

# Prodesse<sup>®</sup> Pro hMPV<sup>™</sup>+

## Instructions for Use

For detection of human Metapneumovirus

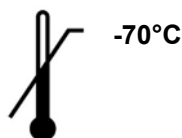


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## Intended Use

The Pro hMPV™+ Assay is a Real-Time PCR (RT-PCR) *in vitro* diagnostic test for the qualitative detection of human Metapneumovirus (hMPV) nucleic acid isolated and purified from nasopharyngeal swab (NP) specimens obtained from individuals exhibiting signs and symptoms of acute respiratory infection. This assay targets a highly conserved region of the Nucleocapsid gene of hMPV. The detection of hMPV nucleic acid from symptomatic patients aids in the diagnosis of human respiratory hMPV infection if used in conjunction with other clinical and laboratory findings. This test is not intended to differentiate the four genetic sub-lineages of hMPV.

Negative results do not preclude hMPV infection and should not be used as the sole basis for diagnosis, treatment or other management decisions.

## Summary and Explanation

Human Metapneumovirus (hMPV) causes acute respiratory illness. Patients infected with hMPV present symptoms of acute upper and/or lower respiratory tract infections and share the same nonspecific symptoms as many other respiratory infectious agents. Coughing, rhinitis, fever, otitis media and dyspnea are the most common symptoms of hMPV. There are two distinct genetic lineages that have been established for hMPV and are designated as subtypes A and B. These lineages have further been divided into subgroups A1, A2, B1 and B2, as determined by performing phylogenetic analysis of sequence data, most often utilizing the fusion protein and G glycoprotein gene. Although information on the mode of transmission and virulence is not definitive, hMPV is likely spread by similar means as common respiratory viruses such as influenza. hMPV has been shown to co-infect with Respiratory Syncytial Virus (up to 30% rate)<sup>1</sup>. hMPV appears to provide only partial immunity following infection and can re-infect individuals potentially leading to repeated episodes of illness. Infections occur mainly during winter months. The prevalence of each subtype of hMPV varies, seemingly both from year to year, and by location. Likewise, the overall incidence of hMPV can vary from year to year and its prevalence has been reported to range from 6.6 to 12% in patients with symptoms of respiratory infection<sup>1</sup>. hMPV is responsible for a significant portion of the 150,000 children hospitalized annually in the United States for bronchiolitis<sup>2</sup>.

## Principles of the Procedure

The Pro hMPV+ Assay enables detection and differentiation of hMPV and Internal Control. An overview of the procedure is as follows:

1. Nasopharyngeal swab specimens are collected from patients with signs and symptoms of respiratory infection using a polyester, nylon or rayon tipped swab and placed into viral transport medium (refer to **Materials Required but not Provided**).
2. An Internal Control (IC) is added to every sample and is carried through all steps of the procedure from nucleic acid extraction through amplification and detection to monitor for inhibitors present in the specimens.
3. Extraction and purification of nucleic acids are performed using either a MagNA Pure LC System (Roche) and the MagNA Pure Total Nucleic Acid Isolation Kit (Roche) or a NucliSENS easyMAG system (bioMérieux) and the Automated Magnetic Extraction Reagents (bioMérieux).
4. The extracted and purified nucleic acids are added to hMPV Mix II along with enzymes included in the kit. The hMPV Mix II contains oligonucleotide primers complementary to a highly conserved region of the Nucleocapsid gene of hMPV and target-specific oligonucleotide probes dual-labeled with a reporter dye attached to the 5'-end and a quencher dye attached to the 3'-end.
5. After initial reverse transcription of RNA into complementary DNA (cDNA), amplification proceeds during which the probe anneals specifically to a region of the template between the forward and reverse primers. As primer extension and amplification occur, the 5' – 3' exonuclease activity of the Taq polymerase cleaves the probe separating the reporter dye from the quencher. This generates an increase in fluorescent signal upon excitation from an LED light source of appropriate wavelength. With each cycle, additional reporter dye molecules are cleaved from their respective probes, further increasing fluorescent intensity. The amount of fluorescence at any given cycle is dependent on the amount of amplification products present at that time. Fluorescent intensity is monitored during each PCR cycle by the real-time instrument.

Analyte	Gene Targeted	Probe Fluorophore	Absorbance Peak	Emission Peak	Instrument Channel
human Metapneumovirus	Nucleocapsid	FAM	495 nm	520 nm	FAM
Internal Control	NA	Quasar 670	647 nm	667 nm	Cy5

**Materials Provided**
**Pro hMPV+ Assay Kit (Cat. # H27VK00)**

Reagents	Description	Quantity/ Tube	Cap Color	Cat. #	Reactions/ Tube
hMPV Mix II	<ul style="list-style-type: none"> <li>⇒ Taq DNA polymerase</li> <li>⇒ 2 oligonucleotide primer pairs</li> <li>⇒ 2 oligonucleotide probes</li> <li>⇒ Buffer containing dNTPs (dATP, dCTP, dGTP, dTTP),</li> <li>⇒ MgCl<sub>2</sub> and stabilizers</li> </ul>	1030 µL	Brown	HSM47	50 (2 tubes provided)
M-MLV Reverse Transcriptase II	<ul style="list-style-type: none"> <li>⇒ 11.4 U/µL</li> </ul>	36 µL	White	GLS32	100
RNase Inhibitor II	<ul style="list-style-type: none"> <li>⇒ 40 U/µL</li> </ul>	120 µL	Green	GLS33	100
Positive Control (PC) - hMPV RNA Control III	<ul style="list-style-type: none"> <li>⇒ Non-infectious <i>in vitro</i> transcribed RNA hMPV specific viral sequences</li> </ul>	500 µL	Purple	HCT47	25
Internal Control (IC) - Internal RNA Control III	<ul style="list-style-type: none"> <li>⇒ Non-infectious <i>in vitro</i> transcribed RNA</li> </ul>	30 µL	Yellow	GCT12	100

**Materials Required But Not Provided**
**Plasticware and consumables**

- Polyester, rayon or nylon tipped nasopharyngeal swabs
- RNase/DNase-free 1.5 mL polypropylene microcentrifuge tubes
- Sterile RNase/DNase-free filter or positive displacement micropipettor tips
- MagNA Pure LC System Disposables (Reagent Tubs, Reaction Tips, Tip Trays, Cartridges) or easyMAG System Disposables (Sample Strips and Tips)
- Biohit Pipette Tips for use with easyMAG System
- Greiner Break Four uncoated plates for use with easyMAG System
- Cepheid PCR reaction tubes, 25 µL
- Parafilm M or MagNA Pure LC Cartridge Seals

**Reagents**

- Roche MagNA Pure LC Total Nucleic Acid Isolation Kit (*Roche Cat. # 03038505001*) for 192 isolations or bioMérieux NucliSENS easyMAG reagents (*Buffer 1 Cat. # 280130, Buffer 2 Cat. # 280131, Buffer 3 Cat. # 280132, Magnetic Silica Cat. # 280133, and Lysis Buffer Cat. # 280134*)
- Micro Test M4 Viral Transport Medium (*Remel, Inc. Cat. # R12500*), Micro Test M4RT Viral Transport Medium (*Remel, Inc. Cat. # R12505*), Micro Test M5 Viral Transport Medium (*Remel, Inc. Cat. # R12515*), Micro Test M6 Viral Transport Medium (*Remel, Inc. Cat. #12530*), BD Universal Viral Transport vial, 3mL (*Becton, Dickinson and Co. Cat. # 220220*) or Copan Universal Transport Medium (*Copan Diagnostics, Inc., Copan Cat. # 330C*)
- Molecular Grade Water (*RNase/DNase Free*)
- Extraction Control (*e.g. previously characterized positive sample or negative sample spiked with a well characterized hMPV strain*)

**Equipment**

- 70°C Freezer
- Roche MagNA Pure LC System with software version 3.0.11 or bioMérieux NucliSENS easyMAG System with Software version 1.0.1 or 2.0
- Biohit multi-channel pipettor for use with easyMAG System
- Cepheid SmartCycler II Real Time Instrument with Dx Software version 1.7b or 3.0a/3.0b
- Micropipettors (range between 1-10 µL, 10-200 µL and 100-1000 µL)
- Mini-centrifuge with adapter for Cepheid Reaction Tubes
- Cepheid cooling block
- Ice/Ice Bucket or -20°C Cold Block
- Biosafety Cabinet

