510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:
K172282

B. Purpose for Submission:
The purpose of this submission is to show that the Panther Fusion Paraflu Assay (for use on the Panther Fusion system) is substantially equivalent to the Prodesse ProParaFlu+ Assay (K153223) and to obtain clearance for the Panther Fusion Paraflu Assay.

C. Measurand:
The Panther Fusion Paraflu Assay detects parainfluenza 1, parainfluenza 2, and parainfluenza 3 hemagglutinin neuraminidase gene RNA and parainfluenza 4 nucleocapsid gene 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 parainfluenza 1, parainfluenza 2, parainfluenza 3, and parainfluenza 4 through nucleic acid extraction, amplification, and detection using real-time 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 Paraflu 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:
   OOU – Parainfluenza Multiplex Nucleic Acid Assay
   OOI – Real Time Nucleic Acid Amplification System

4. Panel:
   Microbiology (83)

H. Intended Use:

1. Intended use(s):
   The Panther Fusion Paraflu assay is a multiplex real-time PCR (RT-PCR) *in vitro* diagnostic test for the rapid and qualitative detection and differentiation of parainfluenza 1 virus, parainfluenza 2 virus, parainfluenza 3 virus and parainfluenza 4 virus (HPIV-1, HPIV-2, HPIV-3, and HPIV- 4). 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 HPIV-1, HPIV-2, HPIV-3, and HPIV-4 infections in humans. Negative results do not preclude HPIV-1, HPIV-2, HPIV-3, and HPIV-4 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 Paraflu 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 parainfluenza 1 virus, parainfluenza 2 virus, parainfluenza 3 virus, and parainfluenza 4 virus directly from the nasopharyngeal swab specimens.

The Panther Fusion Paraflu 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 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. 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):
   Prodesse ProParaflu+ Assay

2. Predicate 510(k) number(s):
   K153223

3. Comparison with predicate:

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

Item	**Panther Fusion Paraflu Assay (Subject Device)**	**Prodesse ProParaflu+ Assay (Predicate Device) K153223**
Technology Principle of Operation	Multiplex Real Time RT-PCR	Same
Organisms Detected	Parainfluenza 1 virus, parainfluenza 2 virus, parainfluenza 3 virus, and parainfluenza 4 virus	Same
Analyte	Viral RNA	Same
Assay Controls	Internal control in each sample. External control processed at periodic interval	Same
Patient Population	Male and female patients with signs/symptoms of respiratory infection	Same
Specimen Types	Nasopharyngeal (NP) swab specimens	Same

Table 2. Comparison of Differences between Predicate Device and Subject Device

Item	**Panther Fusion Paraflu Assay (Subject Device)**	**Prodesse ProParaflu+ Assay (Predicate Device) K153223**
Platform	Automated real-time RT-PCR platform. Uses Panther Fusion system for all steps including nucleic acid extraction, amplification, detection and result processing.	Manual real-time RT-PCR platform. Uses Roche MagNA Pure LC System or bioMerieux NucliSENS easyMAG for nucleic acid extraction and the Cepheid SmartCycler II system for real time RT-PCR.
Intended Use	The Panther Fusion Paraflu assay is a multiplex real-time PCR (RT-PCR) *in vitro* diagnostic test for the rapid and qualitative detection and differentiation of parainfluenza 1 virus, parainfluenza 2 virus, parainfluenza 3 virus, and parainfluenza 4 virus (HPIV- 1, HPIV-2, HPIV-3, and HPIV-4). 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 HPIV-1, HPIV-2, HPIV-3, and HPIV-4 infections in humans. Negative results do not preclude HPIV-1, HPIV-2, HPIV-3, and HPIV-4 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.	The Prodesse ProParaflu+ Assay is a multiplex Real-Time PCR (RT-PCR) *in vitro* diagnostic test for the qualitative detection and discrimination of Parainfluenza 1 Virus, Parainfluenza 2 Virus and Parainfluenza 3 Virus (HPIV-1, HPIV- 2 and HPIV-3) nucleic acids isolated and purified from nasopharyngeal (NP) swab specimens obtained from individuals exhibiting signs and symptoms of respiratory tract infections. This Assay targets the conserved regions of the Hemagglutinin-Neuraminidase (HN) gene of HPIV-1, HPIV-2 and HPIV-3, respectively. The detection and discrimination of HPIV-1, HPIV-2 and HPIV-3 nucleic acids from symptomatic patients aid in the diagnosis of human respiratory tract parainfluenza infections if used in conjunction with other clinical and laboratory findings. This test is not intended to detect Parainfluenza 4a or Parainfluenza 4b Viruses. Negative test results are presumptive and should be confirmed by cell culture. Negative results do not preclude Parainfluenza 1, 2 or 3 virus infections and should not be used as the sole basis for treatment or other management decisions.
Time to Obtain Test Results	Approximately 2.5 hours	Approximately 4 hours

