AdV/hMPV/RV Assay (Panther Fusion™ System)

Instructions for Use
For *in vitro* diagnostic use
For U.S. Export Only

General Information

Intended Use

The Panther Fusion™ AdV/hMPV/RV assay is a multiplex real-time PCR (RT-PCR) *in vitro* diagnostic test for the rapid and qualitative detection and differentiation of Adenovirus (AdV), human Metapneumovirus (hMPV), and Rhinovirus (RV). Nucleic acids are isolated and purified from nasopharyngeal (NP) swab specimens obtained from individuals exhibiting signs and symptoms of a respiratory tract infection.

This assay is intended to aid in the differential diagnosis of Adenovirus, human Metapneumovirus, and Rhinovirus infections in humans. Negative results do not preclude Adenovirus, human Metapneumovirus, and Rhinovirus infections and should not be used as the sole basis for treatment or other management decisions. This assay is designed for use on the Panther Fusion system.

Summary and Explanation of the Test

Respiratory viruses are responsible for a wide range of acute respiratory tract infections including the common cold, influenza, and croup and represent the most common cause of acute illness in the United States. Disease severity can be especially high in the young, the immunocompromised, and elderly patients. Accurate and timely diagnosis of the cause of respiratory tract infections has many benefits. They include improved treatment of the patient by ensuring appropriate antiviral treatment (e.g. oseltamivir for influenza), decreasing the overall cost of care, reducing selection for antimicrobial resistant organisms due to excessive and inappropriate use of antibiotics,[1] assisting infection control personnel in providing appropriate measures to minimize nosocomial spread, and providing valued information to public health authorities regarding which viruses are circulating in the community.[2]

Adenoviruses are members of the *Adenoviridae* family which are medium-sized (90-100 nm), non-enveloped icosohedral viruses with double-stranded DNA.[3] At this time, in humans there are over 50 Adenovirus types in seven species (A to G).[4] Adenoviruses most commonly cause respiratory illness which can range from the common cold to pneumonia, croup, and bronchitis.[3] Depending on the type, Adenoviruses can cause other illnesses such as gastroenteritis, conjunctivitis, cystitis, and, less commonly, neurological disease.[3] Infants and people with weakened immune systems are at high risk for developing severe illness caused by Adenovirus infection.[3] Adenovirus circulates year-round and outbreaks are more common in late winter, spring, and early summer but can occur throughout the year.[5]

Since the discovery of hMPV in 2001, the virus has been identified worldwide. hMPV is a common respiratory pathogen, particularly in infants and young children. The virus is associated with both upper and lower respiratory tract infections and may be a trigger for asthma.[6] Symptoms commonly associated with hMPV include cough, fever, nasal congestion, and shortness of breath. Clinical symptoms of hMPV infection may progress to bronchiolitis or pneumonia and are similar to other viruses that cause upper and lower respiratory infections. The incubation period is estimated to be 3 to 6 days, and the median duration of illness can vary depending upon severity but is similar to other respiratory infections caused by viruses.[7] The peak of incidence of hMPV is mainly in the spring in temperate latitudes.[8]

Rhinoviruses, members of the family Picornaviridae, are the causative pathogens in more than half of viral respiratory infections, and they are associated with acute exacerbations of

respiratory disease, including asthma, sinusitis, otitis media, and COPD.[9] A number of studies have confirmed rhinoviruses as being the most common cause of “the common cold” and affect all age groups.[8] Symptoms usually include sore throat, runny nose, coughing, sneezing, watery eyes, headaches and body aches. Most people recover within about 7-10 days.[8] Rhinoviruses circulate year round and tend to peak in the spring and fall.[8]

Principles of the Procedure

The Panther Fusion AdV/hMPV/RV assay involves the following steps: sample lysis, nucleic acid capture and elution transfer, and multiplex RT-PCR when analytes are simultaneously amplified, detected and differentiated. Nucleic acid capture and elution takes place in a single tube on the Panther Fusion system. The eluate is transferred to the Panther Fusion system reaction tube containing the assay reagents. Multiplex RT-PCR is then performed for the eluted nucleic acid on the Panther Fusion system.

Nucleic acid capture and elution: Prior to processing and testing on the Panther Fusion system, specimens are transferred to a Specimen Lysis Tube containing specimen transport media (STM) that lyses the viral particles, releases target nucleic acid, and protects it from degradation during storage.

The Internal Control-S (IC-S) is added to each test specimen and controls via the working Panther Fusion Capture Reagent-S (wFCR-S). The IC-S in the reagent monitors specimen processing, amplification, and detection.

Capture oligonucleotides hybridize to nucleic acid in the test specimen. Hybridized nucleic acid is then separated from the specimen in a magnetic field.

Wash steps remove extraneous components from the reaction tube. The elution step elutes purified nucleic acid. During the nucleic acid capture and elution step, total nucleic acid is isolated from specimens.

Elution transfer and RT-PCR: During the elution transfer step, eluted nucleic acid is transferred to a Panther Fusion reaction tube already containing oil and reconstituted mastermix.

For RV, hMPV, and internal control targets, amplification occurs via RT-PCR. A reverse transcriptase step generates DNA copies of the target sequence. For AdV, target amplification occurs via PCR. For all targets, specific forward and reverse primers and probes amplify targets while simultaneously detecting and discriminating multiple target types via multiplex PCR.

The Panther Fusion system compares the fluorescence signal to a predetermined cut-off to produce a qualitative result for the presence or absence of the analyte.

The analytes and the channel used for their detection on the Panther Fusion system is summarized in the table below.

Analyte	Gene Targeted	Instrument Channel
Adenovirus	Hexon	HEX
human Metapneumovirus	Nucleocapsid	ROX
Rhinovirus	5’ UTR	FAM
Internal Control	Not applicable	RED677

Warnings and Precautions

A. For *in vitro* diagnostic use.

B. For professional use.

C. Carefully read this entire package insert and the *Panther/Panther Fusion System Operator's Manual*.

Laboratory Related

D. The Panther Fusion Enhancer Reagent-S (FER-S) is corrosive, harmful if swallowed and causes severe skin burns and eye damage.

E. Only personnel adequately trained on the use of this assay and in handling potentially infectious materials should perform these procedures. If a spill occurs, immediately disinfect using appropriate site procedures.

F. Handle all specimens as if infectious, using safe laboratory procedures such as those outlined in CDC/NIH Biosafety in Microbiological and Biomedical Laboratories[10] and in the CLSI Document M29 Protection of Laboratory Workers from Occupationally Acquired Infections.[11]

G. Use only supplied or specified disposable laboratory ware.

H. Wear disposable, powderless gloves, protective eye wear, and laboratory coats when handling specimens and reagents. Wash hands thoroughly after handling specimens and reagents.

I. Dispose of all material that has come into contact with specimens and reagents in accordance with applicable national, international, and regional regulations.

Specimen Related

J. Expiration dates listed on the Panther Fusion Specimen Lysis Tubes pertain to the transfer of sample into the tube and not to testing of the sample. Specimens collected/transferred any time prior to these expiration dates are valid for testing provided they are transported and stored in accordance with the appropriate package insert, even if these expiration dates have passed.

K. Maintain proper storage conditions during specimen shipping to ensure the integrity of the specimen. Specimen stability under shipping conditions other than those recommended has not been evaluated.

L. Avoid cross-contamination during the specimen handling steps. Specimens can contain extremely high levels of virus or other organisms. Ensure that specimen containers do not come in contact with one another, and discard used materials without passing them over any open containers. Change gloves if they come in contact with specimens.

Assay Related

M. Do not use the reagents and controls after the expiration date.

N. Store assay components at the recommended storage condition. See *Reagent Storage and Handling Requirements* and *Panther Fusion System Test Procedure* for more information.

O. Do not combine any assay reagents or fluids. Do not top off reagents or fluids; the Panther Fusion system verifies reagent levels.

P. Avoid microbial and ribonuclease contamination of reagents.

Q. Quality control requirements must be performed in conformance with local/regional or accreditation requirements and your laboratory's standard quality control procedures.

