510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:
K172629

B. Purpose for Submission:
The purpose of this submission is to show that the Panther Fusion AdV/mMPV/RV Assay (for use on the Panther Fusion system) is substantially equivalent to the FilmArray Respiratory Panel 2 (K170604) and to obtain clearance for the Panther Fusion AdV/mMPV/RV Assay.

C. Measurand:
The Panther Fusion AdV/mMPV/RV Assay detects Adenovirus (AdV) hexon gene DNA, human Metapneumovirus (hMPV) nucleocapsid gene RNA, and Rhinovirus (RV) 5' untranslated region (UTR) RNA, isolated from nasopharyngeal swab specimens from patients with signs and symptoms of respiratory infection.

D. Type of Test:
This assay is a multiplex nucleic acid assay that detects and differentiates Adenovirus (AdV) human Metapneumovirus (hMPV), and Rhinovirus (RV) through nucleic acid extraction, amplification, and detection using real-time PCR and RT-PCR. All steps of the assay are automated, after the manual addition of sample into the sample lysis tube (SLT), and performed within the Panther and Panther Fusion system.

E. Applicant:
Hologic, Inc.

F. Proprietary and Established Names:
Panther Fusion AdV/hMPV/RV Assay

G. Regulatory Information:

1. Regulation section:
   21 CFR §866.3890 – Respiratory Viral Panel Multiplex Nucleic Acid Assay

2. Classification:
   Class II

3. Product code:
   OCC- Respiratory Virus Panel Nucleic Acid Assay System
   OEM – Human Metapneumovirus RNA Assay System
   OOI – Real Time Nucleic Acid Amplification System

4. Panel:
   Microbiology (83)

H. Intended Use:

1. Intended use(s):
   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.

2. Indication(s) for use:
   Same as Intended Use

3. Special conditions for use statement(s):
   For Prescription Use Only

4. Special instrument requirements:
   Panther Fusion System

I. Device Description:

The Panther Fusion AdV/hMPV/RV Assay is a multiplex real-time reverse transcriptase PCR (RT-PCR) *in vitro* diagnostic test developed for use on the fully automated Panther Fusion system to detect and differentiate AdV, hMPV and RV directly from the nasopharyngeal swab specimens.

The Panther Fusion AdV/hMPV/RV Assay involves the following steps:
a) Sample lysis; prior to processing and testing on the Panther Fusion system, specimens are transferred to a tube containing specimen transport media (STM) that lyses the cells, releases target nucleic acid, and protects them from degradation during storage.
b) 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. 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 is used to monitor specimen processing, amplification, and detection. Magnetic particles with covalently bound oligonucleotides mediate the nucleic acid capture. Capture oligonucleotides hybridize to total nucleic acid in the test specimen. Hybridized nucleic

acid is then separated from the lysed specimen in a magnetic field. Wash and aspiration steps remove extraneous components debris from the reaction tube. The elution step elutes purified nucleic acid.
c) Elution transfer and multiplex PCR and RT-PCR; Eluted nucleic acid is transferred to a Panther Fusion reaction tube already containing oil and reconstituted master mix. A reverse transcriptase generates a DNA copy of the target sequence for hMPV and RV. Target specific forward and reverse primers and probes then amplify targets while simultaneously detecting and discriminating multiple target types via multiplex RT-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 positive result for each analyte will be accompanied by the cycle threshold (Ct) value.

J. Substantial Equivalence Information:

1. Predicate device name(s):
   FilmArray Respiratory Panel 2

2. Predicate 510(k) number(s):
   K170604

3. Comparison with predicate:

Table 1. Comparison of Similarities between Predicate Device and Subject Device

Item	Panther Fusion AdV/hMPV/RV Assay (Subject Device)	FilmArray Respiratory Panel 2 Assay (Predicate Device)
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	The FilmArray Respiratory Panel 2 (RP2) is a multiplexed nucleic acid test intended for use with FilmArray 2.0 or FilmArray Torch systems for the simultaneous qualitative detection and identification of multiple respiratory viral and bacterial nucleic acids in nasopharyngeal swabs (NPS) obtained from individuals suspected of respiratory tract infections. The following organism types and subtypes are identified using the FilmArray RP2: Adenovirus, Coronavirus 229E, Coronavirus HKU1, Coronavirus NL63, Coronavirus OC43, Human Metapneumovirus, Human Rhinovirus/Enterovirus, Influenza A, Influenza A subtype H1, Influenza A subtype H3, Influenza A subtype H1-2009, Influenza B,

	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.	Parainfluenza Virus 1, Parainfluenza Virus 2, Parainfluenza Virus 3, Parainfluenza Virus 4, Respiratory Syncytial Virus, *Bordetella parapertussis, Bordetella pertussis*, *Chlamydia pneumoniae,* and *Mycoplasma pneumoniae.*
Organisms Detected	Adenovirus, human Metapneumovirus, Rhinovirus (No cross-reactivity with Enterovirus)	Adenovirus, human Metapneumovirus, Rhinovirus. Can also detect Coronavirus 229E, Coronavirus HKU1, Coronavirus NL63, Coronavirus OC43, Influenza A, Influenza A subtype H1, Influenza A subtype H3, Influenza A subtype H1-2009, Influenza B, Parainfluenza Virus 1, Parainfluenza Virus 2, Parainfluenza Virus 3, Parainfluenza Virus 4, Respiratory Syncytial Virus, *Bordetella parapertussis, Bordetella pertussis*, *Chlamydia pneumoniae,* and *Mycoplasma pneumoniae.* Cannot distinguish Rhinovirus and Enterovirus.
Assay Controls	Same	Internal control in each sample. External control processed at periodic intervals.
Patient Population	Same	Male and female patients with signs/symptoms of respiratory infection
Specimen Types	Same	Nasopharyngeal (NP) swab specimens
Analyte	Same	RNA/DNA
Technology Principle of Operation	Same	Multiplex nucleic acid amplification (RT-PCR)
Platform	Automated multiplex RT-PCR platform. Uses Panther Fusion system for all steps including specimen addition, reagent preparation, nucleic acid extraction, amplification, detection and result processing.	Automated nested multiplex PCR platform. Uses the FilmArray Pouch Loading Station to prepare FilmArray pouch (add specimen and hydrate reagents). Uses the FilmArray Instrument to process prepared FilmArray pouch for nucleic acid extraction, amplification, end-point detection and result processing.
Time to Obtain Test Results	Approximately 2.5 hours	About 45 minutes

K. Standard/Guidance Document Referenced (if applicable):

CLSI guidelines: EP15-A3: User Verification of Precision and Estimation of Bias and EP05-A3: Evaluation of Precision of Quantitative Measurement Procedures.

L. Test Principle:

The assay detects viral nucleic acids that have been extracted from a patient respiratory sample. A multiplex Real-time RT-PCR reaction is carried out under optimized conditions generating amplicons for AdV, hMPV and RV. The Internal Control-S (IC-S) is added to each test specimen before processing to act as a control for specimen processing, amplification, and detection. Identification of AdV, hMPV and RV, and the IC-S occurs using target-specific primers and fluorescent-labeled probes that hybridize to conserved regions in the viral genomes.

Table 3. Assay primer and probe targets

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

***** Internal Control-S is a non-infectious synthetic nucleic acid sequence that is extracted and detected through targeted primers and probes.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

   a. Precision/Reproducibility:

   Precision and Reproducibility studies were designed based on the recommendations in the following CLSI guidelines: EP15-A3 and EP05-A3.

   Precision

   The precision study was conducted at 1 site, with 3 operators, 3 lots of reagents, and 3 Panther Fusion instruments. The study was conducted over the course of 46 days with 18 testing days. The table below shows the samples included in the testing panel. Each panel member was tested in triplicate over 2 runs.