**Warnings and Precautions**

- For In Vitro Diagnostic Use only.
- Use of this product should be limited to personnel who have been trained in the techniques of Real-Time PCR.
- Performance characteristics of this assay have only been determined with nasopharyngeal swab specimens.
- Specimens should be handled as if infectious using safe laboratory procedures such as those outlined in CDC/NIH *Biosafety in Microbiological and Biomedical Laboratories* and in the CLSI Document M29 *Protection of Laboratory Workers from Occupationally Acquired Infections*. Thoroughly clean and disinfect all surfaces with 10% bleach. Autoclave any equipment or materials that have contacted clinical specimens before discarding.
- Use pipettes with aerosol barrier or positive displacement tips for all procedures.
- Always pre-plan, organize and segregate workflow. Workflow in the laboratory should proceed in a unidirectional manner, beginning in the Pre-Amplification Area and moving to the Amplification/Detection Area.
  - Begin pre-amplification activities with reagent preparation and proceed to specimen preparation.
  - Always dedicate supplies and equipment to a specified area; no cross-movement allowed between areas.
  - Do not use equipment and supplies used for reagent preparation for specimen preparation activities or for pipeting or processing other sources of target nucleic acid.
  - Keep all amplification supplies and equipment in the Amplification/Detection Area at all times.
  - Always wear disposable gloves in each area and change them before entering a different area.
  - Do not open sample tubes following PCR.
- Take care to preserve the purity of kit reagents. Avoid contamination from Positive Control and specimens by following good laboratory practices.
- Do not use reagent after its expiration date.
- Do not mix reagents with different lot numbers or substitute reagents from other manufacturers.
- Material Safety Data Sheets (MSDS) are available on Gen-Probe Prodesse, Inc.'s website at [www.gen-probe.com](http://www.gen-probe.com) from the **Package Inserts** tab.

## Reagent Storage, Handling and Stability

- Store all reagents (opened and unopened) at  $\leq -70^{\circ}\text{C}$  until the expiration date listed on the kit.
- Always check the expiration date on the reagent tubes. For Intermediate stock of the Internal Control, use the expiration date of the originating stock control tube. Do not expose Controls to more than one (1) freeze-thaw cycle.
- Pro hMPV+ components are shipped frozen, should arrive frozen, and should be stored frozen after receipt. If the contents are not frozen, contact Customer Service for assistance.
- An internal study demonstrated that performance of hMPV Mix II, MMLV II and RNase Inhibitor II are not affected for up to 5 freeze-thaw cycles.
- Visually examine reagents for adequate reagent volume before beginning any test procedures.
- Protect hMPV Mix II from light.
- **Controls and aliquots of controls must be thawed and kept on ice at all times during preparation and use.**



*Aliquoting of kit components to maintain less than 5 freeze/thaw cycles is recommended for labs with smaller batch sizes.*

### Recommendation

## Specimen Collection, Handling and Storage

### Collecting the Specimen

To obtain nasopharyngeal swab samples:

1. Insert a flexible-shaft polyester, rayon or nylon tipped swab containing a dry tip into one nostril and into the nasopharyngeal area.
2. Press the swab gently against the nasal wall to allow the swab to absorb secretions.
3. Rotate the swab two to three times and withdraw it.
4. Place the swab into a tube containing 3 mL of viral transport medium (Remel M4, M4RT, M5, or M6; Becton Dickinson UVT; or Copan UTM).
5. Break off the shaft of the swab and cap the tube.



### Note

Using a smaller volume of the viral transport medium may result in inhibition.

### Transporting Specimens

Ensure that when transporting human respiratory specimens, all applicable regulations for the transport of etiologic agents are met. Transport human respiratory specimens refrigerated at  $2-8^{\circ}\text{C}$ .

### Storing Specimens

Store specimens refrigerated ( $2-8^{\circ}\text{C}$ ) for up to 72 hours before processing. Store any leftover specimens at  $\leq -70^{\circ}\text{C}$ . If retesting a frozen specimen, thaw specimen quickly (1 to 2 minutes) in a  $37^{\circ}\text{C}$  water bath and immediately place on ice or thaw specimen on ice.

### Storing Purified Nucleic Acid

Store purified nucleic acids at  $\leq -70^{\circ}\text{C}$ . They should be tested after no more than one (1) freeze-thaw cycle.



### Note

*Inadequate or inappropriate specimen collection, storage and transport are likely to yield false negative results.*



*Training in specimen collection is highly recommended because of the importance of specimen quality.*

Recommendation

## Reagent and Control Preparation

### Reagents



Note

*Prepare reagents from the Roche MagNA Pure LC Total Nucleic Acid Isolation Kit or the bioMérieux easyMAG Automated Magnetic Extraction Reagents following the manufacturer's instructions.*

### Controls



Recommendation

- ❖ *For aliquots of the Positive Control and Intermediate stock of the Internal Control, use the expiration date of the originating stock control tube.*
- ❖ *Controls and aliquots of controls must be thawed and kept on ice/cold block at all times during preparation and use. It is recommended to prepare controls in a sample prep area, such as a Biological Safety Cabinet.*

### Positive Control (PC)



Note

*Include the Positive Control with each RT-PCR run.*

1. Thaw Positive Control (the purple cap tube) on ice.
2. Make 25 aliquots of 20  $\mu\text{L}$ , label and store at  $\leq -70^{\circ}\text{C}$ . Ensure that aliquots do not undergo more than one (1) freeze-thaw cycle.
3. Dilute the Positive Control just prior to setup of the RT-PCR reaction (see [Step 4 \(a\)](#) of the [Assay Procedure](#)).



Recommendation

*Do not spike Positive Control with the Internal Control. Do not take Positive Control through the nucleic acid isolation procedure.*

### Internal Control (IC)

1. Thaw Internal Control (the yellow cap tube) on ice.
2. Create Intermediate stock tubes of the Internal Control using the following dilution scheme:
 
$$26 \mu\text{L Internal Control} + 65 \mu\text{L RNase Inhibitor} + \underset{\text{water}}{2509 \mu\text{L molecular grade}} = \underset{\text{volume}}{2600 \mu\text{L total}}$$
3. Make aliquots of 110  $\mu\text{L}$ , label, and store at  $\leq -70^{\circ}\text{C}$  (this is enough volume to add to 5 samples at 20  $\mu\text{L}$  per sample). Make aliquots of larger or smaller volumes based on the number of samples expected to be processed in a single run. Ensure that aliquots do not undergo more than one (1) freeze-thaw cycle.
4. Add the appropriate volume of Intermediate stock of the Internal Control to each sample prior to nucleic acid isolation (see [Step 1](#) of the [Assay Procedure](#)).
5. Save RNase Inhibitor II for use in [Step 4](#) of the [Assay Procedure](#).

### Negative Control (NC)

**Use Viral Transport Medium as the Negative Control.** Add the appropriate volume of Intermediate stock of the Internal Control to the Negative Control prior to nucleic acid isolation (see [Step 1](#) of the [Assay Procedure](#)).

**Extraction Control (EC)**

Good laboratory practice recommends including a positive extraction control (e.g. previously characterized positive sample or negative sample spiked with a well characterized hMPV strain) in each nucleic acid isolation run. The extraction control should be treated like a sample during assay performance and analysis.

**Assay Procedure**
**Assay Overview:**

**Get Ready: Create the Assay Protocol for the Cepheid SmartCycler instrument using the Dx Software (first time only).**

1. **Prepare the Samples and Negative Control.**
2. **Isolate the Nucleic Acid** – MagNA Pure LC System using the Total Nucleic Acid Isolation (TNAI) Kit  
OR
3. **Isolate the Nucleic Acid** – NucliSENS easyMAG System using the Automated Magnetic Extraction Reagents.
4. **Set up the RT-PCR Reaction.**
5. **Run the Pro hMPV+ Assay.**
6. **Print report.**


**Note**

- ❖ Instructions provided for the Cepheid SmartCyclerII Instrument with Dx Software version 1.7b and 3.0a/3.0b.
- ❖ Do NOT deviate from the protocol settings defined in this section.