R. Do not use the assay cartridge if the storage pouch has lost its seal or if the assay cartridge foil is not intact. Contact Hologic if either occurs.

S. Do not use the fluid packs if the foil seal is leaking. Contact Hologic if this occurs.

T. Handle the assay cartridges with care. Do not drop or invert assay cartridges. Avoid prolonged exposure to ambient light.

Note: *Hazard communication reflects the EU Safety Data Sheets (SDS) classifications. For hazard communication information specific to your region, refer to the region specific SDS on the Safety Data Sheet Library at www.hologicsds.com. For more information on the symbols, refer to the symbol legend on www.hologic.com/package-inserts.*

EU Hazard Information
 **Panther Fusion Oil** ***POLYDIMETHYLSILOXANE 100%*** ***WARNING*** H315 - Causes skin irritation H319 - Causes serious eye irritation
  **Panther Fusion Enhancer Reagent (FER-S)** ***LITHIUM HYDROXIDE, MONOHYDRATE 5 - 10%*** ***DANGER*** H302 - Harmful if swallowed H314 - Causes severe skin burns and eye damage P260 - Do not breathe dust/fume/gas/mist/vapours/spray P280 - Wear protective gloves/protective clothing/eye protection/face protection P303 + P361 + P353 - IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower P305 + P351 + P338 - IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing P310 - Immediately call a POISON CENTER or doctor/physician P280 - Wear eye protection/ face protection

Reagent Storage and Handling Requirements

A. The following table provides storage and handling requirements for this assay.

Reagent	Unopened Storage	On Board/ Open Stability[1]	Opened Storage
Panther Fusion AdV/hMPV/RV Assay Cartridge	2°C to 8°C	60 days	2°C to 8°C [2]
Panther Fusion Capture Reagent-S (FCR-S)	15°C to 30°C	30 days	15°C to 30°C
Panther Fusion Enhancer Reagent-S (FER-S)	15°C to 30°C	30 days	15°C to 30°C
Panther Fusion Internal Control-S (IC-S)	2°C to 8°C	(In wFCR-S)	Not applicable
Panther Fusion Elution Buffer	15°C to 30°C	60 days	15°C to 30°C
Panther Fusion Oil	15°C to 30°C	60 days	15°C to 30°C
Panther Fusion Reconstitution Buffer I	15°C to 30°C	60 days	15°C to 30°C
Panther Fusion AdV/hMPV/RV Positive Control	2°C to 8°C	Single use vial	Not applicable-single use
Panther Fusion Negative Control	2°C to 8°C	Single use vial	Not applicable-single use

When reagents are removed from the Panther Fusion system, return them immediately to their appropriate storage temperatures.

[1]On board stability starts at the time the reagent is placed on the Panther Fusion system for the Panther Fusion AdV/hMPV/RV assay cartridge, FCR-S, FER-S and IC-S. The on board stability for the Panther Fusion Reconstitution Buffer I, Panther Fusion Elution Buffer and Panther Fusion Oil Reagent starts when the reagent pack is first used.

[2]If removed from the Panther Fusion system, store the assay cartridge in an air-tight container with desiccant at the recommended storage temperature.

B. Working Panther Fusion Capture Reagent-S and Panther Fusion Enhancer Reagent-S are stable for 60 days when capped and stored at 15°C to 30°C. Do not refrigerate.

C. Discard any unused reagents that have surpassed their on board stability.

D. Controls are stable until the date indicated on the vials.

E. Avoid cross-contamination during reagent handling and storage.

F. **Do not freeze reagents.**

Specimen Collection and Storage

Specimens - Clinical material collected from patient placed in an appropriate transport system. For the Panther Fusion AdV/hMPV/RV assay, this includes NP swab specimens in viral transport medium (VTM).

Samples - Represents a more generic term to describe any material for testing on the Panther Fusion system including specimens, specimens transferred into a Panther Fusion Specimen Lysis Tube, and controls.

Note: *Handle all specimens as if they contain potentially infectious agents. Use Universal Precautions.*

Note: *Take care to avoid cross-contamination during specimen handling steps. For example, discard used material without passing over open tubes.*

A. Specimen collection.

Collect NP swab specimens according to standard technique using a polyester-, rayon-, or nylon-tipped swab. Immediately place the swab specimen into 3 mL of VTM.

The following types of VTM were verified for use.

- Remel MicroTest M4, M4RT, M5 or M6 formulations
- Copan Universal Transport Medium
- BD Universal Viral Transport Medium

B. Specimen processing

1. Prior to testing on the Panther Fusion system, transfer specimen* to a Panther Fusion Specimen Lysis Tube.
   - Transfer 500 µL of the NP swab specimens to a Panther Fusion Specimen Lysis Tube.

     Note: *When testing frozen specimen, allow specimen to reach room temperature prior to processing. Do not allow specimen to exceed 3 freeze/thaw cycles.*
2. Storing specimens before testing
   a. After collection, specimens can be stored at 2°C to 8°C up to 96 hours before transfer to the Panther Fusion Specimen Lysis Tube. Remaining specimen volumes can be stored at ≤-70°C for up to 24 months.
   b. Specimen in the Panther Fusion Specimen Lysis Tube may be stored under one of the following conditions:
      - 15°C to 30°C up to 6 days or
      - 2°C to 8°C up to 3 months.

      Note: *It is recommended that specimens transferred to the Panther Fusion Specimen Lysis Tube are stored capped and upright in a rack.*

C. Specimen on board the Panther Fusion system may be archived for additional testing at a later time.

D. Storing samples after testing

1. Samples that have been assayed should be stored upright in the rack under one of the following conditions:
    - 15°C to 30°C up to 6 days or
    - 2°C to 8°C up to 3 months.
2. The samples should be covered with a new, clean plastic film or foil barrier.
3. If assayed samples need to be frozen or shipped, remove the penetrable cap and place a new non-penetrable cap on the specimen tubes. If samples need to be shipped for testing at another facility, recommended temperatures must be maintained. Prior to uncapping previously tested and recapped samples, specimen transport tubes must be centrifuged for 5 minutes at 420 Relative Centrifugal Force (RCF) to bring all of the liquid down to the bottom of the tube. Avoid splashing and cross-contamination.

Specimen Transport

Maintain specimen storage conditions as described in *Specimen Collection and Storage*.

Note: *Specimens must be shipped in accordance with applicable national, international, and regional transportation regulations.*

Panther Fusion System

The Panther Fusion system is an integrated nucleic acid testing system that fully automates all steps necessary to perform various Panther Fusion assays from sample processing through amplification, detection, and data reduction.

Reagents and Materials Provided for Panther Fusion AdV/hMPV/RV Assay

Assay Packaging

Components[1]	Part No.	Storage
Panther Fusion AdV/hMPV/RV Assay Cartridges 96 Tests Panther Fusion AdV/hMPV/RV assay cartridge, 12 tests, 8 per box	PRD-04330	2°C to 8°C
Panther Fusion Internal Control-S 960 Tests Panther Fusion Internal Control-S tube, 4 per box	PRD-04332	2°C to 8°C
Panther Fusion AdV/hMPV/RV Assay Controls Panther Fusion AdV/hMPV/RV Positive Control tube, 5 per box Panther Fusion Negative Control tube, 5 per box	PRD-04338	2°C to 8°C
Panther Fusion Extraction Reagent-S 960 Tests Panther Fusion Capture Reagent-S bottle, 240 tests, 4 per box Panther Fusion Enhancer Reagent-S bottle, 240 tests, 4 per box	PRD-04331	15°C to 30°C
Panther Fusion Elution Buffer 2400 Tests Panther Fusion Elution Buffer pack, 1200 tests, 2 per box	PRD-04334	15°C to 30°C
Panther Fusion Reconstitution Buffer I 1920 Tests Panther Fusion Reconstitution Buffer I pack, 960 tests, 2 per box	PRD-04333	15°C to 30°C
Panther Fusion Oil Reagent 1920 Tests Panther Fusion Oil Reagent pack, 960 tests, 2 per box	PRD-04335	15°C to 30°C

[1]Components can also be ordered in the following bundles:
Panther Fusion Universal Fluids Kit, PRD-04430, contains 1 each Panther Fusion Oil and Panther Fusion Elution buffer.
Panther Fusion Assay Fluids I-S, PRD-04431, contains 2 Panther Fusion Extraction Reagents-S, 2 Panther Fusion Internal Control-S, and 1 Panther Fusion Reconstitution Buffer I.