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 HPIV-1, HPIV-2, HPIV-3, and HPIV-4. 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 HPIV-1, HPIV-2, HPIV-3, and HPIV-4, and the IC-S occurs by the use of 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**
HPIV-1	Hemagglutinin neuraminidase	FAM
HPIV-2	Hemagglutinin neuraminidase	HEX
HPIV-3	Hemagglutinin neuraminidase	ROX
HPIV-4	Nucleocapsid	RED647
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	HPIV -1 (1X LOD)
2	HPIV - 3 (3X LOD) /HPIV - 4 (0.01X LOD)
3	HPIV - 1 (3X LOD) /HPIV - 2 (0.01X LOD)
4	HPIV - 2 (1X LOD)
5	HPIV - 2 (3X LOD) /HPIV - 3 (0.01X LOD)
6	Negative (unspiked)
7	HPIV - 3 (1X LOD)
8	HPIV - 4 (1X LOD)
9	HPIV - 4 (3X LOD) /HPIV - 1 (0.01X LOD)

Out of 67 total runs, there were 5 invalid runs: 4 due to hardware failure, and 1 due to operator error; an additional 8 runs were invalidated by the study lead, 4 due to operator not following protocol, 2 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)
HPIV-1	HPIV-1 3x LOD	3	162	100.0% (162/162)	100.0% (97.7 - 100%)
	HPIV-1 1x LOD	1	160	100.0% (160/160)	100.0% (97.7 - 100%)
	HPIV-1 0.01x LOD	9	161	3.1% (5/161)	96.9% (92.9 - 98.7%)
	Negative	6	162	0.0% (0/162)	100.0% (97.7 - 100%)
HPIV-2	HPIV-2 3x LOD	5	162	100.0% (162/162)	100.0% (97.7 - 100%)
	HPIV-2 1x LOD	4	162	100.0% (162/162)	100.0% (97.7 - 100%)
	HPIV-2 0.01x LOD	3	162	27.8% (45/162)	72.2% (64.9 - 78.5%)
	Negative	6	162	0.0% (0/162)	100.0% (97.7 - 100%)
HPIV-3	HPIV-3 3x LOD	2	162	100.0% (162/162)	100.0% (97.7 - 100%)
	HPIV-3 1x LOD	7	162	97.5% (158/162)	97.5% (93.8 - 99.0%)
	HPIV-3 0.01x LOD	5	162	4.9% (8/162)	95.1% (90.6 - 97.5%)
	Negative	6	162	0.6% (1/162)	99.4% (96.6 - 99.9%)
HPIV-4	HPIV-4 3x LOD	9	161	100.0% (161/161)	100.0% (97.7 - 100%)
	HPIV-4 1x LOD	8	162	98.1% (159/162)	98.1% (94.7 - 99.4%)
	HPIV-4 0.01x LOD	2	162	4.3% (7/162)	95.7% (91.4 - 97.9%)
	Negative	6	162	0.0% (0/162)	100.0% (97.7 - 100%)