**Get Ready: Create the Assay Protocol for the Cepheid SmartCycler instrument using the Dx Software (first time only)**


**Note**

- ❖ The protocol is only created for first time use; it does not need to be recreated with each sample run.
- ❖ Refer to SmartCycler Dx Software Operator Manual for assistance in defining assay protocols.
- ❖ To **Define** and **Edit** Assay protocols, the user must have administrative access rights. Otherwise, the fields will not be accessible for data entry and editing (they will be grayed out).
- ❖ Cepheid Dx Software interprets the data and reports the run as either **VALID** or **INVALID**, based on the results of the Negative Control. Enter the Positive Control and Extraction control as if they were samples.
- ❖ Interpret the control results and determine if the run is **VALID** or **INVALID**. All Control criteria must be met in order for the run to be **VALID** (see **Interpretation of Results** section).

**1. Create the Pro hMPV+ Assay protocol:**

- a. Launch the Cepheid Dx software application.
- b. Click on the **Define Assay** box at the top of the screen.
- c. Click on the **New Assay** box at the bottom of the screen.
- d. Enter **Pro hMPV+ Assay** for the assay protocol in the window that opens.
- e. Click **OK**.
- f. In the middle of the **Define Assay** screen, select **FTTC25** for the **Dye Set**.
- g. Select **NC fails if: Any target criterion is positive** (Dx 3.0a/3.0b: this is default setting in Control Settings Tab).
- h. Check the box to **Require Lot Number** (Dx 3.0a/3.0b: click on Advanced Tab and check Require Lot Number box).
- i. Deselect the box to **Use Patient IDs** (Dx 3.0a/3.0b: click on Advanced Tab, Use Patient IDs box is deselected by default).

- j. Enter **Thermocycler Parameter** in the Protocol section (bottom half of **Define Assay** screen).

Stage 1			Stage 2			Stage 3 Repeat 5 times			Stage 4 Repeat 45 times		
Hold			Hold			2- Temperature Cycle			2- Temperature Cycle		
Temp	Secs	Optics	Temp	Secs	Optics	Temp	Secs	Optics	Temp	Secs	Optics
42	1800	OFF	95	600	OFF	95	30	OFF	95	10	OFF
						55	60	ON	55	60	ON

Stages 5 – 10 remain UNUSED

2. Enter information in **Analysis Settings** tab as follows:



❖ Gray boxes are default settings.

Note

Channel	Dye Name	Target	Usage	Curve Analysis	Thresh Setting	Manual Thresh	Auto Thresh	Auto Min. Cycle	Auto Max. Cycle	Valid Min. Cycle	Valid Max. Cycle	Bkgnd Sub	Bkgnd Min. Cycle	Bkgnd Max. Cycle	Boxcar Avg	EndPt Thresh	NC IC %	IC Delta
1	FAM*	hMPV	Assay**	Primary Curve	Manual Threshold	30	NA	5	10	13	40	On	5	50	0	30	10†	NA
2	TET	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
3	TxR	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
4	Cy5*	Internal Control	Internal Control	Primary Curve	Manual Threshold	22	NA	5	10	17	40	On	5	50	0	22	10†	NA

\* If the Dye Names are incorrect, check that FTTC25 Dye Set is being used.

\*\* Dx 3.0a/3.0b = Target

† Dx 3.0a/3.0b = NA

3. Enter information in the **Control Settings** tab.

- ❖ Enter the Positive Control and/or the Extraction Control as a sample. Do not use the Positive Control Settings. Enter 0 Replicates to inactivate Positive Control PC1, PC2 and PC3. A 3079 error (Fluorescence Signal Too High) in the Positive Control invalidates the run, this is avoided if the Positive Control and/or the Extraction Control is entered as a sample and the results for each of the target channels are individually evaluated.



Note

- ❖ Use only one Negative Control (NC1). Enter 0 Replicates to inactivate the Negative Controls NC2 and NC3.
- ❖ Gray boxes are default settings.

Control ID	Control Name	Replicate	hMPV Valid Min Cycle	hMPV Valid Max Cycle	hMPV EndPt Thresh	IC +/-	IC Valid Min Cycle	IC Valid Max Cycle	IC EndPt Thresh
PC1	Pos Cntrl 1	0	13.0	45.0	10	+	13.0	45.0	10
PC2	Pos Cntrl 2	0	13.0	45.0	10	+	13.0	45.0	10
PC3	Pos Cntrl 3	0	13.0	45.0	10	+	13.0	45.0	10
NC1	Neg Cntrl 1	1	13.0	40.0	30	+	17.0	40.0	22
NC2	Neg Cntrl 2	0	13.0	45.0	10	+	13.0	45.0	10
NC3	Neg Cntrl 3	0	13.0	45.0	10	+	13.0	45.0	10

4. Confirm **Probe Check Settings** tab.


Note

The probe check is not used for any of the Pro hMPV+ Assay protocol.

Channel	Dye Name	Prb1 Min	Prb1 Max	Prb2 Min	Prb2 Max	Prb3 Min	Prb3 Max	Delta 12 Min	Delta 12 Max	Delta 23 Min	Delta 23 Max	Delta 13 Min	Delta 13 Max
1	FAM	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
2	TET	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
3	TxR	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
4	Cy5	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

 5. Select **Save Assay**.

### 1. Prepare the Samples, Extraction Control, and Negative Control (Pre-Amplification Area)

#### a. Add Internal Control to all samples.

- i. Thaw the appropriate number of aliquots of Intermediate stock of the Internal Control (enough volume needed for each sample, Extraction Control, and the Negative Control) on ice.
- ii. Remove 180  $\mu$ L of sample from the original sample tube and pipet into a labeled 1.5 mL microcentrifuge tube. Alternately, pipet 180  $\mu$ L of sample directly into sample cartridge or sample vessel.
- iii. Remove 180  $\mu$ L of Extraction Control from the original sample tube and pipet into a labeled 1.5 mL microcentrifuge tube. Alternately, pipet 180  $\mu$ L of sample directly into sample cartridge or sample vessel.
- iv. Add 20  $\mu$ L of Intermediate stock of the Internal Control to each sample. **Pipet up and down a minimum of 5 times to mix using a new pipet tip for each sample.**
- v. Keep tubes on ice.
- vi. Store any remaining sample at  $\leq -70^{\circ}\text{C}$ .

#### b. Add Internal Control to the Negative Control.

- i. Include one (1) Negative Control in each run.
- ii. Add 180  $\mu$ L of Viral Transport Medium to a labeled 1.5 mL microcentrifuge tube. Alternately, pipet 180  $\mu$ L of Viral Transport Medium directly into sample cartridge or sample vessel.
- iii. Add 20  $\mu$ L of Intermediate Stock of Internal Control to the Viral Transport Medium. **Pipet up and down a minimum of 5 times to mix.**
- iv. Keep tube on ice.
- v. Discard remaining volume of Internal Control – DO NOT reuse.



Do not reuse Internal Control.

### 2. Isolate the Nucleic Acid (Pre-Amplification Area I) – MagNA Pure LC System using the Total Nucleic Acid Isolation (TNAI) Kit

#### a. Start the instrument and software.

- i. Turn power on to the MagNA Pure LC instrument and then turn on the computer.
- ii. Start the MagNA Pure LC software.
- iii. From the *Main Menu* Screen, select **Sample Ordering** and enter sample information in **Sample Name** column.
- iv. Select the **Total NA Variable\_elution\_volume.blk** protocol.
- v. Follow the software instructions and specify the number of samples.

- vi. Type in **200 µL** for the sample volume and verify that **50 µL** elution volume is selected.
- vii. Select **Stage Setup** and the software will automatically calculate the amount of each reagent that is required.

**b. Fill the reagent tubs.**

Before starting the isolation procedure, fill all reagent tubs outside the instrument with the required volume of each reagent listed on the **Start Information** screen.