Table 4. Precision Sample Panel

Panel Member ID	Description
1	AdV (1x LOD)
2	hMPV (3x LOD)/ RV (0.01x LOD)
3A	AdV (3x LOD)/ hMPV (0.01x LOD)
4	hMPV (1x LOD)
5	RV (3x LOD)/ AdV (0.01x LOD)
6	RV (1x LOD)
7	Negative

Out of 61 total runs, there were no invalid runs. Seven runs were invalidated by the study lead, 4 due to operator not following protocol, 1 due to one of the days' 2 runs being invalid (if any run in a day was invalid, both runs were invalidated and retested on a different day), and 2 due to instrument errors. Of the 54 valid runs, 2 results were invalid due to instrument error.

Table 5. Precision study results

Target	Panel Description	Member ID	Valid N	% Positive (pos n/valid n)	% Agreement (95% CI)
RV	RV 3x LOD	5	161	100.0% (161/161)	100.0% (97.7-
	RV 1x LOD	6	162	100.0% (162/162)	100.0% (97.7-
	RV 0.01x	2	160	1.9% (3/160)	98.1% (94.6-
	Negative	7	162	0.6% (1/162)	99.4% (96.6-
AdV	AdV 3x LOD	3A	162	100.0% (162/162)	100.0% (97.7-
	AdV 1x LOD	1	162	100.0% (162/162)	100.0% (97.7-
	AdV 0.01x	5	161	10.6% (17/161)	89.4% (83.7-
	Negative	7	162	0.6% (1/162)	99.4% (96.6-
hMPV	hMPV 3x	2	160	100.0% (160/160)	100.0% (97.7 -
	hMPV 1x	4	161	100.0% (161/161)	100.0% (97.7 -
	hMPV 0.01x	3A	162	17.9% (29/162)	82.1% (75.5-
	Negative	7	162	0.0% (0/162)	100.0% (97.7 -

Note: Results are shown only for the intended targets. Panel members co-spiked with two different targets are presented twice.

All panel members at or above 1X LoD were ≥ 95% positive for the expected target. Negative panel member was 0.0% positive for AdV and hMPV but 0.6% positive for RV (1/162 positive). Results for all panel members at 0.01X LoD were <95% positive as expected. This performance is acceptable and demonstrates acceptable assay precision.

Table 6. Precision study Ct signal variability analysis results*

Target	Member ID	Mean Ct	Between Instrument		Between Reagent Lot		Between Operator		Between Days		Between Runs		Within Run		Total	
			SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
RV	5	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
	6	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
	2	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
AdV	3	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
	1	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
	5	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	2	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
	4	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
	3	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
IC	7	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

*Results are shown only for the intended targets. Panel members co-spiked with two different targets are presented twice.

The mean and variability analysis between instruments, between reagent lots, between operators, between days, between runs, within runs, and overall (total) for Ct values is shown in the table above. Overall %CV was $\leq$ 2.8%. The greatest source of variability was the RV 1X LoD panel member (within-run % CV of 2.6%). Overall variability was low and the study demonstrates assay variability within an acceptable range.

Reproducibility

The reproducibility study was conducted at 3 sites, with two operators at each site. Each operator performed one run per day using one reagent lot. Testing was performed over five non-consecutive days at each site using one Panther Fusion instrument. Each operator tested three replicates of each panel member in every run. The table below shows the sample panel that was used for the reproducibility study.

Table 7. Reproducibility study sample panel

Panel Member	Viral Analyte	Panel Description	Target Analyte Concentration Level
1	AdV	Low Positive	1X LOD
2	AdV	Moderate Positive	3X LOD
3	hMPV	Low Positive	1X LOD

4	hMPV	Moderate Positive	3X LOD
5	RV	Low Positive	1X LOD
6	RV	Moderate Positive	3X LOD
7	Negative	Negative	Negative

Analysis was performed to determine percent agreement with expected results and the associated two-sided 95% confidence interval (CI) as well as sources of variation: 1) within runs, 2) between runs, 3) between operators, 4) between days, and 5) between sites (instruments). A total of 33 runs with 630 samples were tested yielding 30 valid runs and 617 valid results. Two runs were invalid due to operator error and 1 run was invalid due to instrument error.

Table 8. Reproducibility study results

Panel Member		AdV		hMPV		RV	
		Agreement with Expected	**95% Confidence Interval**	**Agreement with Expected**	**95% Confidence Interval**	**Agreement with Expected**	**95% Confidence Interval**
1	Low Pos	88/88	100 (95.8-100)	88/88	100 (95.8-100)	88/88	100 (95.8-100)
2	Mod Pos	89/89	100 (95.8-100)	89/89	100 (95.8-100)	89/89	100 (95.8-100)
3	Low Pos	88/88	100 (95.8-100)	88/88	100 (95.8-100)	88/88	100 (95.8-100)
4	Mod Pos	89/89	100 (95.8-100)	89/89	100 (95.8-100)	89/89	100 (95.8-100)
5	Low Pos	89/89	100 (95.8-100)	89/89	100 (95.8-100)	89/89	98.9 (93.8-99.8)
6	Mod Pos	87/87	100 (95.8-100)	87/87	100 (95.8-100)	87/87	100 (95.8-100)
7	Neg	87/87	100 (95.8-100)	87/87	100 (95.8-100)	87/87	100 (95.8-100)

Agreement was 100% for AdV, hMPV, and RV moderate and low positive panel members and for the negative panel member tested with the Panther Fusion AdV/hMPV/RV Assay.

The table below shows the variability of the Panther Fusion AdV/hMPV/RV Assay between sites, operator, days, and runs. The largest source of variation was the 'within runs' factor with %CV values ranging from 1.49% to 4.53%. All other sources of variation had %CV values less than 1.72%. The total performance variability (%CV) across all assay results was <5%.

Table 9. Reproducibility study Ct signal variability analysis results

Target Analyte	Panel Member	Average CT	Between Sites		Between Operators		Between Days		Between Runs		Within Run		Total	
			SD	CV(%)	SD	CV(%)	SD	CV(%)	SD	CV(%)	SD	CV(%)	SD	CV(%)
AdV	1	35.12	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	2	33.49	<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	3	35.07	0.23	0.64	0.0		0.0	0.0	0.0	0.0	0.0	1.14	3.25	1.16
hMPV	4	33.12	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	5	33.74	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	6	32.32	0.16	0.48	<0.1	0.16	0.38	1.18	<0.1	0.13	0.71	2.20	0.83	2.55

The reproducibility study data presented demonstrates an acceptable reproducibility for this assay.

b. Linearity/assay reportable range:

Not applicable; this is a qualitative assay

c. Expected values (controls, calibrators, or methods), Stability:

Controls:
The assay contains an internal control (IC-S) which is added to each test specimen. The IC-S is first added to the Panther Fusion Capture Reagent-S (FCR-S) to make the working Panther Fusion Capture Reagent-S (wFCR-S). The control material wFCR-S is added to the specimen after lysis. This control monitors specimen processing, amplification, and detection. It does not monitor lysis of the sample.

Two external controls are also included with this assay in a single use vial, the Panther Fusion AdV/hMPV/RV Positive Control and the Panther Fusion Negative Control. The control validity was studied during the Reproducibility Study where a positive and negative control was tested during every run (30 runs total). All controls produced the expected result.

Stability:
Stability studies have been performed to support the following claims:

Sample Stability:
The study data supports specimen stability at the following storage temperatures and storage times:

- Stored neat at 2-8°C for at least 96 hours
- Stored diluted (transferred to Specimen Lysis Tube (SLT)) at 30°C for at least 144 hours (6 days)
- Stored diluted (transferred to SLT) at 2-8°C for at least 3 months.
- 3 Freeze/Thaw cycles (-70°C to Room Temperature = 1 Freeze/Thaw cycle)

Kit Stability:

Stability of the whole kit and all the included reagents was evaluated. The reagents included in the kit are: Panther Fusion AdV/hMPV/RV Assay Cartridge, Panther Fusion Capture Reagent-S, Panther Fusion Enhancer Reagent-S, Panther Fusion Internal Control-S, Panther Fusion Elution Buffer, Panther Fusion Oil, Panther Fusion Reconstitution Buffer I, Panther Fusion AdV/hMPV/RV Assay Positive Control, and Panther Fusion Negative Control. Two types of stability studies were performed: Shelf-Life Stability and In-Use (or on-board) Stability. The study data supports the following storage conditions.