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 HPIV-1, HPIV-2 and HPIV-4 but 0.6% positive for HPIV-3 (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	Between Instruments		Between Reagent Lots		Between Operators		Between Days		Between Runs		Within Run		Total	
			SD	CV (%)	SD	CV	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV	SD	CV (%)
HPIV-1	3	35.2	0.0	0.0	0.1	0.2	0.0	0.0	0.1	0.3	0.0	0.0	0.4	1.1	0.4	1.2
	1	37.0	0.0	0.0	0.1	0.4	0.0	0.0	0.0	0.2	0.0	0.0	0.6	1.7	0.6	1.8
	9	42.3	0.3	0.9	0.4	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.4	1.0	0.7	1.7
HPIV-2	5	32.8	0.0	0.0	0.0	0.1	0.0	0.1	0.0	0.0	0.1	0.3	0.3	0.9	0.3	1.0
	4	34.3	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.5	1.5	0.5	1.5
	3	40.7	0.1	0.3	0.0	0.1	0.0	0.0	0.3	0.8	0.0	0.0	1.1	2.8	1.2	3.0
HPIV-3	2	35.5	0.5	1.4	0.0	0.0	0.0	0.0	0.2	0.7	0.0	0.0	1.5	4.4	1.6	4.7
	7	37.5	0.2	0.6	0.4	1.0	0.0	0.0	0.0	0.0	0.3	1.0	2.0	5.4	2.1	5.7
	5	40.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.3	8.3	0.7	1.7	3.4	8.5
HPIV-4	9	36.2	0.0	0.0	0.0	0.0	0.3	0.9	0.0	0.0	0.5	1.4	1.5	4.3	1.6	4.6
	8	38.1	0.0	0.0	0.0	0.0	0.2	0.7	0.0	0.0	0.0	0.0	1.9	5.0	1.9	5.1
	2	42.5	0.0	0.0	1.1	2.6	0.8	1.9	0.0	0.0	0.0	0.0	0.7	1.8	1.6	3.7
IC	6	32.1	0.0	0.0	0.0	0.1	0.0	0.2	0.0	0.0	0.1	0.5	0.4	1.2	0.4	1.4

*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 ≤ 5.7%. The greatest source of variability was from the HPIV-3 1X LoD panel member (within-run % CV of 5.4%). 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	HPIV-1	Low Positive	1X LOD
2	HPIV-1	Moderate Positive	3X LOD
3	HPIV-2	Low Positive	1X LOD
4	HPIV-2	Moderate Positive	3X LOD
5	HPIV-3	Low Positive	1X LOD
6	HPIV-3	Moderate Positive	3X LOD
7	HPIV-4	Low Positive	1X LOD
8	HPIV-4	Moderate Positive	3X LOD
9	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 810 samples were tested yielding 30 valid runs and 791 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		HPIV-1		HPIV-2		HPIV-3		HPIV-4	
		Agreement with Expected	95% Confidence Interval	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)	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)	89/89	100 (95.8-100)
3	Low Pos	87/87	100 (95.8-100)	87/87	100 (95.8-100)	87/87	100 (95.8-100)	87/87	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)	89/89	100 (95.8-100)
5	Low Pos	87/87	100 (95.8-100)	87/87	100 (95.8-100)	86/87	98.9 (93.8-99.8)	87/87	100 (95.8-100)
6	Mod Pos	89/89	100 (95.8-100)	89/89	100 (95.8-100)	89/89	100 (95.8-100)	89/89	100 (95.8-100)

7	Low Pos	87/87	100 (95.8-100)	87/87	100 (95.8-100)	87/87	100 (95.8-100)	84/87	96.6 (90.3-98.8)
8	Mod Pos	88/88	100 (95.8-100)	88/88	100 (95.8-100)	88/88	100 (95.8-100)	88/88	100 (95.8-100)
9	Neg	87/87	100 (95.8-100)	87/87	100 (95.8-100)	87/87	100 (95.8-100)	87/87	100 (95.8-100)

Agreement was 100% for HPIV-1, HPIV-2, HPIV-3, and HPIV-4 moderate positive panel members and for the negative panel member tested with the Panther Fusion Paraflu Assay.

For low positive panel members tested with the Panther Fusion Paraflu Assay, the agreement was 100% for HPIV-1 and HPIV-2, 98.9% for HPIV-3 detection, and 96.6% for HPIV-4 detection. The lower agreement observed using low positive panel members is to be expected, since these panel members were spiked with a concentration close to the limit of detection (yielding an approximately 95% to 100% detection rate).

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(%)**
HPIV-1	1	37.19	0	0	<0.1	0.26	<0.1	0.21	<0.1	<0.1	0.79	2.13	0.8	2.16
	2	35.29	0.18	0.52	0	0	0.11	0.31	<0.1	<0.1	0.54	1.54	0.59	1.66
HPIV-2	3	34.37	0	0	0	0	0.13	0.38	<0.1	<0.1	0.49	1.43	0.51	1.48
	4	32.72	<0.1	0.16	<0.1	0.24	0	0	0	0	0.35	1.07	0.36	1.11
HPIV-3	5	37.79	0.14	0.37	0.31	0.81	0	0	0	0	1.81	4.78	1.84	4.87
	6	35.46	0	0	0.49	1.4	0	0	0	0	1.83	5.17	1.9	5.36
HPIV-4	7	38.5	0	0	0	0	0.52	1.35	<0.1	<0.1	2.2	5.72	2.26	5.88
	8	36	0	0	0.39	1.08	0	0	0	0	1.6	4.44	1.65	4.57

The table above shows the variability of the Panther Fusion Paraflu Assay between sites, operator, days, and runs. The largest source of variation was the 'within runs' factor with %CV values ranging from 1.07% to 5.72%. All other sources of variation had %CV values less than 1.40%. The total performance variability (%CV) across all assay results was <6%.