**Note**

*Use only the reagent amount needed for the number of samples entered into the software. Reagents are not stable for long-term storage in tubs. Vortex Magnetic Glass Particles (MGPs) and load the exact amount of MGPs (as listed on the **Start Information** Screen) into the instrument just before the run starts.*

**c. Load reagent tubs and disposables into the instrument.**

Use the information on the **Start Information** screen to place all disposable plastics and reagent tubs necessary for the batch run on the Reagent/Sample Stage.



**Recommendation**

*Use a colored "Positioning Frame" (provided with the TNAI kit) on the Reagent Tub Rack to help to correctly load reagents.*

**d. Load the samples, Extraction Control, and Negative Control into the MagNA Pure Sample Cartridge.**

- i. Transfer all 200 µL of each sample to the proper individual wells in the MagNA Pure Sample Cartridge.
- ii. Transfer all 200 µL of the Extraction Control and Negative Control to different wells in the MagNA Pure Sample Cartridge.
- iii. Cover cartridge with Parafilm or MagNA Pure LC Cartridge Seal and keep cartridge on ice until ready to load the instrument.

**e. Load the samples.**

Transfer the Sample Cartridge containing the samples, Extraction Control, and Negative Control into the MagNA Pure LC instrument.

**f. Start the run.**

- i. Start the Batch Run by confirming the correct placement of all disposable plastics and reagents by mouse-clicking the respective text boxes on the **Start Information** screen.
- ii. Select the **OK** button to start the automated isolation procedure. The instrument will automatically dispense all reagents and process the samples.

**g. Store the eluted total nucleic acid.**

After completing the run, place the Storage Cartridge containing the eluted nucleic acids immediately on ice or transfer eluted nucleic acid to 1.5 mL tubes and store for longer durations at  $\leq -70^{\circ}\text{C}$ .



**Note**


*Do not store purified nucleic acids in the Storage Cartridge on the Cooling Unit 1.*

### 3. Isolate the Nucleic Acid (Pre-Amplification Area I) – NucliSENS easyMAG System using the Automated Magnetic Extraction Reagents

#### a. Start Instrument and Software.

Turn power on to the easyMAG instrument and once the LED on the instrument turns green, turn on the computer and log into the software.

#### b. Prepare the software for a run.

To prepare for a run, touch the “**Settings**”  icon in the main toolbar which defaults to the “Application Settings” icon and choose the following run settings:

**Default Request:** Generic 1.0.6 or 2.0.1 (for software version 1.0.1 or 2.0, respectively)

**Run Name Prefix:** N/A (leave as default)

**Sample ID prefix:** N/A (leave as default)

**Sample Type:** Primary (on-board lysis)



**Default On-board Lysis Dispensing:** Yes

**Default On-board Lysis Incubation:** Yes



**Sample Addition Guidance:** Off

**Reagent Tracking:** Off

#### c. Input buffer information

Touch the “**Instrument**”  icon to default to the “**Reagent Inventory**”  icon and input the buffer barcodes by first scanning the instrument position (A, B, C, or D) and then its corresponding buffer. For example, scan position A and then scan the bottle of Lysis buffer in that position and then move on to position B and its corresponding bottle.

#### d. Create a worklist.

i. Touch the “**Daily Use**”  icon which will default to the “**Define Extraction Request**”  icon and select the following settings:

**Sample ID:** Manually enter the sample name.

**Matrix:** Other


**Request:** Generic 1.0.6 or 2.0.1 (for software version 1.0.1 or 2.0, respectively)

**Volume (mL):** 0.200 (input volume of sample)






**Eluate (µL):** 55

**Type:** Primary

**Priority:** Normal

ii. Press **Enter** on the keyboard or touch the “**New Extraction Request**”  icon after each manual sample addition. The settings above will remain as the default settings for each subsequent entry as long as you do not navigate to other pages.


#### e. Create a run and add samples from the worklist.

Touch the “**Organize Runs**”  icon and then the “**Create Run**”  icon which will bring up the *New Run Window*. In this screen, name the run appropriately and verify that the **auto-number** box is left unchecked (NOT SELECTED) and that **Yes** is selected for both the On-Board Lysis Dispensing and On-Board Lysis Incubation options. Touch **OK** and the *New Run Window* will close and you will be in the “**Organize Runs**” screen. Assign samples to run with the positioning (arrow)  icons. Touch the “**Load Run**”  icon and select the run. Print the worklist with the “**Print worklist**”  icon. This worklist will aid in keeping track of the order of the samples to be loaded into the sample vessel wells.

#### f. Load the samples and tips and barcode the sample strip(s).

Add all of the 200 µL from each sample into the proper vessels of the sample strip(s) as noted in the worklist. Insert tips into sample vessel(s) in the correct order as noted in the worklist and scan the sample strip(s) position on the instrument and then the sample strip itself. For example, scan position A and then the sample strip in that position, then B and then C, if necessary. After scanning the sample strip(s), the indicator will change from red to green on the screen.

**g. On-Board Lysis Dispensing.**


Once the samples and tips are loaded and the strip(s) scanned, close the lid and touch the “**Dispense Lysis**”  icon. The instrument will dispense 2 mL of Lysis Buffer and incubate for 10 minutes.

**h. Prepare the magnetic silica to add to the sample vessel.**

During the 10-minute lysis incubation, use the Biohit multi-channel pipettor to prepare the magnetic silica. This procedure will need to be performed for each sample vessel used in the run (1, 2, or 3 times).

- i.** Set the pipettor to **Program 1** and place a Biohit pipette tip on position 1. Program 1 provides the means to aspirate and dispense 550  $\mu$ L of liquid. The magnetic silica is prepared in a 1:1 ratio of Molecular Biology Grade Water to Magnetic Silica.
- ii.** Using Program 1 of the pipettor, press the **start** button to aspirate and then again to dispense 550  $\mu$ L of water into a microcentrifuge tube. Vortex the tube of magnetic silica briefly to mix and use Program 1 of the pipettor to aspirate and then dispense 550  $\mu$ L of magnetic silica into the same microcentrifuge tube as the water. Eject the tip, cap the tube and vortex to mix.
- iii.** Set the pipettor to **Program 2** and place a Biohit pipette tip on position 1. Program 2 will transfer 8 volumes of the previous mix to the 8 vessels of a strip on an ELISA plate (1 strip/sample vessel). Press the **start** button to aspirate the mix. Press the **start** button again to dispense the remaining mixture back into the tube containing the mix to reset the pipette.
- iv.** Press the **start** button 8 separate times to dispense the remaining mix in each of 8 vessels of an ELISA plate strip and eject the tip.
- v.** After the 10 minute lysis incubation is done, set the pipettor to **Program 3** and place 8 Biohit pipette tips on the multichannel pipettor (or however many samples are present in the specific sample strip). Make sure that the filter tips are very well connected with the multichannel pipettor to prevent leakage errors. Program 3 first mixes the magnetic silica mixture in the ELISA plate and then aspirates it to be delivered to the vessels of the sample strip where it will be mixed. Press the **start** button once and the pipette will mix the silica in the ELISA plate and then aspirate it for addition to the sample vessel. Verify that each tip has the same volume of silica mix before placing in the sample vessel. Place the pipettor over the sample vessel strip so the tips are below the liquid level of each sample and press the **start** button again, which will then aspirate 800  $\mu$ L out of each sample vessel and perform 3 mix cycles with 1000  $\mu$ L. At this point be sure to hold the pipette steady below the liquid/air interface as it is mixing so as not to introduce bubbles to the sample.
- vi.** Repeat for each sample strip in the run.

**i. Start the run.**

Touch the “**Start**”  icon to begin the run. The instrument will perform 5 washes and heat and elute. The purified nucleic acids need to be transferred to appropriate storage tubes (1.5 mL microcentrifuge tubes) on ice within 30 minutes of extraction completion to avoid contamination by the magnetic silica stuck to the front wall of the sample vessel(s). Use immediately or store at  $\leq -70^{\circ}\text{C}$ .

#### 4. Set up the RT-PCR Reaction (Pre-Amplification Area II)



Note

Start the SmartCycler Pro hMPV+ Assay run within 1 hour of making the RT-PCR Master mix.