Table 10. Reagent storage conditions

Reagent	Unopened Storage	On-Board/Open Stability	Opened Storage
Panther Fusion AdV/hMPV/RV Assay Cartridge	2°C to 8°C	60 days	2°C to 8°C
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

Shipping Stability:

The purpose of this study was to demonstrate that the exposure to extreme hot or cold temperatures potentially encountered by the Panther Fusion AdV/hMPV/RV Assay components during shipment would not impact the performance of the assay. One lot of components and controls for the Panther Fusion AdV/hMPV/RV Assay were tested in this study. All components were exposed to the extreme temperatures in their final container closure systems.

The Panther Fusion AdV/hMPV/RV Assay components and controls were cycled between the extreme low temperatures (-40 ±5°C), room temperature (28 ±2°C), and extreme high temperature (55±5°C). One stress cycle consisted of incubation for at least 9 hours at extreme high temperatures, then at least 15 hours at room-temperature, followed by at least 9 hours at extreme low temperature, followed by at least 15-hour incubation at room temperature (1 cycle = approx. 48 hours). A total of 5 cycles were performed to evaluate the worst case scenario of shipment to customers.

All runs tested gave the expected result for spiked and un-spiked samples. The Panther Fusion AdV/hMPV/RV Assay and its components are not altered by exposure to extreme hot or cold temperatures that may be encountered during shipping.

Carryover:

This study was conducted to determine the carry-over (cross-contamination) rate of the Panther Fusion AdV/hMPV/RV Assay when high titer AdV/hMPV/RV positive samples are tested interspersed throughout the runs of negative samples on the Panther Fusion system.

One run was comprised of 100 negative samples (baseline run), followed by three runs comprised of 50 positive samples and 50 negative samples in a checkerboard pattern (positive and negative samples loaded in alternate order).

Negative samples were comprised of simulated clinical matrix in VTM. Positive samples consisted of simulated clinical matrix in VTM or pooled lower respiratory tract specimens (LRT) spiked with RV A and AdV-1 at 10^4 TCID50/mL (> 10,000X LoD). Testing was completed on three Panther Fusion systems using one lot of reagents.

Table 11. Carryover study results

Runs	**Samples**	**Detection Channel (Target), % Positive (reactive n/valid n)**				
		Valid N	**FAM (RV)**	**HEX (AdV)**	**ROX (hMPV)**	**RED677 (IC)**
Baseline	Negative	300	**0.7% (2/300)**	0.0% (0/300)	0.0% (0/300)	100% (300/300)
Runs 2-4	Negative	450	**0.2% (1/450)**	0.0% (0/450)	0.0% (0/450)	100% (450/450)
Runs 2-4	Positive	449*	**100% (449/449)**	100% (449/449)	9.1% (41/449)***	100.0% (449/449)**

* one invalid reaction due to result processing error
**% validity is shown.
***All 41 hMPV positive results were from LRT positive samples (n=224) made with pooled clinical specimens of which several were hMPV positive. None of the samples in simulate clinical matrix (SCM) were positive (n=225).

Baseline runs comprised of negative samples showed 0.7% positivity for RV (2/300 positive for RV) and 0.0% positivity for AdV (0/300 positive for AdV) across 3 instruments. One negative sample from the checkerboard runs was RV A positive, resulting in 1/450 or 0.2% overall carry-over contamination rate for RV. This sample was adjacent to positive SCM samples. All negative samples from the checkerboard runs were AdV negative, resulting in 0.0% overall carry-over contamination rate for AdV. All valid positive samples from the checkerboard runs (runs 2-4) were RV and AdV positive (449/449). A subset of the LRT positive samples were low hMPV positive (41/449, with Ct > 38) although they were not spiked with hMPV. This is expected because LRT positive samples were made from pooled clinical specimens, some of which were positive for hMPV. Carryover was detected in this study and this will be noted in the limitations section of the package insert.

d. Detection limit:

The purpose of this study was to evaluate and verify the analytical sensitivity and Limit of Detection (LoD) of Adenovirus (AdV), Human Metapneumovirus (hMPV) and Human Rhinovirus (RV) in pooled negative clinical nasopharyngeal swab specimens when tested with the Panther Fusion AdV/hMPV/RV Assay. Target-specific LoD values were obtained using multiple strains for each targeted virus. The strains tested for each virus are listed in the table 12 below.

Table 12.

Virus	Strain
Adenovirus	Strain 1 Species C
	Strain 3 Species B
	Strain 4 Species E
	Strain 9 Species D
	Strain 12 Species A
	Strain 40 Species F
Human Metapneumovirus	Strain A1-16
	Strain A2-20
	Strain B1-3
	Strain B2-8
Rhinovirus	Strain A-18
	Strain B-26

Dilutions of each virus were prepared in pooled negative clinical nasopharyngeal swab (NP) specimens and tested with 11-13 replicates per concentration, using three reagent lots. Testing was performed on three Panther Fusion systems per concentration and per reagent lot for a total of at least 36 replicates per virus type. Determined LoD value for each virus type was verified by testing newly prepared samples for at least 20 replicates using one lot of reagents. The LoD value for each virus is listed in the table below.

Table 13. LoD study results

Target Type	Strain (Species)	Conc. (TCID50/mL)	Detection Channel (Target)			
			FAM (RV)	HEX (AdV)	ROX(hMPV)	RED677 (IC)
Adenovirus	1 (C)	10^0.0	0.0% (0/20)	**100.0% (20/20)**	0.0% (0/20)	100.0% (20/20)
	3 (B)	10^0.0	0.0% (0/20)	**100.0% (20/20)**	0.0% (0/20)	100.0% (20/20)
	4 (E)	10^-2.0	0.0% (0/20)	**100.0% (20/20)**	0.0% (0/20)	100.0% (20/20)
	9 (D)	10^-0.5	0.0% (0/20)	**100.0% (20/20)**	0.0% (0/20)	100.0% (20/20)
	12 (A)	10^-0.5	0.0% (0/20)	**100.0% (20/20)**	0.0% (0/20)	100.0% (20/20)
	40 (F)	10^-1.5	0.0% (0/20)	**100.0% (20/20)**	0.0% (0/20)	100.0% (20/20)
hMPV	A1-16	10^2.0	0.0% (0/20)	0.0% (0/20)	**100.0% (20/20)**	100.0% (20/20)
	A2-20	10^1.0	0.0% (0/20)	0.0% (0/20)	**100.0% (20/20)**	100.0% (20/20)
	B1-3	10^0.5	0.0% (0/20)	0.0% (0/20)	**100.0% (20/20)**	100.0% (20/20)
	B2-8	10^0.0*	0.0% (0/60)	0.0% (0/60)	**95.0% (57/60)**	100.0% (60/60)
Rhinovirus	A-18	10^-0.5	**100.0% (20/20)**	0.0% (0/20)	0.0% (0/20)	100.0% (20/20)
	B-26	10^0.0	**95.0% (19/20)**	0.0% (0/20)	0.0% (0/20)	100.0% (20/20)

*Initial 20 replicates tested were less than 95% positive for hMPV, therefore additional replicates (n=40) were tested. Positivity result from all the replicates tested (initial n=20 and n=40 additional) is shown.

e. Analytical specificity:

Interfering Substances

This study evaluated the performance of the Panther Fusion AdV/hMPV/RV Assay in the presence of medications, over the counter products, and other potentially interfering substances. Assay results were evaluated to determine if the presence of potentially interfering substances in analyte-negative or analyte-positive samples affected assay performance. Panels made of Simulated Clinical Matrix with potentially interfering substances were divided into two aliquots. One aliquot was tested un-spiked and the other aliquot was tested with intended targets (AdV, hMPV, RV) spiked at 0.5 log above LoD. Panels were tested with 3 reagent lots. The substances tested are listed below:

Table 14. Interfering substances sample panel

Panel	Type	Potentially Interfering Substance	Active Ingredient(s)	Concen-tration
1	Endogenous	Mucin	Purified mucin protein	60 µg/mL
2		Human blood	NA	2% v/v
3	Nasal sprays or drops	Neo-Synephrine®	Phenylephrine	15% v/v
		Anefrin	Oxymetazoline HCl .05%	15% v/v
		Saline	Sodium chloride with preservatives	15% v/v
		Ventolin® HFA	Albuterol (Albuterol Sulfate)	15% v/v
4	Nasal corticosteroids	QVAR®, Beconase AQ	Beclomethasone (Beclomethasone	5% v/v
		Dexacort	Dexamethasone	5% v/v
		AEROSPAN®	Flunisolide	5% v/v
5		Nasacort	Triamcinolone	5% v/v
		Rhinocort	Budesonide	5% v/v
		Nasonex	Mometasone (Mometasone furoate)	5% v/v
		Flonase	Fluticasone (Fluticasone (propionate))	5% v/v
6	Nasal gel	Zicam® (Allergy Relief)	Luffa opperculata, Galphimia, Glauca, Histaminum	5% v/v
7	Throat lozenges	Chloraseptic Throat Lozenges	Benzocaine	0.63 mg/mL
			Menthol	
8	Anti-viral drugs	Relenza®	Zanamivir	3.3 mg/mL
		TamiFlu	Oseltamivir	25 mg/mL
		Rebitol	Ribavirin	20 mg/mL
9	Antibiotic, nasal ointment	Bactroban cream	Mupirocin	10 mg/mL
10	Antibiotic, systemic	Tobramycin	Tobramycin	4.0 µg/mL
11	No interfering Substance (SCM only)	N/A	N/A	N/A

Table 15. Interfering substances study results

Test condition	Panel and Description	Detection Channel (Target), % Positive (reactive n/valid n)			
		FAM (RV)	HEX (AdV)	ROX	RED677 (IC)
Un-spiked	Panel 1 (Mucin)	0.0% (0/9)	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)
	Panel 2 (Blood)	0.0% (0/9)	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)
	Panel 3 (Nasal Sprays or Drops: Neo-synephrine, Anefrin, Saline, Ventolin)	0.0% (0/9)	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)
	Panel 4 (Nasal Corticosteroids: QVAR, Beconase AQ, Dexacort, Aerospan)	0.0% (0/9)	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)
	Panel 5 (Nasal Corticosteroids: Nasacort, Rhinocort, Naonex, [illegible])	0.0% (0/9)	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)
	Panel 6 (Nasal Gel: Zicam)	0.0% (0/9)	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)
	Panel 7 (Throat Lozenges: Chloraseptic Throat Lozenges)	0.0% (0/9)	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)
	Panel 8 (Anti-viral Drugs: Relenza, TamiFlu, Rebitol)	0.0% (0/9)	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)
	Panel 9 (Antibiotic, Nasal Ointment: Bactroban Cream)	0.0% (0/9)	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)
	Panel 10 (Antibiotic Systemic: Tobramycin)	0.0% (0/9)	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)
	Panel 11 (SCM)	0.0% (0/9)	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)
Spiked with RV (Rhinovirus A-18 at 10^0.0 TCID 50/mL)	Panel 1 (Mucin)	100.0% (9/9)	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)
	Panel 2 (Blood)	100.0% (9/9)	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)
	Panel 3 (Nasal Sprays or Drops: Neo-synephrine, Anefrin, Saline, Ventolin)	100.0% (9/9)	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)
	Panel 4 (Nasal Corticosteroids: QVAR, Beconase AQ, Dexacort, Aerospan)	100.0% (9/9)	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)
	Panel 5 (Nasal Corticosteroids: Nasacort, Rhinocort, Naonex, Flonase)	100.0% (9/9)	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)
	Panel 6 (Nasal Gel: Zicam)	100.0% (9/9)	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)
	Panel 7 (Throat Lozenges: Chloraseptic Throat Lozenges)	100.0% (9/9)	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)
	Panel 8 (Anti-viral Drugs: Relenza, TamiFlu, Rebitol)	93.3% (14/15)	0.0% (0/15)	0.0% (0/15)	100.0% (9/15)
	Panel 9 (Antibiotic, Nasal Ointment: Bactroban Cream)	100.0% (9/9)	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)
	Panel 10 (Antibiotic Systemic: Tobramycin)	100.0% (9/9)	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)
	Panel 11 (SCM)	93.3% (14/15)	0.0% (0/15)	0.0% (0/15)	100.0% (9/15)
Spiked with	Panel 1 (Mucin)	0.0% (0/9)	100.0% (9/9)	0.0% (0/9)	100.0% (9/9)
	Panel 2 (Blood)	0.0% (0/9)	100.0% (9/9)	0.0% (0/9)	100.0% (9/9)
	Panel 3 (Nasal Sprays or Drops: Neo-synephrine, Anefrin, Saline, Ventolin)	0.0% (0/9)	100.0% (9/9)	0.0% (0/9)	100.0% (9/9)
	Panel 4 (Nasal Corticosteroids: QVAR, Beconase AQ, Dexacort,	0.0% (0/9)	100.0% (9/9)	0.0% (0/9)	100.0% (9/9)

AdV (Adenovirus 1C at 10^0.5 TCID 50/mL)	Panel 5 (Nasal Corticosteroids: Nasacort, Rhinocort, Naonex, [illegible])	0.0% (0/9)	100.0% (9/9)	0.0% (0/9)	100.0% (9/9)
	Panel 6 (Nasal Gel: Zicam)	0.0% (0/9)	100.0% (9/9)	0.0% (0/9)	100.0% (9/9)
	Panel 7 (Throat Lozenges: Chloraseptic Throat Lozenges)	0.0% (0/9)	100.0% (9/9)	0.0% (0/9)	100.0% (9/9)
	Panel 8 (Anti-viral Drugs: Relenza, TamiFlu, Rebitol)	0.0% (0/9)	100.0% (9/9)	0.0% (0/9)	100.0% (9/9)
	Panel 9 (Antibiotic, Nasal Ointment: Bactroban Cream	0.0% (0/9)	100.0% (9/9)	0.0% (0/9)	100.0% (9/9)
	Panel 10 (Antibiotic Systemic: Tobramycin)	0.0% (0/9)	100.0% (9/9)	0.0% (0/9)	100.0% (9/9)
	Panel 11 (SCM)	0.0% (0/9)	100.0% (9/9)	0.0% (0/9)	100.0% (9/9)
Spiked with hMPV (hMPV A2-20 at 10^1.5 TCID 50/mL)	Panel 1 (Mucin)	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)	100.0% (9/9)
	Panel 2 (Blood)	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)	100.0% (9/9)
	Panel 3 (Nasal Sprays or Drops: Neo-synephrine, Anefrin, Saline, Ventolin)	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)	100.0% (9/9)
	Panel 4 (Nasal Corticosteroids: QVAR, Beconase AQ, Dexacort, [illegible])	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)	100.0% (9/9)
	Panel 5 (Nasal Corticosteroids: Nasacort, Rhinocort, Naonex, [illegible])	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)	100.0% (9/9)
	Panel 6 (Nasal Gel: Zicam)	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)	100.0% (9/9)
	Panel 7 (Throat Lozenges: Chloraseptic Throat Lozenges)	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)	100.0% (9/9)
	Panel 8 (Anti-viral Drugs: Relenza, TamiFlu, Rebitol)	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)	100.0% (9/9)
	Panel 9 (Antibiotic, Nasal Ointment: Bactroban Cream	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)	100.0% (9/9)
	Panel 10 (Antibiotic Systemic: Tobramycin)	0.0% (0/10)	0.0% (0/10)	100.0% [illegible]	100.0% [illegible]
	Panel 11 (SCM)	0.0% (0/10)	0.0% (0/10)	100.0%	100.0%