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 Paraflu 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 of the included reagents was evaluated. The reagents included in the kit are: Panther Fusion Paraflu 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 Paraflu 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 Paraflu 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 Paraflu 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 Paraflu Assay components during shipment would not impact the performance of the assay. One lot of components and controls for the Panther Fusion Paraflu Assay were tested in this study. All components were exposed to the extreme temperatures in their final container closure systems and all of the components of the Panther Fusion Paraflu Assay were exposed to the extreme conditions.

The Panther Fusion Paraflu 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 Paraflu 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 Paraflu Assay when high titer Human Parainfluenza Virus type 2, 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 spiked with HPIV-2 at 10^6 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 (HPIV 1)	HEX (HPIV 2	ROX (HPIV 3)	Red 647 (HPIV 4)	RED677 (IC)
Baseline	Negative	300	0.0% (0/300)	0.0% (0/300)	0.0% (0/300)	0.0% (0/300)	100.0% (300/30
Runs 2-4	Negative	450	0.0% (0/450)	0.0% (0/450)	0.0% (0/450)	0.0% (0/450)	100.0% (450/45
Runs 2-4	Positive	450	0.0% (0/450)	100.0% (450/45	0.0% (0/450)	0.0% (0/450)	100.0% (450/45

Baseline runs comprised of negative samples showed 0% positivity for all targets, for all three instruments. All positive samples from the checkerboard runs (runs 2-4) were HPIV-2 positive. No negative samples from the checkerboard runs were HPIV-2 positive. No carryover was detected in this study.

d. Detection limit:

The purpose of this study was to evaluate and verify the analytical sensitivity and Limit of Detection (LoD) of HPIV-1, HPIV-2, HPIV-3, and HPIV-4 viruses in pooled negative clinical nasopharyngeal swab specimens when tested with the Panther Fusion Paraflu Assay. Target-specific LoD values were obtained using one strain for each targeted virus. Dilutions of each virus were prepared in pooled negative clinical nasopharyngeal swab (NP) specimens and tested with 12 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 12. LoD study results

Target	Confirmed LOD Concentration (TCID50/ml)	Detection Channel (Target)				
		FAM (HPIV-1)	HEX (HPIV-2)	ROX (HPIV-3)	RED647 (HPIV-4)	RED677 (IC)
HPIV-1	10^{-2}	100.0% (20/20)	0.0% (0/20)	0.0% (0/20)	0.0% (0/20)	100.0% (20/20)
HPIV-2	10^{2}	0.0% (0/20)	100.0% (20/20)	0.0% (0/20)	0.0% (0/20)	100.0% (20/20)
HPIV-3	10^{1}	0.0% (0/20)	0.0% (0/20)	95.0% (19/20)	0.0% (0/20)	100.0% (20/20)
HPIV-4	$10^{0.5}$	0.0% (0/20)	0.0% (0/20)	0.0% (0/20)	95.0% (19/20)	100.0% (20/20)

e. Analytical specificity:

Interfering Substances

This study evaluated the performance of the Panther Fusion Paraflu 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 had an effect on 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 (HPIV-1, HPIV-2, HPIV-3 and HPIV-4) spiked at 1-2X 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	Bactroban cream	Mupirocin	10 mg/mL
10	Antibiotic, systemic	Tobramycin	Tobramycin	4.0 µg/mL
11	No interfering Substance	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 (HPIV-1)	HEX (HPIV-2)	ROX (HPIV-3)	RED647 (HPIV-4)	RED677 (IC)
Un-spiked	Panel 1 (Mucin)	0.0% (0/9)	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)	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)	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)	0.0% (0/9)	100.0% (9/9)
	Panel 5 (Nasal Corticosteroids: Nasacort, Rhinocort, Naonex, Flonase)	0.0% (0/9)	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)	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)	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)	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)	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)	0.0% (0/9)	100.0% (9/9)
	Panel 11 (SCM)	0.0% (0/9)	0.0% (0/9)	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)
Spiked with HPIV-1	Panel 1 (Mucin)	100.0% (9/9)	0.0% (0/9)	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)	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)	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)	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)	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)	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)	0.0% (0/9)	100.0% (9/9)
	Panel 8 (Anti-viral Drugs: Relenza, TamiFlu, Rebitol)	100.0% (9/9)	0.0% (0/9)	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)
	Panel 9 (Antibiotic, Nasal Ointment: Bactroban Cream)	100.0% (9/9)	0.0% (0/9)	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)	0.0% (0/9)	100.0% (9/9)
	Panel 11 (SCM)	100.0% (9/9)	0.0% (0/9)	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)
Spiked with HPIV-2	Panel 1 (Mucin)	0.0% (0/9)	100.0% (9/9)	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)
	Panel 2 (Blood)	0.0% (0/9)	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)	0.0% (0/9)	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)	0.0% (0/9)	100.0% (9/9)	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)
	Panel 5 (Nasal Corticosteroids: Nasacort, Rhinocort, Naonex, Flonase)	0.0% (0/9)	100.0% (9/9)	0.0% (0/9)	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)	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)	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)	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)	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)	0.0% (0/9)	100.0% (9/9)
	Panel 11 (SCM)	0.0% (0/9)	100.0% (9/9)	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)
Spiked with HPIV-3	Panel 1 (Mucin)	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)	0.0% (0/9)	100.0% (9/9)
	Panel 2 (Blood)	0.0% (0/9)	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)	0.0% (0/9)	100.0% (9/9)	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)	100.0% (9/9)	0.0% (0/9)	100.0% (9/9)
	Panel 5 (Nasal Corticosteroids: Nasacort, Rhinocort, Naonex, Flonase)	0.0% (0/9)	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)	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)	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)	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)	0.0% (0/9)	100.0% (9/9)	0.0% (0/9)	100.0% (9/9)
	Panel 10 (Antibiotic Systemic: Tobramycin)	0.0% (0/10)	0.0% (0/10)	100.0% (10/10)	0.0% (0/9)	100.0% (10/10)
	Panel 11 (SCM)	0.0% (0/10)	0.0% (0/10)	100.0% (10/10)	0.0% (0/9)	100.0% (10/10)
Spiked with HPIV-4	Panel 1 (Mucin)	0.0% (0/9)	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)	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)	0.0% (0/9)	100.0% (9/9)	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)	100.0% (9/9)
	Panel 5 (Nasal Corticosteroids: Nasacort, Rhinocort, Naonex, Flonase)	0.0% (0/9)	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)	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)	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)	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)	0.0% (0/9)	100.0% (9/9)	100.0% (9/9)
	Panel 10 (Antibiotic Systemic: Tobramycin)	0.0% (0/10)	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)	100.0% (9/9)
	Panel 11 (SCM)	0.0% (0/10)	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)	100.0% (9/9)

For all conditions, all spiked panel members were 100.0% positive for intended targets. All un-spiked panel members were 0.0% positive for all targets other than IC. This study demonstrates that the substances tested do not interfere with the performance of the Panther Fusion Paraflu Assay at the concentrations tested.

Competitive Interference:

The purpose of this study was to demonstrate that samples tested with the Panther Fusion Paraflu Assay that are co-infected with multiple types of targeted organisms do not inhibit the detection of either one (competitive interference). Each targeted organism (HPIV-1, HPIV-2, HPIV-3 and HPIV-4) 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. Additional panel members were made with one target at high concentration (1000X LoD) and another target at low concentration
(5X – 10X LoD), if the competitive interference was observed. 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	HPIV-1	Low (3X LoD)	HPIV-2	High (1000X LoD)
2	HPIV-1	Low (3X LoD)	HPIV-3	High (1000X LoD)
3	HPIV-1	Low (3X LoD)	HPIV-4	High (1000X LoD)
4	HPIV-2	Low (3X LoD)	HPIV-1	High (1000X LoD)
5	HPIV-2	Low (3X LoD)	HPIV-3	High (1000X LoD)
6	HPIV-2	Low (3X LoD)	HPIV-4	High (1000X LoD)
7	HPIV-3	Low (3X LoD)	HPIV-1	High (1000X LoD)
8	HPIV-3	Low (3X LoD)	HPIV-2	High (1000X LoD)
9	HPIV-3	Low (3X LoD)	HPIV-4	High (1000X LoD)
10	HPIV-4	Low (3X LoD)	HPIV-1	High (1000X LoD)
11	HPIV-4	Low (3X LoD)	HPIV-2	High (1000X LoD)
12	HPIV-4	Low (3X LoD)	HPIV-3	High (1000X LoD)
13	HPIV-1	Low (10X LoD)	HPIV-4	High (1000X LoD)
14	HPIV-1	Low (5X LoD)	HPIV-4	High (1000X LoD)