**a. Dilute the Positive Control.**

- i.* Include the Positive Control with each run. Thaw one (1) aliquot of the Positive Control on ice.
- ii.* Add 45  $\mu\text{L}$  of molecular grade water to individual 1.5 mL microcentrifuge tube.
- iii.* Transfer 5  $\mu\text{L}$  of the Positive Control to the tube. **Pipet up and down a minimum of 5 times to mix.**
- iv.* Keep tubes on ice.
- v.* Discard remaining volume of Positive Control – DO NOT reuse.



*Do not reuse Positive Control.*

**b. Prepare the RT-PCR Master mix.**

- i.* Calculate the amount of each reagent needed based on the number of reactions (samples + controls):
 

19.45 $\mu\text{L}$	hMPV Mix II
+0.30 $\mu\text{L}$	M-MLV Reverse Transcriptase
+0.25 $\mu\text{L}$	RNase Inhibitor
20.00 $\mu\text{L}$	per reaction
- ii.* Thaw the hMPV Mix II on ice and **mix by pipetting up and down a minimum of 5 times before use.**
- iii.* Remove M-MLV Reverse Transcriptase and RNase Inhibitor enzymes from the freezer and keep on ice during use. M-MLV Reverse Transcriptase should be spun down and pipetted from the top.
- iv.* Prepare the RT-PCR Master mix by combining the reagents listed above in a 1.5 mL microcentrifuge tube. **Pipet up and down a minimum of 5 times to mix.**
- v.* Keep the RT-PCR Master mix on ice and protected from light before adding to SmartCycler tubes.

**c. Add 20  $\mu\text{L}$  of RT-PCR Master mix to the SmartCycler tubes.**

- i.* Load the required number of tubes into the Cepheid Cooling Block.
- ii.* Pipet the RT-PCR Master mix into the upper part of the SmartCycler tubes. Discard any unused RT-PCR Master mix.

**d. Add 5  $\mu\text{L}$  of each sample's nucleic acid to individual SmartCycler tubes containing RT-PCR Master mix.**

- i.* After adding the sample's nucleic acid to the SmartCycler tube, **pipet up and down 2 to 3 times in the upper part of the tube.**
- ii.* Close the tube. Use a new pipette tip for each sample.

**e. Add 5  $\mu\text{L}$  of diluted Positive Control to a separate SmartCycler tube containing RT-PCR Master mix.**

- i.* After adding the diluted Positive Control to the SmartCycler tube, **pipet up and down 2 to 3 times in the upper part of the tube** using a new pipet tip for each control tube.
- ii.* Close the tube.
- iii.* Discard remaining volume of diluted Positive Control – DO NOT reuse.



*Do not reuse diluted Positive Control.*

**f. Add 5  $\mu\text{L}$  of the Extraction Control nucleic acid to a separate SmartCycler tube containing RT-PCR Master mix.**

- i.* After adding the extraction control nucleic acid to the SmartCycler tube, **pipet up and down 2 to 3 times in the upper part of the tube.**
- ii.* Close the tube.

- g. Add 5  $\mu$ L of the Negative Control nucleic acid to the last SmartCycler tube containing RT-PCR Master mix.**
  - i.** After adding the Negative Control nucleic acid to the SmartCycler tube, **pipet up and down 2 to 3 times in the upper part of the tube.**
  - ii.** Close the tube.
- h. Centrifuge all tubes.**
  - i.** Appropriately label the SmartCycler tubes on the caps.
  - ii.** Centrifuge all tubes for 5 to 10 seconds using the Cepheid microcentrifuge specially adapted to fit the SmartCycler tubes.
  - iii.** Return tubes to the cooling block.
- i. Keep the tubes on the Cooling Block before loading them into the SmartCycler instrument.**

### 5. Run the Pro hMPV+ Assay (Amplification/Detection Area)

- a.** Create a new run by clicking on the **Create Run** icon at the top of the screen. This will open the *Create Run* screen.
- b.** Under **Run Name** in the left panel of the *Create Run* screen, enter a unique run identifier.
- c.** Click on the **Assay** arrow in the left panel of the *Create Run* screen and select the **Pro hMPV+ Assay** protocol from the drop-down menu.
- d.** Under **Assay Information** in the left panel of the *Create Run* screen, enter the **Lot Number** and **Expiration Date** (YYYY/MM/DD) of the Pro hMPV+ Assay Kit.
- e.** In the left panel of the *Create Run* screen, enter the number of specimens (including the Positive Control, but excluding the Negative Control) and click on **Apply**. This will display the **Site Table** and the SmartCycler Dx Software will automatically select the **I-Core** sites.
- f.** In the **Site Table** under the **Sample ID** column, enter the Sample Identifier or Positive Control Identifier for the appropriate I-Core sites.
- g.** Insert each reaction tube into an I-Core site of the SmartCycler by pressing down firmly on all tubes and close each lid. Verify that the Negative Control (NC1) is loaded into the correct I-Core site.
- h.** Select the **Start Run** button located at the bottom left corner of the screen. Verify that the LED is on for the appropriate I-Core sites.

### 6. Print report

- a.** Click on **Report** at bottom of screen to open the **Report Preview** screen.
- b.** Click on the **Print Icon** at the top of the screen.

## Interpretation of Control Results

### Validation of Run



Note

The user must interpret the Positive Control and the Extraction Control (if included) results to determine whether the RT-PCR run and/or the extraction run are VALID; the SmartCycler Dx software will automatically interpret the Negative Control results.

For a **VALID** Extraction run, the following conditions must be met:

Sample ID <sup>1</sup>	Assay Result	IC Result	Warning / Error Code	Sample Type	IC Ct	hMPV Result	hMPV Ct
Extraction Control	Positive	NA	**	SPEC	NA	Positive	13-40
Neg Control	Valid <sup>2</sup>	Pass		NC1	17-40	Valid	0

<sup>1</sup> Columns and data not used for interpretation are not included.

<sup>2</sup> (Typical) An Invalid assay will display Error Code 4098.

\*\* Error Code 3079: Warning/Error Code 3079 is periodically observed with hMPV positives (Extraction Control, hMPV positive NP swab samples). Warning/Error Code 3079 occurs when the fluorescence (RFU) signal is too high. In this case, results for that sample are reported by the Dx software as ND (Not Determined). If a Ct value between 13-40 is reported in the "hMPV Ct" column for the Extraction Control, results can be recorded as positive and the Extraction run considered VALID.

For a **VALID** RT-PCR run, the conditions in the table below must be met. If the run is valid, specimens should be interpreted using the next section *Interpretation of Specimen Results*.

Sample ID <sup>1</sup>	Assay Result	IC Result	Warning / Error Code	Sample Type	IC Ct	hMPV Result	hMPV Ct
Pos Control	Positive	NA	**	SPEC	NA	Positive	25-40
Neg Control	Valid <sup>2</sup>	Pass		NC1	17-40	Valid	0

<sup>1</sup> Columns and data not used for interpretation are not included.

<sup>2</sup> (Typical) An Invalid assay will display Error Code 4098.

\*\* Error Code 3079: Warning/Error Code 3079 is periodically observed with hMPV positives (hMPV Positive Control). Warning/Error Code 3079 occurs when the fluorescence (RFU) signal is too high. In this case, results for that sample are reported by the Dx software as ND (Not Determined). If a Ct value of 25-40 is reported in the "hMPV Ct" column for the Positive Control, results can be recorded as positive and the RT-PCR run considered VALID.

### Invalid Extraction Run

If the conditions for a Valid Extraction run are not met (i.e. the Extraction Control is not positive or the Negative Control is invalid), repeat the entire extraction run. Start from original sample(s) using a new Extraction Control and a new Negative Control (starting at **Step 1** of the *Assay Procedure*).

### Invalid RT-PCR Run

If the Positive Control is not positive within the specified Ct range but the Negative Control is valid, prepare all new reactions using remaining purified nucleic acids and a new Positive Control (starting with PCR at **Step 4** of the *Assay Procedure*).

False positives have been known to occur in the Negative Control. If the Negative Control is invalid (i.e. positive in the hMPV channel), prepare new reactions using remaining purified nucleic acids from the Negative Control and sample(s) as appropriate.