For panel members 1-7 and 9-10, all spiked samples were 100.0% positive for intended targets. All un-spiked panel members were 0.0% positive for all targets other than IC. For panel members 8 and 11 spiked with RV, both were less than 100% positive (88.9% or 8/9 positive). One negative was from 1 reagent lot (Reagent lot B) for both cases. Additional 6 replicates for both panel members were tested with reagent lot B, and all were RV positive (combined 14/15 or 93.5% positive). The negative result is likely caused by either lower than intended spiked titer (because of LoD variation with different RV strains); this is supported by the results from panel member 11 (control) which also had 14/15 positive. Sensitivity for RV was deemed not negatively affected by the interfering substances added to Panel member 8 because the one positive result for a panel member with no interfering substances added was also erroneously called negative. This study demonstrates that the substances tested do not interfere with the performance of the Panther Fusion AdV/hMPV/RV Assay at the concentrations tested.

Competitive Interference:

The purpose of this study was to demonstrate that samples tested with the Panther Fusion AdV/hMPV/RV Assay that are co-infected with multiple types of targeted organisms do not inhibit the detection of either one (competitive interference). Each targeted organism (AdV, hMPV and RV) was tested. Co-infection Panels were made by spiking one target organism at high concentration (1000X LoD) and another target organism at low concentration (3X LoD) in simulated clinical matrix. Testing was completed on one Panther Fusion system using one lot of reagents. The test panel members are shown below.

Table 16. Competitive Interference study sample panel composition

Panel member	Target 1		Target 2	
	Description	Concentration	Description	Concentration*
1	AdV	Low (3X LoD)	hMPV	High (1000X LoD)
2	AdV	Low (3X LoD)	RV	High (1000X LoD)
3	hMPV	Low (3X LoD)	AdV	High (1000X LoD)
4	hMPV	Low (3X LoD)	RV	High (1000X LoD)
5	RV	Low (3X LoD)	AdV	High (1000X LoD)
6	RV	Low (3X LoD)	hMPV	High (1000X LoD)

There was no interference observed. All panel members were 100% positive for both targets. Results are summarized below.

Table 17. Competitive interference study results

Panel Member	Target 1 (Low)*	Target 2 (High)*	Detection Channel (Target) , % Positive (reactive n/valid n)			
			FAM (RV)	HEX (AdV)	ROX (hMPV)	RED677 (IC)
1	AdV	hMPV	0.0% (0/6)	100.0% (6/6)	100.0% (6/6)	100.0% (6/6)
2	AdV	RV	100.0% (6/6)	100.0% (6/6)	0.0% (0/6)	100.0% (6/6)
3	hMPV	AdV	0.0% (0/6)	100.0% (6/6)	100.0% (6/6)	100.0% (6/6)
4	hMPV	RV	100.0% (6/6)	0.0% (0/6)	100.0% (6/6)	100.0% (6/6)
5	RV	AdV	100.0% (6/6)	100.0% (6/6)	0.0% (0/6)	100.0% (6/6)
6	RV	hMPV	100.0% (6/6)	0.0% (0/6)	100.0% (6/6)	100.0% (6/6)

Cross-Reactivity:

This study evaluated the analytical specificity and sensitivity of the Panther Fusion AdV/hMPV/RV Assay in the presence of non-targeted microorganisms that could be present in the clinical specimen. Assay results were evaluated to determine if the presence of spiked microbes in analyte negative or analyte positive specimens affected assay performance. Panel members were composed of 3-5 different microorganisms spiked into simulated clinical matrix at either 10^3-10^7 TCID50/mL (for virus) or 10^4-10^7 CFU/mL or IFU/mL (for bacteria). Each panel member was split into two aliquots. One aliquot was spiked with one representative strain of intended targets (AdV, hMPV, RV) to a final concentration of 0.5 log above LoD to assess interference (negative effect on

sensitivity). The other aliquot was left un-spiked to assess specificity. Panels were tested with 3 reagent lots. Panel descriptions are shown below.

Table 18. Cross-reactivity study microorganisms tested

Panel members	Microorganisms	Concentration	Units
1	*Bordetella bronchiseptica*	1.00E+07	CFU/ml
	Bordetella parapertussis	1.00E+07	CFU/ml
	Bordetella pertussis	1.00E+07	CFU/ml
	Candida albicans	1.00E+07	CFU/ml
	Candida glabrata	1.00E+06	CFU/ml
	Chlamydia pneumoniae	1.00E+05	CFU/ml
	Chlamydia trachomatis	1.00E+04	CFU/ml
2	CMV Strain AD 169	1.00E+04	$TCID_{50}$/ml
	Coronavirus 229E	1.00E+05	$TCID_{50}$/ml
	Coronavirus OC43	1.00E+05	$TCID_{50}$/ml
	Coxsackievirus A10	1.00E+04	$TCID_{50}$/ml
	Coxsackievirus A21	1.00E+04	$TCID_{50}$/ml
	Coxsackie B3	1.00E+06	$TCID_{50}$/ml
	Coxsackie B4	1.00E+04	$TCID_{50}$/ml
	Coxsackie B5/10/2006	1.00E+05	$TCID_{50}$/ml
3	*Corynebacterium diphtheria*	1.00E+07	$TCID_{50}$/ml
	Escherichia. coli	1.00E+07	CFU/ml
	Haemophilus influenzae	1.00E+07	$TCID_{50}$/ml
4	Echovirus 11	1.00E+06	TCID50/mL
	Echovirus 2	1.00E+06	TCID50/mL
	Echovirus 3	1.00E+04	$TCID_{50}$/ml
	Echovirus 6	1.00E+06	$TCID_{50}$/ml
5	Enterovirus 68	1.00E+05	$TCID_{50}$/ml
	Enterovirus 70	1.00E+04	$TCID_{50}$/ml
	EBV	1.00E+06	$TCID_{50}$/ml
	Varicella Zoster Virus	1.00E+04	$TCID_{50}$/ml
6	IA/California/07/2009 H1N1	1.00E+03	$TCID_{50}$/ml
	IA/Switzerland/9715293/2013 H3N2	1.00E+03	$TCID_{50}$/ml
	IB/Brisbane/33/08	1.00E+03	$TCID_{50}$/ml
	RSV A	1.00E+05	$TCID_{50}$/ml
	RSV B	1.00E+05	$TCID_{50}$/ml
7	HPIV-1	1.00E+04	$TCID_{50}$/ml
	HPIV-2	1.00E+05	$TCID_{50}$/ml
	HPIV-3 *	1.00E+05	$TCID_{50}$/ml