Interference was observed for HPIV-1 in Panel member 3 with HPIV-1 spiked at 3X LoD (Low) and HPIV-4 spiked at 1000X LoD (high). Panel member 3 was 100% positive for HPIV-4 (6/6) but 50.0% positive for HPIV-1 (3/6). A second lot of the Panel member 3 was made and tested for additional n=20 replicates. Two new panel members were made and tested where HPIV-1 was spiked at a slightly higher concentration: Panel member 13 (HPIV-1 at 10X LoD and HPIV-4 at 1000X LoD) and Panel member 14 (HPIV-1 at 5X LoD and HPIV-4 at 1000X LoD) both produced 100% positive for HPIV-1 and for HPIV-4 (6/6), demonstrating that the competitive interference for the HPIV-1 by HPIV-4 is not observed when HPIV-1 is present at least at 5X LOD in the presence of HPIV-4 at 1000X LoD. All the other panel members were 100% positive for both targets. This is acceptable as the four viruses are very similar and treatment is the same for all. here is minimal risk associated with interference from different human parainfluenza viruses. 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 (HPIV-1)	HEX HPIV-2)	ROX (HPIV-3)	RED647 (HPIV-4)	RED677 (IC)
1	HPIV-1	HPIV-2	100.0% (6/6)	100.0% (6/6)	0.0% (0/6)	0.0% (0/6)	100.0% (6/6)
2	HPIV-1	HPIV-3	100.0% (6/6)	0.0% (0/6)	100.0% (6/6)	0.0% (0/6)	100.0% (6/6)
3	HPIV-1	HPIV-4	50.0% (3/6)	0.0% (0/6)	0.0% (0/6)	100.0% (6/6)	100.0% (6/6)
4	HPIV-2	HPIV-1	100.0% (6/6)	100.0% (6/6)	0.0% (0/6)	0.0% (0/6)	100.0% (6/6)
5	HPIV-2	HPIV-3	0.0% (0/6)	100.0% (6/6)	100.0% (6/6)	0.0% (0/6)	100.0% (6/6)
6	HPIV-2	HPIV-4	0.0% (0/6)	100.0% (6/6)	0.0% (0/6)	100.0% (6/6)	100.0% (6/6)
7	HPIV-3	HPIV-1	100.0% (6/6)	0.0% (0/6)	100.0% (6/6)	0.0% (0/6)	100.0% (6/6)
8	HPIV-3	HPIV-2	0.0% (0/6)	100.0% (6/6)	100.0% (6/6)	0.0% (0/6)	100.0% (6/6)
9	HPIV-3	HPIV-4	0.0% (0/6)	0.0% (0/6)	100.0% (6/6)	100.0% (6/6)	100.0% (6/6)
10	HPIV-4	HPIV-1	100.0% (6/6)	0.0% (0/6)	0.0% (0/6)	100.0% (6/6)	100.0% (6/6)
11	HPIV-4	HPIV-2	0.0% (0/6)	100.0% (6/6)	0.0% (0/6)	100.0% (6/6)	100.0% (6/6)
12	HPIV-4	HPIV-3	0.0% (0/6)	0.0% (0/6)	100.0% (6/6)	100.0% (6/6)	100.0% (6/6)
13	HPIV-1	HPIV-4	100.0% (6/6)	0.0% (0/6)	0.0% (0/6)	100.0% (6/6)	100.0% (6/6)
14	HPIV-1	HPIV-4	100.0% (6/6)	0.0% (0/6)	0.0% (0/6)	100.0% (6/6)	100.0% (6/6)

*Spiked concentration at 3X LoD and 1000X LoD for Low and High, respectively, except for the Panel Members 13 and 14: Panel Member 13 Low sample was spiked at 10X LoD and Panel Member 14 Low sample was spiked at 5X LoD.

