repeatability SD	Reproducibility SD	Total SD	Repeatability CV	Reproducibility CV	Total CV
IAV	6	*	*	0.1	*	*	0.0%
HRV	6	0.1	0.1	0.1	0.2%	0.4%	0.5%
IBV	6	*	*	0.2	*	*	0.0%
IAV (H1N1) swl	6	0.0	0.1	0.1	0.2%	0.3%	0.4%
HCoV 229E	6	0.1	0.4	0.4	0.2%	1.3%	1.3%
HCoV NL63	6	0.0	0.9	0.9	0.2%	3.2%	3.3%
HCoV HKU1	6	0.1	0.2	0.2	0.4%	0.7%	0.8%
HCoV OC43	6	0.1	0.5	0.5	0.3%	1.8%	1.9%

Table 15: Reproducibility Study for FTD Respiratory pathogens 21 (Continued)

Pathogen	n	Repeatability SD	Reproducibility SD	Total SD	Repeatability CV	Reproducibility CV	Total CV
HPIV-3	6	0.1	0.1	0.1	0.2%	0.2%	0.3%
HPIV-2	6	0.0	1.1	1.1	0.2%	4.4%	4.4%
HPIV-4	6	0.1	0.1	0.1	0.2%	0.2%	0.3%
HPIV-1	6	0.1	0.6	0.6	0.4%	2.3%	2.3%
HMPV	6	0.1	0.4	0.4	0.5%	1.4%	1.4%
HBoV	6	0.2	0.4	0.4	0.6%	1.5%	1.6%
M. pneumoniae	6	0.1	0.3	0.3	0.3%	1.0%	1.0%
HRSV	6	0.1	0.1	0.1	0.2%	0.4%	0.5%
HPeV	6	0.1	0.6	0.6	0.4%	2.3%	2.3%
EV	6	0.0	0.1	0.1	0.2%	0.4%	0.4%
HAdV	6	0.0	0.9	0.9	0.2%	3.4%	3.4%

Legend: n = total sample size, SD = standard deviation, CV = Coefficient of variation, * = repeatability imprecision equals zero

Data demonstrated a repeatability imprecision between 0.2% to 0.6% and a reproducibility imprecision between 0.2% to 4.4% when testing high positive material.

Interfering Substances

An interference study was conducted to evaluate the susceptibility of FTD Respiratory pathogens 21 to provide erroneous results in presence of potential interfering substances in the clinical sample. Artificial nasopharyngeal swab medium was spiked with all pathogens targeted within one PP mix at low concentrations and then aliquoted. Each tube was further spiked with an interfering substance, the solvent or left untreated. The pools were extracted using NucliSENS® easyMAG® and evaluated using technical triplicates and one lot of FTD Respiratory pathogens 21 on the Applied Biosystems® 7500 (Thermo Fisher Scientific). The list of the tested substances and potential interference are documented in Table 16.

Table 16: Potential Interfering Substance Involved

Substance	Name	Source	Tested Concentration	Results
IS-1	Whole blood	Biomex	10% v/v	Moderate interference
IS-2	Mucin (bovine)	Sigma	60 µg/mL	No interference
IS-3	Salbutamol	Sigma	1.7 µmol/L	No interference
IS-4	Nasal spray (Xylo)	Ratiopharm	10% v/v	No interference
IS-5	Nasal spray (NaCl)	Emsa	10% v/v	No interference
IS-6	Guaifenesin	Sigma	15.2 mmol/L	No interference
IS-7	Acetylcystein	Sigma	920 µmol/L	No interference
IS-8	Nicotine	Sigma	6.2 µmol/L	No interference
IS-9	Benzocaine	Sigma	0.63 mg/mL	No interference
IS-10	Oseltamivir	Sigma	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

Data shows a moderate interference of blood demonstrated by a Ct shift varying from 1.07 to 1.97 Ct depending on the pathogen (data not shown).

Clinical Performance

A systematic literature review was performed to establish clinical claims from post-market data.

All publications reporting results of method comparison studies from 2017 to 2019 were selected and diagnostic sensitivity and specificity were calculated across all studies. These external performance studies tested nasopharyngeal swabs and other sample types (bronchoalveolar lavage, nasal and throat swabs, nasopharyngeal aspirates, nasopharyngeal washes) and compared the results to other PCR-based diagnostic methods. The selected studies were performed on different platforms, including NucliSENS® easyMAG® (bioMérieux) and the Applied Biosystems® 7500 (Thermo Fisher Scientific). An overall diagnostic sensitivity for FTD Respiratory pathogens 21 of 93.94% and an overall diagnostic specificity of 98.93% was reached (see Table 17).