	HPIV-4a	1.00E+04	$TCID_{50}$/ml
8	HSV-1 Macinytre Strain	1.00E+05	$TCID_{50}$/ml
	HSV-2 Type 2G Strain	1.00E+05	TCID50/ml
9	*Klebsiella pneumonia*	1.00E+07	CFU/ml
	Lactobacillus acidophilus Z048	1.00E+06	CFU/ml
	Lactobacillus plantarum	1.00E+06	CFU/ml
	Tatlockia micdadei (Legionella micdadei)	1.00E+06	CFU/ml
	Legionella pneumophila	1.00E+07	CFU/ml
10	Measles/7/2000	1.00E+04	$TCID_{50}$/ml
	Mumps virus	1.00E+05	CFU/ml
	Polio virus 1	1.00E+06	$TCID_{50}$/ml
11	*Mycobacterium intracellulare*	5.00E+10	rRNA copies/ml, estimated to be equivalent to 1.00E+07 CFU/mL
	Mycobacterium tuberculosis	5.00E+09	rRNA copies/ml, estimated to be equivalent to 1.00E+06 CFU/mL
	Mycoplasma pneumoniae	1.00E+06	CFU/ml
	Moraxella catarrhalis	1.00E+07	CFU/ml
12	*Neisseria gonorrhea*	1.00E+07	CFU/ml
	Neisseria meningitides	1.00E+07	CFU/ml
	Neisseria mucosa	1.00E+07	CFU/ml
13	*Proteus mirabilis*	1.00E+07	CFU/ml
	Proteus vulgaris	1.00E+07	CFU/ml
	Pseudomonas aeruginosa	1.00E+07	CFU/ml
14	*Staphlycoccus aureus*	1.00E+07	CFU/ml
	Staphlycoccus epidermidis	1.00E+07	CFU/ml
	Streptococcus agalactiae	1.00E+07	CFU/ml
	Streptococcus pneumoniae	1.00E+07	CFU/ml
	Streptococcus pyogenes	1.00E+07	CFU/ml
	Streptococcus salivarius	1.00E+07	CFU/ml
15	*Acinetobacter baumannii* 307-0294	1.00E+07	CFU/ml
	Burkholderia cepacia Z066	1.00E+06	CFU/ml
	Serratia marcescens Z053	1.00E+07	CFU/ml
16	No microorganisms (SCM only)	N/A	N/A

Un-spiked -- specificity

When the panel member 1 was tested un-spiked (0% positive expected), it was 0% positive for hMPV and AdV but 1/9 positive for RV. Additional 6 replicates were

tested with the same reagent lot. Additional test results were all RV negative resulting in combined RV positivity of 6.7% (1/15). Additionally, new SCM was prepared and spiked with seven different organisms included in Panel 1, all new samples were 0.0% positive for RV. These results suggest that one RV positive result was likely due to cross-contamination during sample handling and not due to cross-reactivity. For all the rest of panel members, when tested un-spiked, all were 0% positive for AdV, hMPV and RV.

Spiked with RV – Sensitivity for RV

When the panel members 2, 4, 5, 10 and 15 were spiked with RV at 0.5 log above LOD and tested for total 9 replicates (3 replicates per each of 3 reagent lots), result was 0.0% positive for AdV and hMPV as expected but less than 100.0% positive for RV (55.6%, 66.7%, 88.9%, 77.8% and 77.8% respectively). For the panel member 5, only 1/9 replicates were negative for RV. Additional 9 replicates were tested with the reagent lot which generated one negative (lot B); all new samples were RV positive. The combined RV positivity of all samples tested was 94.4% (17/18). These results suggest that one RV negative result was likely due to cross-contamination during sample handling and not due to cross-reactivity.

For the panel members 2, 4, 10 and 15, since more than one replicate was RV negative, they were tested again with RV added at higher concentration. When RV was spiked at 1 log above LOD and tested with 3 reagent lots (3 replicates per each of 3 reagent lots), panel members 2, 4, 10 and 15 all generated 100.0% RV positive and 0.0% AdV and hMPV positive. Result suggest that one more organisms included in those panel members do exert slight inhibition against RV detection when RV is present at low concentration (less than 1 log above LOD).

Spiked with AdV – Sensitivity for AdV

When the panel member 15 was spiked with AdV at 0.5 log above LOD and tested for total 9 replicates, result was 0.0% positive for RV and hMPV as expected but was 22.2% positive (expected 100% positive) for AdV (2/9). The panel member 15 was tested again with AdV added at higher concentration. When AdV was spiked at 1 log above LOD it was 100.0% AdV positive and 0.0% hMPV and RV positive. Result suggest that one more organisms included in those panel members do exert slight inhibition against AdV detection when AdV is present at low concentration (less than 1 log above LOD).

Spiked with hMPV – Sensitivity for hMPV

Cross-Reactivity panel members tested with hMPV spiked at 0.5 log above LOD were 100% positive for hMPV and 0.0% positive for RV and AdV. No inhibition is observed for hMPV detection.

The final results for all conditions tested are detailed in the table below.

Table 19. Cross-reactivity study results

Panel Membe	Spiked organism*	Detection Channel (Target), % Positive (reactive n/valid n)			
		FAM (RV)	HEX (AdV)	ROX (hMPV)	RED677(IC)
1	RV	100.0% (9/9)	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)
	AdV	0.0% (0/9)	100.0% (9/9)	0.0% (0/9)	100.0% (9/9)
	hMPV	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)	100.0% (9/9)
	Un-spiked	6.7% (1/15)	0.0% (0/15)	0.0% (0/15)	100.0% (15/15)
1-follow up^	Bordetella bronchiseptica	0.0% (0/3)	0.0% (0/3)	0.0% (0/3)	100.0% (3/3)
	Bordetella parapertussis	0.0% (0/3)	0.0% (0/3)	0.0% (0/3)	100.0% (3/3)
	Bordetella pertussis	0.0% (0/3)	0.0% (0/3)	0.0% (0/3)	100.0% (3/3)
	Candida albicans	0.0% (0/3)	0.0% (0/3)	0.0% (0/3)	100.0% (3/3)
	Candida glabrata	0.0% (0/3)	0.0% (0/3)	0.0% (0/3)	100.0% (3/3)
	Chlamydia trachomatis	0.0% (0/3)	0.0% (0/3)	0.0% (0/3)	100.0% (3/3)
	Chlamydophila pneumoniae	0.0% (0/3)	0.0% (0/3)	0.0% (0/3)	100.0% (3/3)
2	RV	55.6% (5/9)	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)
	RV, spiked at 1 log above LOD (RV A-18 at 10^0.5 TCID50/mL)	100.0% (9/9)	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)
	AdV	0.0% (0/9)	100.0% (9/9)	0.0% (0/9)	100.0% (9/9)
	hMPV	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)	100.0% (9/9)
	Un-spiked	0.0% (0/9)	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)
3	RV	100.0% (9/9)	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)
	AdV	0.0% (0/9)	100.0% (9/9)	0.0% (0/9)	100.0% (9/9)
	hMPV	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)	100.0% (9/9)
	Un-spiked	0.0% (0/9)	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)
4	RV	66.7% (6/9)	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)
	RV, spiked at 1 log above LOD (RV A-18 at 10^0.5 TCID50/mL)	100.0% (9/9)	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)
	AdV	0.0% (0/9)	100.0% (9/9)	0.0% (0/9)	100.0% (9/9)
	hMPV	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)	100.0% (9/9)
	Un-spiked	0.0% (0/9)	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)
5	RV	94.4% (17/18)	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)
	AdV	0.0% (0/9)	100.0% (9/9)	0.0% (0/9)	100.0% (9/9)
	hMPV	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)	100.0% (9/9)
	Un-spiked	0.0% (0/9)	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)
6	RV	100.0% (9/9)	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)
	AdV	0.0% (0/9)	100.0% (9/9)	0.0% (0/9)	100.0% (9/9)
	hMPV	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)	100.0% (9/9)
	Un-spiked	0.0% (0/9)	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)
7	RV	100.0% (9/9)	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)
	AdV	0.0% (0/9)	100.0% (9/9)	0.0% (0/9)	100.0% (9/9)
	hMPV	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)	100.0% (9/9)
	Un-spiked	0.0% (0/9)	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)