Individually Packaged Items

Items	Part No.
Panther Fusion Specimen Lysis Tubes, 100 per bag	PRD-04339

Materials Required and Available Separately

Note: *Materials available from Hologic have catalog numbers listed, unless otherwise specified.*

Material	Cat. No.
Panther System	303095
Panther Fusion Module Upgrade	PRD-04173
Panther Fusion System	PRD-04172
Aptima Assay Fluids Kit (Aptima Wash Solution, Aptima Buffer for Deactivation Fluid, and Aptima Oil Reagent)	303014 (1000 tests)
Multi-tube units (MTUs)	104772-02
Panther Waste Bag Kit	902731
Panther Waste Bin Cover	504405
Or Panther System Run Kit for Real Time Assays contains MTUs, waste bags, waste bin covers, and assay fluids	PRD-03455 (5000 tests)
Or Panther System Run Kit (when running TMA assays in parallel with real time-TMA assays) contains MTUs, waste bags, waste bin covers, auto detect*, and assay fluids	303096 (5000 tests)
Panther Fusion Tube Trays, 1008 tests, 18 trays per box	PRD-04000
Tips, 1000 µL filtered, conductive, liquid sensing, and disposable. *Not all products are available in all regions. Contact your representative for region-specific information*	901121(10612513 Tecan) 903031 (10612513 Tecan) MME-04134 (30180117 Tecan) MME-04128
Aptima penetrable caps (optional)	105668
Replacement non-penetrable caps (optional)	103036A
Replacement extraction reagent bottle caps	CL0040
P1000 pipettor and tips with hydrophobic plugs	-
Bleach, 5% to 8.25% (0.7 M to 1.16 M) sodium hypochlorite solution	-
Disposable powderless gloves	-
Plastic-back laboratory bench covers	-
Lint-free wipes	-

*Needed only for Panther Aptima TMA assays.

Panther Fusion System Test Procedure

Note: *Refer to the Panther/Panther Fusion System Operator's Manual for additional procedural information.*

A. Work Area Preparation

1. Wipe down work surfaces with 2.5% to 3.5% (0.35 M to 0.5 M) sodium hypochlorite solution. Allow the sodium hypochlorite solution to contact surfaces for at least 1 minute and follow with a deionized (DI) water rinse. Do not allow the sodium hypochlorite solution to dry. Cover the bench surface with clean, plastic-backed absorbent laboratory bench covers.
2. Clean a separate work surface where samples will be prepared using the procedure described in step A.1.
3. Clean any pipettors. Use the cleaning procedure described above (Step A.1).

B. Reagent Preparation

1. Remove the bottles of IC-S, FCR-S and FER-S from storage.
2. Open the bottles of IC-S, FCR-S and FER-S, and discard the caps. Open the TCR door on the upper bay of the Panther Fusion system.
3. Place the IC-S, FCR-S and FER-S bottles in the appropriate positions on the TCR carousel.
4. Close the TCR door.

Note: *The Panther Fusion system adds the IC-S to the FCR-S. After the IC-S is added to the FCR-S, it is referred to as wFCR-S (working FCR-S). If the FCR-S and FER-S are removed from the system, use new caps and immediately store according to the proper storage conditions.*

C. Specimen Handling

Note: *Prepare specimens per the Specimen Processing instructions in Specimen Collection and Storage before loading specimens onto the Panther Fusion system.*

1. **Do not vortex samples.**
2. Inspect sample tubes before loading into the rack. If a sample tube contains bubbles or has a lower volume than is typically observed, gently tap the bottom of the tube to bring contents to the bottom.

Note: *To avoid a processing error, ensure adequate specimen volume is added to the Panther Fusion Specimen Lysis Tube. When 500 µL of NP swab specimen is added to the Panther Fusion Specimen Lysis Tube, there is sufficient volume to perform 3 nucleic acid extractions.*

D. System Preparation

For instructions on setting up the Panther Fusion system including loading samples, assay cartridges, and universal fluids, refer to the *Panther/Panther Fusion System Operator's Manual.*

Procedural Notes

A. Controls

1. The Panther Fusion AdV/hMPV/RV Positive Control and Panther Fusion Negative Control can be loaded in any rack position, in any Sample Bay lane on the Panther Fusion system.
2. Once the control tubes are pipetted and are processed for the Panther Fusion AdV/hMPV/RV assay, they are active for up to 30 days (control frequency configured by an administrator) unless control results are invalid or a new assay cartridge lot is loaded.
3. Each control tube can be tested once.
4. Patient specimen pipetting begins when one of the following two conditions is met:
   a. Valid results for the controls are registered on the system.
   b. A pair of controls is currently in process on the system.

Quality Control

A run or specimen result may be invalidated by the Panther Fusion system if problems occur while performing the assay. Specimens with invalid results must be retested.

Negative and Positive Controls

To generate valid results, a set of assay controls must be tested. One replicate of the negative assay control and positive assay control must be tested each time a new lot of assay cartridges is loaded on the Panther Fusion system or when the current set of valid controls for an active cartridge lot have expired.

The Panther Fusion system is configured to require assay controls run at an administrator-specified interval of up to 30 days. Software on the Panther Fusion system alerts the operator when assay controls are required and does not start new tests until the assay controls are loaded and have started processing.

During processing, criteria for acceptance of the assay controls are automatically verified by the Panther Fusion system. To generate valid results, the assay controls must pass a series of validity checks performed by the Panther Fusion system.

If the assay controls pass all validity checks, they are considered valid for the administrator-specified time interval. When the time interval has passed, the assay controls are expired by the Panther Fusion system and requires a new set of assay controls to be tested prior to starting any new samples.

If any one of the assay controls fails the validity checks, the Panther Fusion system automatically invalidates the affected samples and requires a new set of assay controls be tested prior to starting any new samples.

Internal Control

An internal control is added to each sample during the extraction process. During processing, the internal control acceptance criteria is automatically verified by the Panther Fusion system software. Detection of the internal control is not required for samples that are positive for AdV, hMPV, and/or RV. The internal control must be detected in all samples that are negative for AdV, hMPV, and RV targets; samples that fail to meet that criteria will be reported as Invalid. Each sample with an Invalid result must be retested.

The Panther Fusion system is designed to accurately verify processes when procedures are performed following the instructions provided in this package insert and the *Panther/Panther Fusion System Operator's Manual.*

Interpretation of Results

The Panther Fusion system automatically determines the test results for samples and controls. Results for AdV, hMPV, and RV detection are reported separately. A test result may be negative, positive, or invalid.

Table 1 shows the possible results reported in a valid run with result interpretations.

Table 1: Result Interpretation

AdV Result	hMPV Result	RV Result	IC Result	Interpretation
Neg	Neg	Neg	Valid	AdV, hMPV, and RV not detected.
POS	Neg	Neg	Valid	AdV detected. hMPV and RV not detected.
Neg	POS	Neg	Valid	hMPV detected. AdV and RV not detected.
Neg	Neg	POS	Valid	RV detected. AdV and hMPV not detected.
POS	POS	Neg	Valid	AdV and hMPV detected. RV not detected.
Neg	POS	POS	Valid	hMPV and RV detected. AdV not detected.
POS	Neg	POS	Valid	AdV and RV detected. hMPV not detected.
POS	POS	POS	Valid	AdV, hMPV, and RV detected. Triple infections are rare. Retest to confirm result.
Invalid	Invalid	Invalid	Invalid	Invalid. There was an error in the generation of the result; retest sample.

Note: POS result will be accompanied by cycle threshold (Ct) values.

Limitations

A. Use of this assay is limited to personnel who are trained in the procedure. Failure to follow these instructions may result in erroneous results.