In addition, External Quality Assessment (EQA) tests performed annually at Fast Track Diagnostics from 2017 to 2019 supported the performance characteristics.

Table 17: Diagnostic Sensitivity and Specificity Obtained for Pathogens Detected by FTD Respiratory pathogens 21 Across Four Selected Studies[35-38] (Confirmed by PPA and NPA from EQA Tests)

Pathogen	Diagnostic Sensitivity in % (CI)	EQA PPA in % (CI)	Diagnostic Specificity in % (CI)	EQA NPA in % (CI)
HAdV	**86.32** (77.74–92.51)	100 (82.35–100)	**96.67** (93.54–98.55)	100 (2.5–100)
HMPV	**83.33** (51.59–97.91)	100 (83.89–100)	**98.46** (96.45–99.5)	66.7 (9.43–99.16)
IAV	**97.85** (95.63–99.13)	100 (69.15–100)	**99.6** (98.82–99.92)	100 (69.15–100)
IAV (H1N1) swl	**100** (97.05–100)	100 (29.24–100)	**99.34** (98.32–99.82)	100 (80.49–100)
IBV	**98.27** (95.02–99.64)	100 (63.06–100)	**98.87** (97.93–99.46)	100 (73.54–100)
HPIV-1	**77.78** (52.36–93.59)	100 (54.07–100)	**99.68** (98.24–99.99)	100 (84.56–100)
HPIV-2	**88.89** (51.75–99.72)	90 (55.5–99.75)	**99.69** (98.28–99.99)	100 (81.47–100)
HPIV-3	**80.77** (60.65–93.45)	100 (47.82–100)	**98.06** (95.82–99.28)	100 (85.18–100)
HPIV-4	**25** (0.63–80.59)[a]	100 (29.24–100)	**96.33** (92.9–98.4)	100 (86.28–100)
HRSV	**97.44** (92.69–99.47)	100 (81.47–100)	**99.58** (98.93–99.89)	100 (15.81–100)
EV	**62.5** (24.49–91.48)	100 (87.23–100)	**99.08** (94.99–99.98)	100 (29.24–100)
HRV	**88.64** (80.09–94.41)	100 (85.75–100)	**95.33** (91.99–97.56)	83.3 (35.88–99.58)
HPeV[b]	*96.2* (81.03–99.91)	96.2 (81.03–99.91)	*100* (29.24–100)	100 (29.24–100)
HBoV	**87.5** (47.35–99.68)	Not Available	**95.58** (89.98–98.55)	Not Available
HCoV 229E	**100** (69.15–100)	100 (54.07–100)	**99.69** (98.3–99.99)	100 (84.56–100)
HCoV OC43	**64.71** (38.33–85.79)[c]	100 (63.06–100)	**99.37** (97.73–99.92)	100 (83.16–100)
HCoV NL63	**91.67** (61.52–99.79)	100 (66.37–100)	**100** (98.87–100)	100 (82.35–100)
HCoV HKU1	**66.67** (9.43–99.16)[d]	100 (15.81–100)	**100** (98.88–100)	100 (87.23–100)
M. pneumoniae	**85.71** (42.13–99.64)	100 (73.54–100)	**98.18** (93.59–99.78)	100 (29.24–100)
Overall	**93.94** (92.33–95.3)		**98.93** (98.65–99.16)	

Legend: CI = 95% Confidence Interval

[a] Low sample size and low prevalence of pathogen.

[b] Values obtained exclusively from EQA tests because HPeV was not covered by the published studies due to low prevalence.

[c] A PPA of 92.9% was obtained in the study by Sanz, *et al.*[39]

[d] Low sample size and low prevalence of pathogen, 100% PPA in the study by Sanz, *et al.*[39]

Troubleshooting

Table 18 describes a non-exhaustive list of control errors that a user may observe with FTD Respiratory pathogens 21 and suggested corrective actions.

Table 18: 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 controls last. • Decontaminate the work space and instruments after each use.

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

Technical Assistance

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

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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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