8	RV	100.0% (9/9)	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)
	AdV	0.0% (0/9)	100.0% (9/9)	0.0% (0/9)	100.0% (9/9)
	hMPV	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)	100.0% (9/9)
	Un-spiked	0.0% (0/9)	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)
9	RV	100.0% (9/9)	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)
	AdV	0.0% (0/9)	100.0% (9/9)	0.0% (0/9)	100.0% (9/9)
	hMPV	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)	100.0% (9/9)
	Un-spiked	0.0% (0/9)	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)
10	RV	77.8% (7/9)	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)
	RV, spiked at 1 log above LOD (RV A-18 at 10^0.5 TCID50/mL)	100.0% (9/9)	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)
	AdV	0.0% (0/9)	100.0% (9/9)	0.0% (0/9)	100.0% (9/9)
	hMPV	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)	100.0% (9/9)
	Un-spiked	0.0% (0/9)	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)
11	RV	100.0% (9/9)	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)
	AdV	0.0% (0/9)	100.0% (9/9)	0.0% (0/9)	100.0% (9/9)
	hMPV	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)	100.0% (9/9)
	Un-spiked	0.0% (0/9)	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)
12	RV	100.0% (9/9)	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)
	AdV	0.0% (0/9)	100.0% (9/9)	0.0% (0/9)	100.0% (9/9)
	hMPV	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)	100.0% (9/9)
	Un-spiked	0.0% (0/9)	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)
13	RV	100.0% (9/9)	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)
	AdV	0.0% (0/9)	100.0% (9/9)	0.0% (0/9)	100.0% (9/9)
	hMPV	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)	100.0% (9/9)
	Un-spiked	0.0% (0/9)	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)
14	RV	100.0% (9/9)	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)
	AdV	0.0% (0/9)	100.0% (9/9)	0.0% (0/9)	100.0% (9/9)
	hMPV	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)	100.0% (9/9)
	Un-spiked	0.0% (0/9)	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)
15	RV	77.8% (7/9)	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)
	RV, spiked at 1 log above LOD (RV A-18 at 10^0.5 TCID50/mL)	100.0% (9/9)	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)
	AdV	0.0% (0/9)	22.2% (2/9)	0.0% (0/9)	100.0% (9/9)
	AdV, spiked at 1 log above LOD (AdV 1C at 10^1.0 TCID50/mL)	0.0% (0/9)	100.0% (9/9)	0.0% (0/9)	100.0% (9/9)
	hMPV	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)	100.0% (9/9)
	Un-spiked	0.0% (0/9)	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)
16	RV	100.0% (9/9)	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)
	AdV	0.0% (0/9)	100.0% (9/9)	0.0% (0/9)	100.0% (9/9)
	hMPV	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)	100.0% (9/9)
	Un-spiked	0.0% (0/9)	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)

f. Assay cut-off:

The relative fluorescence units (RFU) range is the difference between maximum and minimum fluorescent signal seen in a sample during amplification. For Panther Fusion AdV/hMPV/RV positive samples, the amplification curve rises above the background florescence RFU resulting in a high RFU range for the intended targets. The IC resembles a positive target and also has a high RFU range. For the Panther Fusion AdV/hMPV/RV negative samples the amplification curve remains flat resulting in a low RFU range for the intended targets. This difference in RFU range is the primary criteria used to distinguish positive and negative specimens. RFU range thresholds were set at 500 AdV, 600 for RV, and hMPV for determination of positivity in their specific channels (HEX, FAM, ROX, respectively). RFU range threshold for IC was set at 500 for determination of validity in its specific channel (RED677). The RFU range thresholds were set based on data from multiple RFU readings from known positive samples. The cycle number at which the amplification curve crosses target specific assigned RFU range threshold value is called Ct. Target specific Ct will be generated only for positive valid reaction for that target.

2. Comparison studies:

a. Method comparison with predicate device:

The results of the assay were compared to shell vial culture followed by Direct Fluorescence Antibody Testing (DFA) for adenovirus, Prodesse Pro hMPV+ for human metapneumovirus, and PCR followed by sequencing for rhinovirus. For additional details on the comparator please see the "Clinical Studies" section below.

b. Matrix comparison:

The following five commonly used Viral Transport Media (VTM) types were evaluated: M4, M4RT, M5, M6, and BD UTM (identical to Copan Universal Transport Medium). Five types of simulated clinical matrix (SCM) were prepared by spiking $2x10^4$ cells/mL of HeLa cells in to each of five VTM types listed above. Cultured AdV strain 1 species C, hMPV Strain A2-20, and RV strain A-18 were then spiked into each VTM type at concentrations 1 log below LoD, 0.5 log above LoD, and 1 log above LoD to prepare the VTM panel. Panel was tested once right after sample spiking (T=0) and again after being stored at 2-8°C for at least 72 hours (T>72 hrs). Testing was completed on four Panther Fusion systems using one lot of reagents.

Two aberrant results were detected; one AdV was positive for RV and one hMPV was positive for RV. Because the false positives were observed only once there was no further investigation of the results. The aberrant result appears to be an anomaly with an unknown cause.
All panel members for AdV, hMPV, and RV tested positive in all VTM types.

The testing results support use of all 5 Viral Transport Media types evaluated.

Table 20. Matrix Comparison Results for AdV Positivity

Concentration and Sample Type	Time point	VTM Type	Valid N	RV (Fam)		Adv (Hex)		hMPV (Rox)		IC	
				Pos N	% Positive	Pos N	% Positive	Pos N	% Positive	Pos N	% Positive
- 1 log LOD	T= 0	M4	6	0	0.0	6	100.0	0	0.0	6	100.0
		M4RT	6			5	83.3			6	100.0
		M5	6			5	83.3			6	100.0
		M6	6			6	100.0			6	100.0
		BD UT	6			5	83.3			6	100.0
	T>72 hr	M4	6	0	0.0	6	100.0	0	0.0	6	100.0
		M4RT	6			3	50.0			6	100.0
		M5	6			3	50.0			6	100.0
		M6	6			6	100.0			6	100.0
		BD UT	6			5	83.3			6	100.0
+ 0.5log LOD	T= 0	M4	24	0	0.0	24	100.0	0	0.0	24	100.0
		M4RT	24			24	100.0			24	100.0
		M5	24			24	100.0			24	100.0
		M6	24			24	100.0			24	100.0
		BD UT	24			24	100.0			24	100.0
	T>72 hr	M4	24	0	0.0	24	100.0	0	0.0	24	100.0
		M4RT	24			24	100.0			24	100.0
		M5	24			24	100.0			24	100.0
		M6	24			24	100.0			24	100.0
		BD UT	24			24	100.0			24	100.0
+ 1 log LOD	T= 0	M4	6	0	0.0	6	100.0	0	0.0	6	100.0
		M4RT	6			6	100.0			6	100.0
		M5	6			6	100.0			6	100.0
		M6	6			6	100.0			6	100.0
		BD UT	6			6	100.0			6	100.0
	T>72 hr	M4*	6	1	16.7	6	100.0	0	0.0	6	100.0
		M4RT	6	0	0.0	6	100.0			6	100.0
		M5	6	0	0.0	6	100.0			6	100.0
		M6	6	0	0.0	6	100.0			6	100.0
		BD UT	6	0	0.0	6	100.0			6	100.0

Table 21. Matrix Comparison Results for hMPV Positivity

Concentration and Sample Type	Time point	VTM Type	Valid N	RV (Fam)		Adv (Hex)		hMPV (Rox)		IC	
				Pos N	% Positive	Pos N	% Positive	Pos N	% Positive	Pos N	% Positive
- 1 log LOD of hMPV	T= 0	M4	6	0	0.0	0	0.0	4	66.7	6	100.0
		M4RT	6					5	83.3	6	100.0
		M5	6					3	50.0	6	100.0
		M6	6					5	83.3	6	100.0
		BD UT	6					6	100.0	6	100.0
	T>72 hr	M4	6	0	0.0	0	0.0	6	100.0	6	100.0
		M4RT	6					5	83.3	6	100.0
		M5	6					6	100.0	6	100.0
		M6	6					5	83.3	6	100.0
		BD UT	6					6	100.0	6	100.0
+ 0.5log LOD of hMPV	T= 0	M4	24	0	0.0	0	0.0	24	100.0	24	100.0
		M4RT	24	0	0.0			24	100.0	24	100.0
		M5	24	0	0.0			24	100.0	24	100.0
		M6*	24	1	4.2			24	100.0	24	100.0
		BD UT	24	0	0.0			24	100.0	24	100.0
	T>72 hr	M4	24	0	0.0	0	0.0	24	100.0	24	100.0
		M4RT	24					24	100.0	24	100.0
		M5	24					24	100.0	24	100.0
		M6	24					24	100.0	24	100.0
		BD UT	24					24	100.0	24	100.0
+ 1 log LOD of hMPV	T= 0	M4	6	0	0.0	0	0.0	6	100.0	6	100.0
		M4RT	6					6	100.0	6	100.0
		M5	6					6	100.0	6	100.0
		M6	6					6	100.0	6	100.0
		BD UT	6					6	100.0	6	100.0
	T>72 hr	M4	6	0	0.0	0	0.0	6	100.0	6	100.0
		M4RT	6					6	100.0	6	100.0
		M5	6					6	100.0	6	100.0
		M6	6					6	100.0	6	100.0
		BD UT	6					6	100.0	6	100.0