B. Reliable results are dependent on adequate specimen collection, transport, storage, and processing.

C. Avoid contamination by adhering to good laboratory practices and to the procedures specified in this package insert.

D. Negative results do not preclude adenovirus, human metapneumovirus, or rhinovirus infections and should not be used as the sole basis for treatment or other management decisions.

E. There is a risk of hMPV or AdV false positive results in samples containing high Rhinovirus viral content. When there are dual-positive results with the Panther Fusion AdV/hMPV/RV assay where the RV positive result has a Ct value $\leq$ 26 with an RFU signal $\geq$ 28,000 and the positive result for hMPV and/or AdV has a Ct value $\geq$ 39, it is possible that the positive result for hMPV and/or AdV is a false positive result.

F. This test does not differentiate Adenovirus subtypes (i.e., 1-58), human Metapneumovirus subtypes (i.e., A1, A2, B1, B2) or Rhinovirus species (i.e., Rhinovirus A, Rhinovirus B, or Rhinovirus C); additional testing is required to differentiate any specific Adenovirus subtypes, human Metapneumovirus subtypes, or specific Rhinovirus species in consultation with local public health departments.

G. A positive result indicates the detection of nucleic acid from the relevant virus. Nucleic acid may persist even after the virus is no longer viable.

Panther Fusion System Assay Performance

Clinical Performance: Retrospective Study

A total of 546 retrospectively collected NP swabs from patients in the US were used for evaluation with the Panther Fusion AdV/hMPV/RV assay. The results are shown in Table 2, Table 3, and Table 4.

For NP swab specimens, 500 µL was diluted into a Panther Fusion Specimen Lysis Tube containing 780 µL of Specimen Transport Media (STM) and a single replicate was tested with the Panther Fusion AdV/hMPV/RV assay. The result for each specimen was compared to reference testing using a commercial nucleic acid test (NAT). The sensitivity and specificity for the detection of AdV, hMPV, and RV nucleic acid compared to reference NAT results was determined.

Table 2: AdV Results

Specimen Type	N	AdV+		AdV-		Sensitivity 95% CI	Specificity 95% CI	Overall Agreement 95% CI
		Fusion AdV +	Fusion AdV -	Fusion AdV +	Fusion AdV -			
Nasopharyngeal Swab	546	175	3*	11**	357	98.3%	97.0%	97.4%
						95.2 - 99.4%	94.7 - 98.3%	95.7 - 98.5%

*Two out of three discordant specimens were tested with an FDA cleared assay. AdV was not detected in both specimens. Untested discordant specimens had insufficient volumes.
**Six out of eleven discordant specimens were tested with an FDA cleared assay. AdV was detected in five specimens. Untested discordant specimens had insufficient volumes.

Table 3: hMPV Results

Specimen Type	N	hMPV+		hMPV-		Sensitivity 95% CI	Specificity 95% CI	Overall Agreement 95% CI
		Fusion hMPV +	Fusion hMPV -	Fusion hMPV +	Fusion hMPV -			
Nasopharyngeal Swab	546	104	0	24*	418	100.0%	94.6%	95.6%
						96.4 - 100.0%	92.0- 96.3%	93.5 - 97.0%

*Nineteen out of 24 discordant specimens were tested with in-house developed and validated RT-PCR assay. hMPV was detected in four specimens. Untested discordant specimens had insufficient volumes.

Table 4: RV Results

Specimen Type	N	RV+		RV-		Sensitivity 95% CI	Specificity 95% CI	Overall Agreement 95% CI
		Fusion RV +	Fusion RV -	Fusion RV +	Fusion RV -			
Nasopharyngeal Swab	546	255	28*	12**	251	90.1% 86.1 - 93.1%	95.4% 92.2 - 97.4%	92.7% 90.2 - 94.6%

*Twenty three out of 28 discordant specimens were tested with an in-house developed and validated bidirectional sequencing assay. RV was not detected in 16 out of 23 tested. Untested discordant specimens had insufficient volumes.
**All 12 discordant specimens were tested with an in-house developed and validated bidirectional sequencing assay. RV was detected in nine specimens.

Clinical Performance: Prospective Study

This study was performed to demonstrate clinical performance characteristics for the Panther Fusion AdV/hMPV/RV assay. A prospective multicenter study was conducted with leftover, remnant nasopharyngeal (NP) swab specimens from male and female individuals of all ages exhibiting signs and/or symptoms of a respiratory tract infection. Four participating US pediatric/adolescent, private and/or university hospitals obtained 2961 leftover, remnant NP swab specimens. The samples were tested with the Panther Fusion AdV/hMPV/RV assay, with reference viral culture followed by direct fluorescent antibody (DFA) identification (for AdV), with an FDA-cleared assay for hMPV, and with 2 reverse transcriptase PCR assays followed by bi-directional sequencing (PCR/sequencing, for RV). FDA-cleared or validated PCR-based assays were used for discordant resolution testing for AdV and hMPV; no discordant resolution testing was performed for RV. Performance characteristics were estimated relative to reference results for each sample. Sensitivity and specificity (for AdV and hMPV) and negative and positive percent agreement (for RV) were estimated with corresponding 2-sided 95% Score CIs. Analyses were performed separately for each target analyte (AdV, hMPV, RV).

Of the 2961 specimens, 31 specimens/samples were withdrawn (due to incomplete reference testing results, insufficient volumes for testing, expiration prior to testing, or mishandling), 2930 samples were processed in valid Panther Fusion AdV/hMPV/RV runs, 2875 (98.1%) had final valid results, and 55 (1.9%) had final invalid results. Of the 2875 samples with valid Panther Fusion results, 1358 samples were from females and 1517 samples were from males (see Table 5). Of the samples with valid Panther Fusion AdV/hMPV/RV results, 11 samples with invalid reference results for AdV (n=6) or RV (n=5) were excluded from the performance analyses, leaving 2869 samples evaluable for AdV, 2875 for hMPV, and 2870 for RV.

Table 5: Summary of Subject Demographics for Prospective Samples in the Panther Fusion AdV/hMPV/RV Assay Evaluation

		N (%)
Total		2875 (100)
Sex	Female	1358 (47.2)
	Male	1517 (52.8)
Age Group	0 to 28 days	82 (2.9)
	29 days to < 2 years	757 (26.3)
	2 to 5 years	407 (14.2)
	6 to 11 years	259 (9.0)
	12 to 17 years	184 (6.4)
	18 to 21 years	73 (2.5)
	22 to 64 years	694 (24.1)
	≥ 65 years	419 (14.6)

Of the 2875 evaluable samples tested using the Panther Fusion AdV/hMPV/RV assay, 5.6% (160/2869) were positive for AdV, 3.6% (103/2875) were positive for hMPV, and 21.0% (604/2870) were positive for RV. Table 6 shows the positivity for each analyte by age group.

Table 6: Panther Fusion AdV/hMPV/RV Assay Positivity by Analyte and Age Group

	% Positivity (n/N)		
Analyte	**AdV**	**hMPV**	**RV**
All	5.6% (160/2869)	3.6% (103/2875)	21.0% (604/2870)
0 to 28 days	1,2% (1/82)	1.2% (1/82)	17.1% (14/82)
29 days to <2 years	8.7% (66/757)	5.8% (44/757)	31.5% (238/756)
2 to 5 years	11.5% (47/407)	6.9% (28/407)	28.3% (115/406)
6 to 11 years	12.4% (32/258)	2.3% (6/259)	21.3% (55/258)
12 to 17 years	2.8% (5/181)	0.5% (1/184)	16.8% (31/184)
18 to 21 years	2.7% (2/73)	1.4% (1/73)	12.3% (9/73)
22 to 64 years	0.9% (6/692)	2.2.% (15/694)	13.4% (93/692)
≥ 65 years	0.2% (1/419)	1.7% (7/419)	11.7% (49/419)

Performance characteristics for detection of AdV, hMPV, and RV in prospective NP samples were calculated (see Table 7).