**Interpretation of Specimen Results**

The SmartCycler Dx software automatically determines the specimen results. The interpretation of the assay specimen results is as follows:

Sample ID <sup>1</sup>	Assay Result	IC Result	Warning / Error Code	hMPV Result	Interpretation of Results
Sample ID	Negative	Pass	NONE	<b>NEG</b>	hMPV nucleic acid <b>not detected</b>
Sample ID	Positive	NA*	NONE	<b>POS</b> <sup>2</sup>	hMPV nucleic acid <b>detected</b>
Sample ID	Unresolved	Fail	NONE	<b>NEG</b>	Unresolved – PCR inhibition or reagent failure. Repeat testing from the purified nucleic acid or collect and test a new sample.
Sample ID	ND	ND	3079 <sup>2</sup>	ND	Not Determined – error code 3079
Sample ID	Invalid		4098 <sup>3</sup>	ND	Not Determined – error code 4098

<sup>1</sup> Columns and data not used for interpretation are not included

<sup>2</sup> It is recommended to repeat testing of samples with hMPV Ct values of 35-40 to confirm a positive result.

<sup>3</sup> Error Code 3079: Warning/Error Code 3079 is periodically observed with hMPV positive samples. Warning/Error Code 3079 occurs when the fluorescence (RFU) signal is too high. In this case, all results for that sample are reported by the Dx software as ND (Not Determined). If a Ct value  $\geq 13$  is reported in the “hMPV Ct” column, the sample results can be recorded as POS for hMPV.

<sup>4</sup> An Invalid assay run will display Error Code 4098

\* Detection of the Internal Control in the Cy5 detection channel is not required for positive result. High viral load can lead to reduced or absent Internal Control signal.

### Not Determined Samples

If an assay result of **ND** (Not Determined) is reported with an instrument failure other than Warning/Error Code 3079, repeat testing from the purified nucleic acids (starting with PCR, see [Step 4 \(a\)](#) of the [Assay Procedure](#)). Refer to the Cepheid Dx Software Operator Manual for interpretation of Warning Codes.

## Quality Control

- Quality control requirements must be performed in conformance with local, state, and/or federal regulations or accreditation requirements and your laboratory's standard quality control procedures. It is recommended that the user refer to CLSI document C24-A3, *Statistical Quality Control for Quantitative Measurements: Principles and Definitions*: [Approved Guideline – Third Edition] or other published guidelines for general quality control recommendations. For further guidance on appropriate quality control practices, refer to 42 CFR 493.1202(c).
- Quality control procedures are intended to monitor reagent and assay performance.

Control Type	Used to Monitor
Positive	Substantial reagent failure including primer and probe integrity
Negative	Reagent, environmental, or carry-over contamination
Extraction	Failure in lysis and extraction procedure
Internal	PCR inhibition in individual samples and Reagent failure or process error

- Dilute and test the Positive Control and the Internal Control prior to running samples with each new kit lot to ensure all reagents and kit components are working properly.
- Good laboratory practice recommends including a positive Extraction Control and a Negative Control in each nucleic acid isolation run. The Extraction Control should be treated as a sample.
- Never run the Positive Control through nucleic acid isolation.
- Always include a Negative Control (*containing Internal Control*) and a Positive Control in each amplification/detection run performed.
- Failure of Controls (Positive, Negative and/or Extraction) invalidates the run and results should not be reported.
- If the Positive Control is not positive within the specified Ct range but the Negative Control is valid, repeat testing should be done starting from the purified nucleic acid and using a new aliquot of the Positive Control. If repeat results are still invalid, results should not be reported and testing should be repeated from the original sample or a new sample should be collected and tested.
- If the Extraction Control is not positive within the specified Ct range or the Negative Control is invalid, repeat testing should be done starting from the original sample and using a new Extraction Control and a new Negative Control. If repeat results are still invalid, results should not be reported and a new sample should be collected and tested.

## Limitations

- This test does not differentiate hMPV subtypes (i.e., A1, A2, B1, and B2); additional testing is required to identify and differentiate hMPV subtypes.
- Negative results do not preclude hMPV infection and should not be used as the sole basis for diagnosis, treatment or other management decisions.
- False negative results may occur due to loss of nucleic acid. The Internal Control has been added to the test to aid in the identification of specimens that contain inhibitors to PCR amplification. The Internal Control does not indicate whether or not nucleic acid has been lost due to inadequate collection, transport or storage of specimens.
- A trained health care professional should interpret assay results in conjunction with the patient's medical history, clinical signs and symptoms, and the results of other diagnostic tests.
- Analyte target (hMPV RNA sequence) may be present persistently in vivo, independent of virus viability. Detection of hMPV RNA does not imply that the hMPV is infectious, nor is the causative agent for clinical symptoms.
- The detection of hMPV RNA sequences is dependent upon proper specimen collection, handling, transportation, storage, and preparation (including extraction). Failure to observe proper procedures in any one of these steps can lead to incorrect results.
- **Once the RT-PCR Master mix has been made, the run must be started within one hour.**
- Optimal assay performance requires strict adherence to the assay procedure described in this insert.
- Reliable results are dependent on adequate specimen collection, transport, storage and processing procedures.
- There is a risk of false negative values resulting from improperly collected, transported, or handled specimens.
- There is a risk of false positive values resulting from cross-contamination by target organism or its nucleic acids.
- There is a risk of false negative values due to the presence of sequence variants in the viral target of the assay, procedural errors, amplification inhibitors in specimens, or inadequate numbers of organisms for amplification.
- The performance of the Pro hMPV+ Assay has not been established in immunocompromised patients.
- Positive and negative predictive values are highly dependent on prevalence. The assay performance was established during the 2008 respiratory virus season (January – March). The performance may vary depending on the prevalence and population tested.

## Expected Values

hMPV appears to have a similar seasonality as RSV in the US occurring during the winter/early spring months of the year<sup>3</sup>. Incidences of lower respiratory and upper respiratory infections in children have been reported between 5% - 15%<sup>4</sup>. In the Pro hMPV+ multicenter prospective study hMPV prevalence varied from 1% to 9% by site and averaged 5% overall. The number and percentage of hMPV RNA positive cases determined by the Pro hMPV+ Assay during this study, stratified by patient age group, are presented in the following table:

Age Group	Total (N)	Total # hMPV Positive By the Pro hMPV+ Assay	Observed Prevalence
< 1 year	296	25	8.4%
1-5 years	300	30	10.0%
6-10 years	115	7	6.1%
11-15 years	57	3	5.3%
16-21 years	82	1	1.2%
> 21 years	425	6	1.4%
Total	1275	72	5.6%

**Performance Characteristics**
**Clinical Performance**

Performance characteristics of the Pro hMPV+ Assay were established during a prospective study at 4 U.S. clinical laboratories during the 2008 respiratory virus season (January - March). Specimens used in the study represented excess nasopharyngeal (NP) swab specimens that were prospectively collected from symptomatic individuals suspected of respiratory infection, and were submitted for routine care or analysis by each site. Demographic details for this patient population are summarized in the following table:

Gender	Number of Subjects (Percentage of Total)
Female	617 (48.4%)
Male	654 (51.3%)
Not Determined	4 (0.3%)
Age	
≤ 5 years	596 (46.7%)
6 - 21 years	254 (19.9%)
22 – 59 years	219 (17.2%)
≥ 60 years	206 (16.2%)

Performance of the Pro hMPV+ Assay was assessed and compared to a predetermined algorithm that used composite reference methods. The composite reference methods consisted of two independent molecular (RT-PCR) tests for two separate gene targets of hMPV followed by bi-directional genetic sequencing. The two comparator methods targeted the Nucleocapsid gene (different region of the gene than targeted by the Pro hMPV+ Assay) and the Fusion gene. True hMPV RNA positives were considered as any sample that had bi-directional sequencing data meeting pre-defined quality acceptance criteria for one or both gene targets that matched hMPV sequences deposited in the National Center for Biotechnology Information (NCBI) GenBank database ([www.ncbi.nlm.nih.gov](http://www.ncbi.nlm.nih.gov)). True hMPV RNA negatives were considered as any sample that was tested negative by both of the comparator methods. Nucleic acid extractions on the clinical samples were carried out using either the Roche MagNA Pure LC System or the bioMérieux NucliSENS easyMAG System during the clinical study.