Table 22. Matrix Comparison Results for RV Positivity

Concentration and Sample Type	Time point	VTM Type	Valid N	RV (Fam)		Adv (Hex)		hMPV (Rox)		IC	
				Pos N	% Positive	Pos N	% Positive	Pos N	% Positive	Pos N	% Positive
- 1 log LOD of RV	T= 0	M4	6	2	33.3	0	0.0	0	0.0	6	100.0
		M4RT	6	5	83.3					6	100.0
		M5	6	5	83.3					6	100.0
		M6	6	6	100.0					6	100.0
		BD UTM	6	6	100.0					6	100.0
	T>72 hr	M4	6	5	83.3	0	0.0	0	0.0	6	100.0
		M4RT	6	4	66.7					6	100.0
		M5	6	5	83.3					6	100.0
		M6	6	5	83.3					6	100.0
		BD UTM	6	4	66.7					6	100.0
+ 0.5log LOD of RV	T= 0	M4	24	24	100.0	0	0.0	0	0.0	24	100.0
		M4RT	24	24	100.0					24	100.0
		M5	24	24	100.0					24	100.0
		M6	24	24	100.0					24	100.0
		BD UTM	24	24	100.0					24	100.0
	T>72 hr	M4	24	24	100.0	0	0.0	0	0.0	24	100.0
		M4RT	24	24	100.0					24	100.0
		M5	24	24	100.0					24	100.0
		M6	24	24	100.0					24	100.0
		BD UTM	24	24	100.0					24	100.0
+ 1 log LOD of RV	T= 0	M4	6	6	100.0	0	0.0	0	0.0	6	100.0
		M4RT	6	6	100.0					6	100.0
		M5	6	6	100.0					6	100.0
		M6	6	6	100.0					6	100.0
		BD UTM	6	6	100.0					6	100.0
	T>72 hr	M4	6	6	100.0	0	0.0	0	0.0	6	100.0
		M4RT	6	6	100.0					6	100.0
		M5	6	6	100.0					6	100.0
		M6	6	6	100.0					6	100.0
		BD UTM	6	6	100.0					6	100.0

3. Clinical studies:

a. Clinical Sensitivity and Specificity:

Four clinical sites participated in this study. Each site obtained, processed, and tested prospectively collected specimens with the Panther Fusion AdV/hMPV/RV Assay as well as performed the comparator shell vial culture/DFA testing (AdV), Prodesse Pro hMPV+ (hMPV), and dual reverse transcriptase-PCR (RT-PCR) followed by bi-directional sequencing (RV). The study was conducted from October 2016 to March 2017 and 3 lots of reagents were used during the study. Fresh and frozen samples were tested during this clinical study.

Specimens included in this study were leftover, remnant nasopharyngeal swabs collected from patients with signs and symptoms of respiratory infection. The specimen inclusion criteria were:

- A minimum volume of 1.75mL
- Stored at 2C to 8C for less than 72 hours after collection
- Specimen was collected in appropriate VTM (M4, M4RT, M5, M6, Copan Universal Transport Medium or BD Universal Transport Medium)

Specimens were excluded if they were deemed unsuitable for reference culture or Panther Fusion testing. Examples of unsuitable samples are: specimen leakage, unacceptable VTM, storage time or temperature exceeded, etc.

This study collected 2961 clinical specimens, 6 were withdrawn after it was determined that they did not meet the specimen inclusion criteria and further 25 were withdrawn by patient request. A total of 55 specimens had invalid results on the Panther Fusion AdV/hMPV/RV Assay. Additional samples were removed from analysis due to invalid reference testing, 6 invalid results from the reference testing for AdV and 5 for RV. The overall invalid rate for the Panther Fusion AdV/hMPV/RV Assay was 2.8% (80/2875).

The tables below show the performance of the Panther Fusion AdV/hMPV/RV Assay, the data are stratified by analyte: AdV, hMPV, and RV. All data below represent performance for prospectively collected specimens. The data includes 1118 freshly collected and tested specimens and 1757 samples prospectively collected and stored frozen until testing.

Table 23. Panther Fusion AdV/hMPV/RV Assay Performance Relative to Comparator Assay for Prospective Nasopharyngeal (NP) Samples

Analyte	N	TP	FP	TN	FN	Prevalence (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
AdV	2869	93	67	2707	2	3.3% (2.7-4.0)	97.9 % (92.6-99.4)	97.6 % (96.9-98.1)
hMPV	2875	74	29	2771	1	2.6 % (2.1-3.3)	98.7 % (92.8-99.8)	99.0% (98.5-99.3)
RV	2870	552	52	2182	84	22.2% (20.7-23.7)	86.8 % (83.9-89.2)	97.7% (97.0-98.2)

N= total number of specimens, FN=false negative, FP=false positive, TP=true positive, TN=true negative

Fresh vs. frozen performance was analyzed by comparing the sensitivity and specificity of fresh vs. frozen samples for each analyte. The data below does not suggest a significant difference in performance between fresh and frozen samples for any analyte using the Panther Fusion AdV/hMPV/RV Assay.

Table 24. Fresh vs. frozen study samples

	Fresh					Frozen				
Analyte	**N**	**TP**	**FP**	**TN**	**FN**	**N**	**TP**	**FP**	**TN**	**FN**
AdV	1112	32	19	1060	1	1757	61	48	1647	1
hMPV	1118	12	7	1098	1	1757	62	22	1673	0
RV	1118	181	29	880	28	1752	371	23	1302	56

Table 25. Fresh vs. frozen study performance

	Sensitivity (95% CI)		Specificity (95%)	
Analyte	**Fresh**	**Frozen**	**Fresh**	**Frozen**
AdV	97.0 (84.7-99.5)	98.4 (91.4-99.7)	98.2 (97.3-98.9)	97.2 (96.3-97.9)
hMPV	92.3 (66.7-98.6)	100 (94.2-100)	99.4 (98.7-99.7)	98.7 (98.0-99.1)
RV	86.6 (81.3-90.6)	86.9 (83.4-89.8)	96.8 (95.5-97.8)	98.3 (97.4-98.8)

4. Expected values/Reference range:

The Panther Fusion AdV/hMPV/RV Assay clinical study included a total of 2869 prospectively collected fresh NP swab specimens. The number and percentage of cases positive for one or more viruses AdV, hMPV and RV, as determined by the Panther Fusion AdV/hMPV/RV Assay are shown by age category below.

Table 26. Observed prevalence for AdV, hMPV, and RV during the clinical study

	% 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	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)

N. Instrument Name:

Panther System and Panther Fusion System, software version 6.1

O. System Descriptions:

The software and instrument were reviewed in submission K171963, no changes or additions were made to the software or the instrument since the clearance of the Panther Fusion Flu A/B/RSV Assay (K171963).

P. Other Supportive Instrument Performance Characteristics Data Not Covered In the "Performance Characteristics" Section above:

Not Applicable

Q. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

R. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.