Table 7: Panther Fusion AdV/hMPV/RV Assay Performance Relative to Reference Testing

Analyte	N	TP	FP	TN	FN	Prevalence[1] (95% CI)[2]	Sensitivity/ PPA[3](95% CI)[2]	Specificity/ NPA[3] (95% CI)[2]
AdV	2869	93	67[4]	2707	2[4]	3.3 (2.7-4.0)	97.9 (92.6-99.4)	97.6 (96.9-98.1)
hMPV	2875	74	29[5]	2771	1[5]	2.6 (2.1-3.3)	98.7 (92.8-99.8)	99.0 (98.5-99.3)
RV	2870	552	52[6]	2182	84[6]	22.2 (2.7-23.7)	86.8 (83.9-89.2)	97.7 (97.0-98.2)

FN= false negative, FP= false positive, NPA= negative percent agreement, PPA= positive percent agreement, TP= true positive, TN= true negative.

[1]Study prevalence reported.

[2]Score Confidence Interval.

[3]PPA and NPA apply to RV.

[4]54/67 false positive results were confirmed positive and 2/2 false negative results were confirmed negative for AdV by an FDA-cleared assay.

[5]20/29 false positive results were confirmed positive and 0/1 false negative result was confirmed negative for hMPV by PCR.

[6]No discordant resolution testing was performed for the 52 false positive and 84 false negative results for RV.

Analytical Sensitivity

The analytical sensitivity (limit of detection or LoD) of the Panther Fusion AdV/hMPV/RV assay for the NP swab specimen type was determined by testing pooled AdV/hMPV/RV negative clinical specimens spiked with the following virus cultures at various concentrations: Adenovirus (1, 3, 4, 9, 12, 40), hMPV (A1, A2, B1, B2), and RV (A-18 and B-26). At least twelve replicates were tested with each of the three reagent lots for a combined total of 36 replicates. Target specific LoD concentrations were verified by testing an additional 20 replicates with one reagent lot. Analytical sensitivity (LoD) is defined as the lowest concentration at which ≥95% of all replicates tested positive, as summarized in Table 8.

Table 8: NP Swab Sensitivity

Viral Strain	LoD Concentration
Adenovirus 1 (Species C)	$1x10^{0}$ $TCID_{50}$/mL
Adenovirus 3 (Species B)	$1x10^{0}$ $TCID_{50}$/mL
Adenovirus 4 (Species E)	$1x10^{-2}$ $TCID_{50}$/mL
Adenovirus 9 (Species D)	$1x10^{-0.5}$ $TCID_{50}$/mL
Adenovirus 12 (Species A)	$1x10^{-0.5}$ $TCID_{50}$/mL
Adenovirus 40 (Species F)	$1x10^{-1.5}$ $TCID_{50}$/mL
hMPV A1-16	$1x10^{2}$ $TCID_{50}$/mL
hMPV A2-20	$1x10^{1}$ $TCID_{50}$/mL
hMPV B1-3	$1x10^{0.5}$ $TCID_{50}$/mL
hMPV B2-8	$1x10^{0}$ $TCID_{50}$/mL
Rhinovirus A-18	$1x10^{-0.5}$ $TCID_{50}$/mL
Rhinovirus B-26	$1x10^{0}$ $TCID_{50}$/mL

Reactivity

The reactivity of the Panther Fusion AdV/hMPV/RV assay was evaluated against multiple strains of AdV, hMPV, and RV. Simulated reactivity evaluation was performed *in silico* for the types that are not available for testing. Reactivity was predicted for the AdV type 52-58 and RV type C.

Table 9: Reactivity Results

Target	Description	Concentration	AdV	hMPV	RV
Adenovirus	AdV 1	$1x10^3$ $TCID_{50}$/mL	+	-	-
	AdV 2	$1x10^3$ $TCID_{50}$/mL	+	-	-
	AdV 3	$1x10^3$ $TCID_{50}$/mL	+	-	-
	AdV 4	$1x10^3$ $TCID_{50}$/mL	+	-	-
	AdV 5	$1x10^3$ $TCID_{50}$/mL	+	-	-
	AdV 6	$1x10^3$ $TCID_{50}$/mL	+	-	-
	AdV 7	$1x10^3$ $TCID_{50}$/mL	+	-	-
	AdV 8	$1x10^3$ $TCID_{50}$/mL	+	-	-
	AdV 9	$1x10^3$ $TCID_{50}$/mL	+	-	-
	AdV 10	$1x10^3$ $TCID_{50}$/mL	+	-	-
	AdV 11	$1x10^3$ $TCID_{50}$/mL	+	-	-
	AdV 12	$1x10^3$ $TCID_{50}$/mL	+	-	-
	AdV 13	$1x10^3$ $TCID_{50}$/mL	+	-	-
	AdV 14	$1x10^3$ $TCID_{50}$/mL	+	-	-
	AdV 15	$1x10^3$ $TCID_{50}$/mL	+	-	-
	AdV 16	$1x10^3$ $TCID_{50}$/mL	+	-	-
	AdV 17	$1x10^3$ $TCID_{50}$/mL	+	-	-
	AdV 19	$1x10^3$ $TCID_{50}$/mL	+	-	-
	AdV 20	$1x10^3$ $TCID_{50}$/mL	+	-	-
	AdV 21	$1x10^3$ $TCID_{50}$/mL	+	-	-
	AdV 22	$1x10^3$ $TCID_{50}$/mL	+	-	-
	AdV 23	$1x10^3$ $TCID_{50}$/mL	+	-	-
	AdV 24	$1x10^3$ $TCID_{50}$/mL	+	-	-
	AdV 25	$1x10^3$ $TCID_{50}$/mL	+	-	-
	AdV 26	$1x10^3$ $TCID_{50}$/mL	+	-	-
	AdV 27	$1x10^3$ $TCID_{50}$/mL	+	-	-
	AdV 28	$1x10^3$ $TCID_{50}$/mL	+	-	-
	AdV 29	$1x10^3$ $TCID_{50}$/mL	+	-	-
	AdV 30	$1x10^3$ $TCID_{50}$/mL	+	-	-
	AdV 31	$1x10^3$ $TCID_{50}$/mL	+	-	-
	AdV 32	$1x10^3$ $TCID_{50}$/mL	+	-	-
	AdV 33	$1x10^3$ $TCID_{50}$/mL	+	-	-
	AdV 34	$1x10^3$ $TCID_{50}$/mL	+	-	-
	AdV 35	$1x10^3$ $TCID_{50}$/mL	+	-	-

Table 9: Reactivity Results (continued)