A total of 1275 eligible NP swab samples were tested with the Pro hMPV+ Assay at the four clinical sites and by the composite reference methods at Prodesse. Of the Pro hMPV+ Assay run on all eligible specimens, 98.1% (1273/1298) of these specimens were successful on the first attempt. The remaining 25 specimens gave “Unresolved” results on the first attempt. Unresolved results occur when the sample is negative for both hMPV and the Internal Control, indicating potentially PCR-inhibiting samples. Of the 25 “Unresolved” specimens on the first attempt with sufficient sample for retest, 8.0% (2/25) gave a valid “negative” result on the second attempt. The remaining 23 samples were “Unresolved” on the second attempt, therefore, were not included in the analysis below. All 23 samples were tested negative by the composite reference methods.

		Composite Reference Methods			Comments
		Positive	Negative	Total	
Pro hMPV+ Assay	Positive	64	8	72	Percent Positive Agreement 95.5% (87.6% - 98.5%) 95% CI
	Negative	3	1200	1203	Percent Negative Agreement 99.3% (98.7% - 99.7%) 95% CI
	Total	67	1208	1275	

## Reproducibility

The reproducibility of the Pro hMPV+ Assay was evaluated at 3 laboratory sites. Reproducibility was assessed using a panel of 9 simulated samples that included medium positive, low positive (near the assay limit of detection) and “high negative” hMPV samples. Panels and controls were tested at each site by 2 operators for 5 days (9 samples and 3 controls X 2 operators X 5 days X 3 sites = 360). Nucleic acid extraction on the test panel samples were carried out using either the Roche MagNA Pure LC System (Clinical Trial Site #4) or the bioMérieux NucliSENS easyMAG System (Site #1 and Site #2). The overall percent agreement with the expected result for the Pro hMPV+ Assay was 99.2%.

Panel Member ID	hMPV A2 High Negative <sup>a</sup>	hMPV A2 Low Positive	hMPV A2 Moderate Positive	hMPV B2 High Negative <sup>a</sup>	hMPV B2 Low Positive	hMPV B2 Moderate Positive	hMPV RNA Control	Negative Control <sup>a</sup>	Extraction Control hMPV A2	Total % Agreement	
	Concentration	0.01 X LoD	2 X LoD	10 X LoD	0.01 X LoD	2 X LoD	10 X LoD	NA	NA		NA
	1 x 10 <sup>0</sup> TCID <sub>50</sub> /mL	2 x 10 <sup>2</sup> TCID <sub>50</sub> /mL	1 x 10 <sup>3</sup> TCID <sub>50</sub> /mL	1 x 10 <sup>-1</sup> TCID <sub>50</sub> /mL	2 x 10 <sup>1</sup> TCID <sub>50</sub> /mL	1 x 10 <sup>2</sup> TCID <sub>50</sub> /mL					
Site 1	Agreement with Expected result	15/15 (100%)	15/15 (100%)	15/15 (100%)	15/15 (100%)	15/15 (100%)	15/15 (100%)	10/10 (100%)	10/10 (100%)	10/10 (100%)	<b>120/120 (100%)</b>
	Average Ct Value	26.6	29.2	27.1	27.5	29.3	26.6	32.5	26.2	33.1	
	% CV	1.53	2.84	1.21	1.97	2.20	1.68	0.81	0.80	2.73	
Site 2	Agreement with Expected result	15 /15 (100%)	14/15 (93.3%)	15/15 (100%)	15/15 (100%)	13/15 (86.7%)	15/15 (100%)	10/10 (100%)	10/10 (100%)	10/10 (100%)	<b>117/120 (97.5%)</b>
	Average Ct Value	25.8	30.7	26.9	26.9	30.7	26.3	32.8	25.6	32.9	
	% CV	0.54	3.95	2.88	1.44	4.14	1.25	1.37	0.98	4.86	
Site 4	Agreement with Expected result	15/15 (100%)	15/15 (100%)	15/15 (100%)	15/15 (100%)	15/15 (100%)	15/15 (100%)	10/10 (100%)	10/10 (100%)	10/10 (100%)	<b>120/120 (100%)</b>
	Average Ct Value	27.4	30.5	27.8	28.5	29.4	27.0	33.6	27.6	28.8	
	% CV	1.45	2.15	2.13	3.00	3.80	2.50	1.09	1.87	3.08	
Overall	Total Agreement with Expected result	45/45 (100%)	44/45 (97.8%)	45/45 (100%)	45/45 (100%)	43/45 (95.6%)	45/45 (100%)	30/30 (100%)	30/30 (100%)	30/30 (100%)	<b>357/360 (99.2%)</b>
	95% CI	92.1% - 100%	88.4% - 99.6%	92.1% - 100%	92.1% - 100%	85.2% - 98.8%	92.1% - 100%	88.6% - 100%	88.6% - 100%	88.6% - 100%	<b>97.6% - 99.7%</b>
	Overall Average Ct Value	<b>26.6</b>	<b>30.1</b>	<b>27.63</b>	<b>27.6</b>	<b>29.7</b>	<b>26.6</b>	<b>33.0</b>	<b>26.5</b>	<b>31.6</b>	
	Overall %CV	<b>2.85</b>	<b>3.73</b>	<b>2.57</b>	<b>3.29</b>	<b>3.97</b>	<b>2.16</b>	<b>1.72</b>	<b>3.49</b>	<b>7.36</b>	

<sup>a</sup>Average Ct value calculated for the Internal Control (IC)

### Analytical Sensitivity

The analytical sensitivity (limit of detection or LoD) of the Pro hMPV+ Assay was determined using quantified (TCID<sub>50</sub>/mL) cultures of 2 hMPV (subtype A2 and subtype B2) strains serially diluted in nasopharyngeal clinical matrix. Each viral strain was extracted using the Roche MagNA Pure LC instrument and tested in replicates of 20 per concentration of virus. Analytical sensitivity (LoD) as defined as the lowest concentration at which ≥ 95% of all replicates tested positive, ranged from 10<sup>2</sup> – 10<sup>1</sup> TCID<sub>50</sub>/mL.

Viral Strain	LoD Concentration
hMPV subtype A2	10 <sup>2</sup> TCID <sub>50</sub> /mL
hMPV subtype B2	10 <sup>1</sup> TCID <sub>50</sub> /mL

### Reactivity

The reactivity of the Pro hMPV+ Assay was evaluated against four strains of hMPV (subtypes A1, A2, B1 and B2). Each viral strain was extracted using the Roche MagNA Pure LC instrument and tested in triplicate. All viral cultures of the panel were detected by the Pro hMPV+ Assay.

Viral Strain	Concentration	hMPV (FAM)
hMPV subtype A1	10 <sup>1</sup> TCID <sub>50</sub> /mL	+
hMPV subtype A2	10 <sup>1</sup> TCID <sub>50</sub> /mL	+
hMPV subtype B1	10 <sup>1</sup> TCID <sub>50</sub> /mL	+
hMPV subtype B2	10 <sup>1</sup> TCID <sub>50</sub> /mL	+

### Analytical Specificity

The analytical specificity of the Pro hMPV+ Assay was evaluated by testing a panel of 52 cultures consisting of 28 viruses, 23 bacteria, and 1 yeast strain representing common respiratory pathogens or flora commonly present in the nasopharynx. Bacteria and yeast were tested at concentrations of  $10^6$  to  $7.4 \times 10^7$  CFU/mL. Viruses were tested at concentrations of  $10^2$  to  $10^6$  TCID<sub>50</sub>/mL. Samples were extracted using the Roche MagNA Pure LC instrument and tested in triplicate. Analytical specificity of the Pro hMPV+ Assay was 100%.