Target	Description	Concentration	AdV	hMPV	RV
Adenovirus	AdV 36	$1x10^3$ $TCID_{50}$/mL	+	-	-
	AdV 37	$1x10^3$ $TCID_{50}$/mL	+	-	-
	AdV 38	$1x10^3$ $TCID_{50}$/mL	+	-	-
	AdV 39	$1x10^3$ $TCID_{50}$/mL	+	-	-
	AdV 40	$1x10^3$ $TCID_{50}$/mL	+	-	-
	AdV 41	$1x10^3$ $TCID_{50}$/mL	+		-
	AdV 42	$1x10^3$ $TCID_{50}$/mL	+	-	-
	AdV 43	$1x10^3$ $TCID_{50}$/mL	+	-	-
	AdV 44	$1x10^3$ $TCID_{50}$/mL	+	-	-
	AdV 45	$1x10^3$ $TCID_{50}$/mL	+	-	-
	AdV 46	$1x10^3$ $TCID_{50}$/mL	+	-	-
	AdV 47	$1x10^3$ $TCID_{50}$/mL	+	-	-
	AdV 48	$1x10^3$ $TCID_{50}$/mL	+	-	-
	AdV 49	$1x10^3$ $TCID_{50}$/mL	+	-	-
	AdV 50	$1x10^3$ $TCID_{50}$/mL	+	-	-
	AdV 51	$1x10^3$ $TCID_{50}$/mL	+	-	-
Human Metapneumovirus	hMPV A1-16	$1x10^3$ $TCID_{50}$/mL	-	+	-
	hMPV A1-9	$1x10^3$ $TCID_{50}$/mL	-	+	-
	hMPV A2-20	$1x10^3$ $TCID_{50}$/mL	-	+	-
	hMPV A2-27	$1x10^3$ $TCID_{50}$/mL	-	+	-
	hMPV B1-3	$1x10^3$ $TCID_{50}$/mL	-	+	-
	hMPV B1-5	$1x10^3$ $TCID_{50}$/mL	-	+	-
	hMPV B2-18	$1x10^3$ $TCID_{50}$/mL	-	+	-
	hMPV B2-4	$1x10^3$ $TCID_{50}$/mL	-	+	-
	hMPV B2-8	$1x10^3$ $TCID_{50}$/mL	-	+	-
Rhinovirus*	RV A1	$1x10^2$ $TCID_{50}$/mL	-	-	+
	RV A16	$1x10^2$ $TCID_{50}$/mL	-	-	+
	RV A18	$1x10^2$ $TCID_{50}$/mL	-	-	+
	RV A32	$1x10^2$ $TCID_{50}$/mL	-	-	+
	RV A33	$1x10^2$ $TCID_{50}$/mL	-	-	+
	RV A39	$1x10^2$ $TCID_{50}$/mL	-	-	+
	RV A40	$1x10^2$ $TCID_{50}$/mL	-	-	+
	RV A44	$1x10^2$ $TCID_{50}$/mL	-	-	+
	RV A51	$1x10^2$ $TCID_{50}$/mL	-	-	+
	RV A59	$1x10^2$ $TCID_{50}$/mL	-	-	+
	RV A61	$1x10^2$ $TCID_{50}$/mL	-	-	+
	RV A65	$1x10^2$ $TCID_{50}$/mL	-	-	+

Table 9: Reactivity Results (continued)

Target	Description	Concentration	AdV	hMPV	RV
Rhinovirus*	RV A76	$1x10^2$ $TCID_{50}$/mL	-	-	+
	RV A78	$1x10^2$ $TCID_{50}$/mL	-	-	+
	RV A89	$1x10^2$ $TCID_{50}$/mL	-	-	+
	RV A100	$1x10^2$ $TCID_{50}$/mL	-	-	+
	RV B26	$1x10^2$ $TCID_{50}$/mL	-	-	+
	RV B52	$1x10^2$ $TCID_{50}$/mL	-	-	+
	RV B69	$1x10^2$ $TCID_{50}$/mL	-	-	+
	RV B70	$1x10^2$ $TCID_{50}$/mL	-	-	+
	RV B79	$1x10^2$ $TCID_{50}$/mL	-	-	+
	RV B86	$1x10^2$ $TCID_{50}$/mL	-	-	+

*Simulated reactivity evaluation performed in-silico predicted reactivity with multiple Rhinovirus C strains.

Analytical Specificity

The analytical specificity of the Panther Fusion AdV/hMPV/RV assay was evaluated by testing a panel of 64 organisms, consisting of 30 viral, 32 bacterial, and 2 yeast strains representing common respiratory pathogens or flora commonly present in nasopharynx.

Analytical specificity of the Panther Fusion AdV/hMPV/RV assay was 100% for AdV, hMPV, and RV. The list of organisms and concentrations tested is shown in Table 10.

Table 10: Specificity Results

Organism	Concentration	AdV	hMPV	RV
Acinetobacter baumannii 307-0294	$1x10^7$ CFU/mL	-	-	-
Bordetella bronchiseptica	$1x10^7$ CFU/mL	-	-	-
Bordetella parapertussis	$1x10^7$ CFU/mL	-	-	-
Bordetella pertussis	$1x10^7$ CFU/mL	-	-	-
Burkholderia cepacia Z066	$1x10^6$ CFU/mL	-	-	-
Candida albicans	$1x10^7$ CFU/mL	-	-	-
Candida glabrata	$1x10^6$ CFU/mL	-	-	-
Chlamydia pneumoniae	$1x10^5$ CFU/mL	-	-	-
Chlamydia trachomatis	$1x10^4$ CFU/mL	-	-	-
CMV Strain AD 169	$1x10^4$ $TCID_{50}$/mL	-	-	-
Coronavirus 229E	$1x10^5$ $TCID_{50}$/mL	-	-	-
Coronavirus OC43	$1x10^5$ $TCID_{50}$/mL	-	-	-
Corynebacterium diphtheria	$1x10^7$ CFU_{50}/mL	-	-	-
Coxsackie B3	$1x10^6$ $TCID_{50}$/mL	-	-	-
Coxsackie B4	$1x10^4$ $TCID_{50}$/mL	-	-	-
Coxsackie B5/10/2006	$1x10^5$ $TCID_{50}$/mL	-	-	-
Coxsackievirus A10	$1x10^4$ $TCID_{50}$/mL	-	-	-

Table 10: Specificity Results (continued)

Organism	Concentration	AdV	hMPV	RV
Coxsackievirus A21	$1x10^4$ $TCID_{50}$/mL	-	-	-
E. coli	$1x10^7$ CFU/mL	-	-	-
EBV	$1x10^6$ $TCID_{50}$/mL	-	-	-
Echovirus 11	$1x10^6$ $TCID_{50}$/mL	-	-	-
Echovirus 2	$1x10^6$ $TCID_{50}$/mL	-	-	-
Echovirus 3	$1x10^4$ $TCID_{50}$/mL	-	-	-
Echovirus 6	$1x10^6$ $TCID_{50}$/mL	-	-	-
Enterovirus 68	$1x10^5$ $TCID_{50}$/mL	-	-	-
Enterovirus 70	$1x10^4$ $TCID_{50}$/mL	-	-	-
Haemophilus Influenzae	$1x10^7$ $TCID_{50}$/mL	-	-	-
HPIV-1	$1x10^4$ $TCID_{50}$/mL	-	-	-
HPIV-2	$1x10^5$ $TCID_{50}$/mL	-	-	-
HPIV-3	$1x10^5$ $TCID_{50}$/mL	-	-	-
HPIV-4a	$1x10^4$ $TCID_{50}$/mL	-	-	-
HSV-1 Macinytre Strain	$1x10^5$ $TCID_{50}$/mL	-	-	-
HSV-2 Type 2G Strain	$1x10^5$ $TCID_{50}$/mL	-	-	-
Influenza A (H1N1)	$1x10^3$ $TCID_{50}$/mL	-	-	-
Influenza A (H3N2)	$1x10^3$ $TCID_{50}$/mL	-	-	-
Influenza B	$1x10^3$ $TCID_{50}$/mL	-	-	-
Klebsiella pneumonia	$1x10^7$ CFU/mL	-	-	-
Lactobacillus acidophilus Z048	$1x10^6$ CFU/mL	-	-	-
Lactobacillus plantarum	$1x10^6$ CFU/mL	-	-	-
Legionella pneumophila	$1x10^7$ CFU/mL	-	-	-
Measles/7/2000	$1x10^4$ $TCID_{50}$/mL	-	-	-
Moraxella catarrhalis	$1x10^7$ CFU/mL	-	-	-
Mumps virus	$1x10^5$ CFU/mL	-	-	-
Mycobacterium intracellulare	$5x10^{10}$ rRNA copies/mL	-	-	-
Mycobacterium tuberculosis	$5x10^9$ rRNA copies/mL	-	-	-
Mycoplasma pneumoniae	$1x10^6$ CFU/mL	-	-	-
Neisseria gonorrhea	$1x10^7$ CFU/mL	-	-	-
Neisseria meningitides	$1x10^7$ CFU/mL	-	-	-
Neisseria mucosa	$1x10^7$ CFU/mL	-	-	-
Polio virus 1	$1x10^6$ $TCID_{50}$/mL	-	-	-
Proteus mirabilis	$1x10^7$ CFU/mL	-	-	-
Proteus vulgaris	$1x10^7$ CFU/mL	-	-	-
Pseudomonas aeruginosa	$1x10^7$ CFU/mL	-	-	-

Table 10: Specificity Results (continued)

Organism	Concentration	AdV	hMPV	RV
RSV A	$1x10^5$ $TCID_{50}$/mL	-	-	-
RSV B	$1x10^5$ $TCID_{50}$/mL	-	-	-
Serratia marcescens Z053	$1x10^7$ CFU/mL	-	-	-
Staphlycoccus aureus	$1x10^7$ CFU/mL	-	-	-
Staphlycoccus epidermidis	$1x10^7$ CFU/mL	-	-	-
Streptococcus agalactiae	$1x10^7$ CFU/mL	-	-	-
Streptococcus pneumoniae	$1x10^7$ CFU/mL	-	-	-
Streptococcus pyogenes	$1x10^7$CFU/mL	-	-	-
Streptococcus salivarius	$1x10^7$ CFU/mL	-	-	-
Tatlockia micdadei (Legionella micdadei)	$1x10^6$ CFU/mL	-	-	-
Varicella Zoster Virus	$1x10^4$ $TCID_{50}$/mL	-	-	-

Competitive Interference

Competitive Interference of the Panther Fusion AdV/hMPV/RV assay was evaluated using a simulated clinical matrix with pairs of target viruses at two different concentrations. One of the concentrations was near the Limit of Detection (3X LoD) while the other concentration was high (1000X LoD). The presence of two viruses at varying concentrations in a single sample had no effect on the analytical sensitivity (100% detection for both targets) at the concentration noted in Table 11.

Table 11: Competitive Interference

Condition	Target 1		Target 2		AdV Result	hMPV Result	RV Result
	Description	Concentration	Description	Concentration			
1	AdV	3X LoD	hMPV	1000X LoD	+	+	-
2	AdV	3X LoD	RV	1000X LoD	+	-	+
3	hMPV	3X LoD	AdV	1000X LoD	+	+	-
4	hMPV	3X LoD	RV	1000X LoD	-	+	+
5	RV	3X LoD	AdV	1000X LoD	+	-	+
6	RV	3X LoD	hMPV	1000X LoD	-	+	+

Interference

Mucin, whole blood, and other potentially interfering substances (medications and over-the-counter or OTC products) that may be present in the samples were evaluated in the Panther Fusion AdV/hMPV/RV assay. Clinically relevant amounts of the potentially interfering substances were added to simulated clinical matrix and tested unspiked or spiked with cultured AdV, hMPV, and RV at their respective 3X LoD concentrations. The substances consisted of nasal sprays (liquid and powder), ingestible pills, lozenges, injectable and endogenous substances, as shown in Table 12.

All of the substances tested were found to have no impact on the performance of the Panther Fusion AdV/hMPV/RV assay.

Table 12: Potentially Interfering Substances

Type	Substance Name	Active Ingredient(s)	Concentration
Endogenous	Mucin	Purified mucin protein	60 µg/mL
	Human blood	Blood	2% v/v
Nasal sprays or drops	Neo-Synephrine®	Phenylephrine	15% v/v
	Anefrin	Oxymetazoline	15% v/v
	Saline	Sodium chloride	15% v/v
	Ventolin® HFA	Albuterol	15% v/v
Nasal corticosteroids	QVAR®, Beconase AQ	Beclomethasone	5% v/v
	Dexacort	Dexamethasone	5% v/v
	AEROSPAN®	Flunisolide	5% v/v
	Nasacort	Triamcinolone	5% v/v
	Rhinocort	Budesonide	5% v/v
	Nasonex	Mometasone	5% v/v
	Flonase	Fluticasone	5% v/v
Nasal gel	Zicam® (Allergy Relief)	Luffa opperculata, Galphimia, Glauca, Histaminum hydrochloricum, Sulfur	5% v/v
Throat lozenges	Chloraseptic Throat Lozenges	Benzocaine Menthol	0.63 mg/mL
Anti-viral drugs	Relenza®	Zanamivir	3.3 mg/mL
	TamiFlu	Oseltamivir	25 mg/mL
	Rebitol	Ribavirin	20 mg/mL
Antibiotic, nasal ointment	Bactroban cream	Mupirocin	10 mg/mL
Antibiotic, systemic	Tobramycin	Tobramycin	4.0 µg/mL

Carryover/Contamination

The carryover/cross-contamination study was performed with negative samples alternately placed between high positive samples and tested. High positive samples were prepared by spiking (over 10,000X LoD). Nine separate runs with negative samples and positive samples placed in a checkerboard pattern were tested over three different instruments for a combined total of 449 positive and 450 negative samples. The carryover rate was 0.2%.

Assay Precision

Panther Fusion AdV/hMPV/RV assay precision was evaluated with a 7-member panel. The panel was tested by three operators on two separate runs per day, using three reagent lots on three Panther Fusion systems over 45 days.

The panel members are described in Table 13, along with a summary of the agreement with expected results for each targets. Table 14 presents the mean and variability analysis between

instruments, between reagent lots, between operators, between days, between runs and within runs, and overall (total) for Ct.

Table 13: Panel Description and % Agreement

Target	Panel Member	% Positive	% Total Agreement (95% CI)
AdV	AdV 3x LoD	100.0% (162/162)	100.0% (97.7 - 100%)
	AdV 1x LoD	100.0% (162/162)	100.0% (97.7 - 100%)
	AdV 0.01x LoD	10.6% (17/161)	89.4% (83.7 - 93.3%)
	Negative	0.6% (1/162)	99.4% (96.6 - 99.9%)
hMPV	hMPV 3x LoD	100.0% (160/160)	100.0% (97.7 - 100%)
	hMPV 1x LoD	100.0% (161/161)	100.0% (97.7 - 100%)
	hMPV 0.01x LoD	17.9% (29/162)	82.1% (75.5 - 87.2%)
	Negative	0.0% (0/162)	100.0% (97.7% - 100.0%)
RV	RV 3x LoD	100.0% (161/161)	100.0% (97.7 - 100%)
	RV 1x LoD	100.0% (162/162)	100.0% (97.7 - 100%)
	RV 0.01x LoD	1.9% (3/160)	98.1% (94.6 - 99.4%)
	Negative	0.6% (1/162)	99.4% (96.6 - 99.9%)

Table 14: Signal Variability

Target	Panel Member	Mean Ct	Between Instrument		Between Reagent Lots		Between Operator		Between Days		Between Runs		Within Runs		Total	
			SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
AdV	AdV 3x LoD	33.5	0.1	0.4	0.0	0.1	0.0	0.0	0.1	0.3	0.2	0.7	0.4	1.2	0.5	1.5
	AdV 1x LoD	35.2	0.2	0.6	0.0	0.0	0.0	0.2	0.1	0.3	0.3	0.8	0.5	1.5	0.6	1.9
	AdV 0.01x LoD	40.4	0.3	0.8	0.0	0.0	0.0	0.0	0.0	0.0	0.9	2.4	0.7	1.9	1.3	3.2
hMPV	hMPV 3x LoD	33.5	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.8	0.8	2.4	0.8	2.5
	hMPV 1x LoD	35.1	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.7	2.0	0.7	2.0
	hMPV 0.01x LoD	40.3	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.7	0.5	1.4	1.2	3.1	1.4	3.5
RV	RV 3x LoD	32.5	0.1	0.5	0.1	0.3	0.0	0.1	0.0	0.0	0.3	1.0	0.6	2.0	0.7	2.4
	RV 1x LoD	33.8	0.1	0.5	0.1	0.5	0.0	0.0	0.1	0.4	0.0	0.0	0.8	2.6	0.9	2.8
	RV 0.01x LoD	40.6	1.9	4.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.6	1.6	2.0	5.0
IC	Negative	30.7	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.6	0.5	1.7	0.5	1.8

Reproducibility

Panther Fusion AdV/hMPV/RV assay reproducibility was evaluated at three US sites using seven panel members. Testing was performed using one lot of assay reagents and six operators (two at each site). At each site, testing was performed for at least five days. Each run had three replicates of each panel member.

A negative panel member was created using a matrix of simulated nasal swab specimen in viral transport medium (VTM). Positive panel members were created by spiking 1-2X limit of detection (LoD, low-positive) or 2-3X LoD (moderate-positive) concentrations of the target analyte into a matrix of simulated nasal swab specimen, composed of cultured human cells suspended in VTM.