Strains	Concentration	hMPV (FAM)
hMPV A2	$10^3$ TCID <sub>50</sub> /mL	+
hMPV B2	$10^2$ TCID <sub>50</sub> /mL	+
Adenovirus 1/Adenoid 71	$10^6$ TCID <sub>50</sub> /mL	-
Coronavirus 229E	$10^6$ TCID <sub>50</sub> /mL	-
Coxsackie B4	$10^4$ TCID <sub>50</sub> /mL	-
Coxsackie B5/10/2006	$10^5$ TCID <sub>50</sub> /mL	-
Cytomegalovirus	$10^4$ TCID <sub>50</sub> /mL	-
Echovirus 2	$10^6$ TCID <sub>50</sub> /mL	-
Echovirus 3	$10^5$ TCID <sub>50</sub> /mL	-
Echovirus 6	$10^5$ TCID <sub>50</sub> /mL	-
Echovirus 11	$10^5$ TCID <sub>50</sub> /mL	-
Enterovirus 68	$10^3$ TCID <sub>50</sub> /mL	-
Enterovirus 70	$10^3$ TCID <sub>50</sub> /mL	-
HSV Type 1 MacIntyre Strain	$10^5$ TCID <sub>50</sub> /mL	-
HSV Type 2 G strain	$10^5$ TCID <sub>50</sub> /mL	-
Human Rhinovirus 39	$10^3$ TCID <sub>50</sub> /mL	-
Human Rhinovirus	$10^4$ TCID <sub>50</sub> /mL	-
Influenza A/Port Chalmers	$10^4$ TCID <sub>50</sub> /mL	-
Influenza B/Wisconsin	$10^4$ TCID <sub>50</sub> /mL	-
Measles/7/2000	$10^4$ TCID <sub>50</sub> /mL	-
Mumps Virus	$10^4$ TCID <sub>50</sub> /mL	-
Parainfluenza Type 1	$10^4$ TCID <sub>50</sub> /mL	-
Parainfluenza Type 2	$10^5$ TCID <sub>50</sub> /mL	-
Parainfluenza Type 3	$10^5$ TCID <sub>50</sub> /mL	-
Parainfluenza Type 4	$10^4$ TCID <sub>50</sub> /mL	-
RSV A Strain Long	$10^4$ TCID <sub>50</sub> /mL	-
RSV B Strain Wash	$10^4$ TCID <sub>50</sub> /mL	-
Varicella Zoster Virus	$10^4$ TCID <sub>50</sub> /mL	-
<i>Bordetella pertussis</i>	$10^6$ CFU/mL	-
<i>Chlamydia pneumoniae</i>	$10^4$ TCID <sub>50</sub> /mL	-
<i>Chlamydia trachomatis</i>	$10^4$ TCID <sub>50</sub> /mL	-
<i>Legionella pneumophila</i>	$10^6$ CFU/mL	-
<i>Mycobacterium intracellulare</i>	$10^7$ CFU/mL	-
<i>Mycobacterium tuberculosis</i>	$10^7$ CFU/mL	-
<i>Haemophilus influenza</i>	$10^6$ CFU/mL	-
<i>Pseudomonas aeruginosa</i>	$10^6$ CFU/mL	-
<i>Proteus vulgaris</i>	$10^6$ CFU/mL	-
<i>Proteus mirabilis</i>	$10^6$ CFU/mL	-
<i>Neisseria gonorrhoeae</i>	$10^6$ CFU/mL	-
<i>Neisseria meningitidis</i>	$10^6$ CFU/mL	-
<i>Neisseria mucosa</i>	$7.4 \times 10^7$ CFU/mL	-
<i>Klebsiella pneumoniae</i>	$10^6$ CFU/mL	-
<i>Escherichia coli</i>	$10^6$ CFU/mL	-
<i>Moraxella catarrhalis</i>	$10^7$ CFU/mL	-
<i>Corynebacterium diphtheriae</i>	$3 \times 10^7$ CFU/mL	-
<i>Lactobacillus plantarum</i>	$10^6$ CFU/mL	-
<i>Streptococcus pneumoniae</i>	$10^6$ CFU/mL	-
<i>Streptococcus pyogenes</i>	$10^6$ CFU/mL	-
<i>Streptococcus salivarius</i>	$2 \times 10^6$ CFU/mL	-
<i>Staphylococcus epidermidis</i>	$10^6$ CFU/mL	-
<i>Staphylococcus aureus</i>	$10^6$ CFU/mL	-
<i>Candida albicans</i>	$10^6$ CFU/mL	-

Note: Fresh cultured and titered stocks of organisms were used for the analytical specificity study, except for *C. pneumoniae* and *C. trachomatis*. ATCC frozen cultures and ATCC supplied titers were used for both due to technical difficulties in re-growing and re-titering these organisms.

### Interference

Whole blood and a number of other potentially interfering substances (medications and over the counter (OTC) products) that may be present naturally or artificially introduced in the nasopharynx were evaluated in the Pro hMPV+ Assay. Two subtypes of hMPV (A2 and B2) were used and spiked into NP pools at either 2X LoD or 10X LoD. The hMPV strains were re-titered prior to interference testing. Clinically relevant amounts of the potential interfering substances were added to spiked samples. An Internal Control (IC) was also added to each sample. Nucleic acid from the samples was extracted with the Roche MagNA Pure LC instrument. The Pro hMPV+ Assay was performed in triplicate reactions for each sample on the Cepheid SmartCycler II. The following table shows the potential interfering substances used for this study. The substances consisted of nasal sprays (liquid and powder), ingestible pills and lozenges, injectables, and endogenous substances:

Substance Name	Active Ingredient	Concentration Tested
Mucin	Purified mucin protein	60µg/mL
Blood (human)	N/A	2% (volume/volume)
Neo-Syneprine	Phenylephrine HCl	15% (volume/volume)
Anefrin Nasal Spray	Oxymetazoline Hydrochloride	15% (volume/volume)
Zicam Nasal gel	Luffa Operculata, Galphimia Glauca, Histaminum Hydrochloricum,	5% (volume/volume)
Saline Nasal Spray	Sodium chloride with preservatives	15% (volume/volume) of dose
Chloraseptic Throat Lozenges	Oral anesthetic/analgesic	0.63mg/mL; active ingredients: 1.0mg/mL benzocaine, 1.7mg/mL menthol
Relenza	Zanamivir	3.3mg/mL
Tobramycin	Tobramycin	4.0µg/mL
Mupirocin	Mupirocin	6.6mg/mL
Rebitol	Ribavirin	20mg/mL
TamiFlu	Oseltamivir	25mg/mL
Beconase AQ	Beclomethasone dipropionate	5% (volume/volume)

All of the exogenous or endogenous potentially interfering substances tested were found to have no effect on Pro hMPV+ Assay results when present in simulated respiratory samples at the concentrations indicated.

### Extraction Equivalency

Extraction equivalency of the bioMérieux NucliSENS easyMAG and Roche MagNA Pure LC instruments was evaluated by performing a limit of detection study. Single cultured and titered strains of hMPV A2 and hMPV B2 were spiked (along with an IC) into individual aliquots of negative NP matrix pools at concentrations of 1 log above, at, and 1 log below the previously determined LoD. Each viral strain dilution was extracted in replicates of 10 on each automated extractor and tested using the Pro hMPV+ Assay.

The bioMérieux NucliSens easyMAG instrument and the Roche MagNA Pure LC instrument performed equivalently with respect to limit of detection.

### Carry-over/Contamination

To evaluate the degree of carry-over/cross-contamination that occurs with the use of the Pro hMPV+ Assay in association with nucleic acid extraction on the Roche MagNA Pure LC and the bioMérieux NucliSens easyMAG instruments and RT-PCR on the Cepheid SmartCycler II thermocycler, an internal Carry-Over study was carried out by testing simulated human Metapneumovirus (hMPV) high positive samples run in series alternating with hMPV high negative samples. The hMPV high positive samples in this study represented the lower Cycle Threshold (Ct) range (higher sample titer range) obtained in the Pro hMPV+ clinical trials (lowest Ct = 17.9). The high-negative samples include a low amount of hMPV that is detectable no more than 5% of the time. One out of twenty-two high-negative samples tested showed potential hMPV contamination when extracted using the MagNA Pure extraction system in the study. Potential contamination could have occurred during creation of the sample, during sample preparation for extraction, during extraction or when transferring the purified nucleic acid samples from the sample cartridge to microfuge tubes. The possibility that this sample falls in the 5% category could not be ruled out.

### Disposal

Dispose of hazardous or biologically contaminated materials according to the practices of your institution.

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






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