The agreement with expected results was 100% for all panel members containing AdV, hMPV, or RV, as shown in Table 15.

Table 15: Agreement of Panther Fusion AdV/hMPV/RV Assay Results with Expected Results

Panel			Expected Results			Agreement with Expected Results					
						AdV		hMPV		RV	
Description	Composition	Concentration (TCID 50/mL)	AdV	hMPV	RV	N[1]	(%) 95% CI	N[1]	(%) 95% CI	N[1]	(%) 95% CI
AdV Low Pos	1-2X LoD	1.00E+00	+	-	-	88/88	100 (95.8-100)	88/88	100 (95.8-100)	88/88	100 (95.8-100)
AdV Mod Pos	2-3X LoD	3.00E+00	+	-	-	89/89	100 (95.9-100)	89/89	100 (95.9-100)	89/89	100 (95.9-100)
hMPV Low Pos	1-2X LoD	1.00E+01	-	+	-	88/88	100 (95.8-100)	88/88	100 (95.8-100)	88/88	100 (95.8-100)
hMPV Mod Pos	2-3X LoD	3.00E+01	-	+	-	89/89	100 (95.9-100)	89/89	100 (95.9-100)	89/89	100 (95.9-100)
RV Low Pos	1-2X LoD	3.16E-01	-	-	+	89/89	100 (95.9-100)	89/89	100 (95.9-100)	89/89	100 (95.9-100)
RV Mod Pos	2-3X LoD	9.48E-01	-	-	+	87/87	100 (95.8-100)	87/87	100 (95.8-100)	87/87	100 (95.8-100)
Neg	N/A	N/A	-	-	-	87/87	100 (95.8-100)	87/87	100 (95.8-100)	87/87	100 (95.8-100)

CI= Score confidence interval, Mod= moderate, N/A= not applicable, Neg= negative, Pos= positive, $TCID_{50}$/mL= 50% tissue culture infective dose (measure of virus titer)

[1]A total of 13 samples had final invalid results and were not included in the calculation of overall agreement.

The total AdV, hMPV, and RV signal variability, measured as %CV, ranged from 1.70% to 4.90% in low and moderate positive panel members. For the sources of variation except the 'within-run' factor, %CV values were ≤1.72% as shown in Table 16.

Table 16: Signal Variability of the Panther Fusion AdV/hMPV/RV Assay by Panel Member

			Between Sites		Between Operators		Between Days		Between Runs		Within Runs		Total	
Panel Description	N	Mean Ct	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
AdV Low Pos	88	35.1	0.35	0.99	0.13	0.38	0.0	0.0	0.0	0.0	0.58	1.65	0.69	1.96
AdV Mod Pos	89	33.5	<0.1	0.18	0.17	0.49	0.21	0.63	<0.1	<0.1	0.50	1.49	0.57	1.70
hMPV Low Pos	88	35.1	0.23	0.64	0.0	0.0	0.0	0.0	0.0	0.0	1.14	3.25	1.16	3.32
hMPV Mod Pos	89	33.1	0.0	0.0	0.24	0.71	0.57	1.72	<0.1	<0.1	1.50	4.53	1.62	4.90
RV Low Pos	89	33.7	0.14	0.43	0.24	0.72	0.22	0.66	<0.1	<0.1	0.83	2.45	0.90	2.67
RV Mod Pos	87	32.3	0.16	0.48	<0.1	0.16	0.38	1.18	<0.1	0.13	0.71	2.20	0.83	2.55

Ct= cycle threshold, CV= coefficient of variation, Mod= moderate, Pos= positive, SD= standard deviation
Note: If variability from some factors was numerically negative, SD and CV are shown as 0.0.

The signal variability, measured as %CV, was ≤1.94% between sites, between operators, between days, or overall for the Panther Fusion AdV/hMPV/RV assay positive controls (see Table 17).

Table 17: Signal Variability of the Panther Fusion AdV/hMPV/RV Assay Controls

				Between Sites		Between Operators		Between Days		Between Runs		Within Runs		Total	
Control	**Analyte**	**N**	**Mean Ct**	**SD**	**CV (%)**	**SD**	**CV (%)**	**SD**	**CV (%)**	**SD**	**CV (%)**	**SD**	**CV (%)**	**SD**	**CV (%)**
Pos	AdV	30	33.0	0.0	0.0	0.0	0.0	<0.1	0.24	0.0	0.0	0.27	0.82	0.28	0.85
	hMPV	30	34.0	<0.1	0.21	<0.1	0.18	0.0	0.0	0.0	0.0	0.30	0.89	0.32	0.93
	RV	30	31.8	0.0	0.0	0.0	0.0	0.32	1.02	0.0	0.0	0.53	1.65	0.62	1.94

Ct= cycle threshold, CV= coefficient of variation, Pos= positive, SD= standard deviation
Note: If variability from some factors was numerically negative, SD and CV are shown as 0.0.

Bibliography

1. Centers for Disease Control and Prevention. National Respiratory and Enteric Virus Surveillance System. Centers for Disease Control and Prevention Web site. http://www.cdc.gov/surveillance/nrevss/. Accessed October, 2015.
2. Kahn, J.S. 2006. Epidemiology of human metapneumovirus. Clin. Microbiol. Rev. 19:546-557.
3. http://www.cdc.gov/adenovirus/hcp/clinical-overview.html. Accessed June 2016.
4. Martin, Malcolm A.; Knipe, David M.; Fields, Bernard N.; Howley, Peter M.; Griffin, Diane; Lamb, Robert (2007). Fields' virology. Philadelphia: Wolters Kluwer Health/Lippincott Williams & Wilkins. p. 2395.
5. http://www.cdc.gov/adenovirus/outbreaks.html. Accessed June 2016.
6. Kahn, J.S., Epidemiology of human metapneumovirus. Clin Microbiol Rev, 2006. 19(3): p. 546-57.
7. http://www.cdc.gov/surveillance/nrevss/hmpv/clinical.html. Accessed June 2016.
8. Park, J. Y., Yun, K. W., Lim, J. W., Lee, M. K., Lim, I. S., and Choi, E. S. (2016) Clinical and genetic features of human metapneumovirus infection in children. Pediatrics International, 58: 22–26. doi: 10.1111/ped.12782.
9. Anzueto, A. and M.S. Niederman. 2003. Diagnosis and treatment of rhinovirus respiratory infections. Chest 123:1664-1672.
10. Centers for Disease Control and Prevention/National Institutes of Health. Biosafety in Microbiological and Biomedical Laboratories (BMBL) 6th Edition; Web site. https://www.cdc.gov/labs/BMBL.html. November, 2020.
11. Clinical and Laboratory Standards Institute (CLSI). Document M29. Protection of Laboratory Workers from Occupationally Acquired Infections. CLSI Web site https://clsi.org/standards/products/microbiology/documents/m29/ (April 4, 2022).

Contact Information and Revision History

Hologic, Inc.
10210 Genetic Center Drive
San Diego, CA 92121 USA

Australian Sponsor Address:
Hologic (Australia & New Zealand) Pty Ltd
Macquarie Park NSW 2113

EC REP
Hologic BV
Da Vincilaan 5
1930 Zaventem
Belgium

For country-specific Technical Support and Customer Service email address and telephone number, visit www.hologic.com/support.

Serious incidents occurring in relation to the device in the European Union should be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

AW-23710-001 Rev. 001
2022-07

Revision History	Date	Description
AW-23710 Rev. 001	July 2022	• Created Panther Fusion AdV/hMPV/RV assay IFU AW-23710 Rev. 001 based on AW-16164 Rev. 005 for regulatory compliance with IVDR. • Updated EU hazard information. • Updated sections of Clinical Performance: Retrospective and Prospective, Analytical Specificity and Reproducibility study information, Materials Required and Available Separately, and Bibliography. • Added information regarding specimen stability. • Updated contact information including: EC Rep, CE Mark, Australian Rep information, and technical support. • Miscellaneous style and formatting updates.