Cross-Reactivity:

This study evaluated the analytical specificity and sensitivity of the Panther Fusion Paraflu 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 had an effect on assay performance. Panels members were composed of 3-5 different microorganisms spiked into simulated clinical matrix at either 10^1-10^7 TCID50/mL (for virus) or 10^5-10^8 CFU/mL or IFU/mL (for bacteria). Each panel member was split into two parts. One part was spiked with one representative strain of intended targets (HPIV-1, HPIV-2, HPIV-3, HPIV-4) to a final concentration of 0.5 log above LoD to assess interference (negative effect on sensitivity). The other part 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	Organism	Concentration	Units
1	Adenovirus 1	1.00E+05	$TCID_{50}$/ml
	Adenovirus 7a	1.00E+05	$TCID_{50}$/ml
	CMV Strain AD 169	1.00E+04	$TCID_{50}$/ml
2	*Bordetella bronchiseptica*	1.00E+07	CFU/ml
	Bordetella pertussis	1.00E+08	CFU/ml
	Candida albicans	1.00E+07	CFU/ml
	Chlamydophila pneumoniae (formerly *Chlamydia pneumoniae*)	1.00E+05	IFU/ml
	Chlamydia trachomatis	1.00E+05	CFU/ml
	Coronavirus 229E	1.00E+04	$TCID_{50}$/ml

3	Coxsackie B4	1.00E+06	$TCID_{50}$/ml
	Coxsackie B5/10/2006	1.00E+05	$TCID_{50}$/ml
4	*Corynebacterium diphtheria*	1.00E+07	CFU/ml
	E. coli	1.00E+07	CFU/ml
	Haemophilus influenzae	1.00E+07	CFU/ml
5	Echovirus 11	1.00E+05	$TCID_{50}$/ml
	Echovirus 2	1.00E+04	$TCID_{50}$/ml
	Echovirus 3	1.00E+05	$TCID_{50}$/ml
	Echovirus 6	1.00E+04	$TCID_{50}$/ml
6	Enterovirus 68	1.00E+05	$TCID_{50}$/ml
	Enterovirus 70	1.00E+04	TCID50/ml
	EBV	1.00E+07	$TCID_{50}$/ml
	Rhinovirus 1A	1.00E+05	$TCID_{50}$/ml
	Varicella Zoster Virus	1.00E+03	$TCID_{50}$/ml
7	Flu A/California/07/2009 (2009 H1N1)	1.00E+03	$TCID_{50}$/ml
	Flu A/Victoria/361/2011 (H3N2)	1.00E+03	$TCID_{50}$/ml
	RSV A	1.00E+02	TCID50/ml
	RSV B	1.00E+03	$TCID_{50}$/ml
	IB Massachusetts	1.00E+01	TCID50/ml
8	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-4	1.00E+04	$TCID_{50}$/ml
9	HSV-1 Macinytre Strain	1.00E+05	$TCID_{50}$/ml
	HSV-2 Type 2G Strain	1.00E+05	$TCID_{50}$/ml
	hMPV Subtype A2	1.00E+06	$TCID_{50}$/ml
10	*Klebsiella pneumonia*	1.00E+07	CFU/ml
	Lactobacillus plantarum	1.00E+07	CFU/ml
	Tatlockia micdadei (formerly *Legionella micdadei*)	1.00E+07	CFU/ml
	Legionella pneumophila	1.00E+07	CFU/ml
11	Measles/7/2000	1.00E+05	$TCID_{50}$/ml
	Polio virus	1.00E+06	$TCID_{50}$/ml
	Mumps virus	1.00E+04	$TCID_{50}$/ml
12	*Moraxella catarrhalis*	1.00E+06	CFU/ml
	Mycobacterium intracellulare	1.00E+10	rRNA copies/ml, estimated to be equivalent to 2.00E+06 CFU/mL
	Mycobacterium tuberculosis	1.00E+10	rRNA copies/ml, estimated to be equivalent to 2.00E+06 CFU/mL
13	*Mycoplasma pneumoniae*	1.00E+06	CFU/ml
	Neisseria gonorrhea	1.00E+07	CFU/ml
	Neisseria meningitides	1.00E+07	CFU/ml
	Neisseria mucosa	1.00E+07	CFU/ml
14	*Proteus mirabilis*	1.00E+07	CFU/ml

	Proteus vulgaris	1.00E+07	CFU/ml
	Pseudomonas aeruginosa	1.00E+07	CFU/ml
15	*Staphylococcus aureus*	1.00E+07	CFU/ml
	Staphylococcus epidermidis	1.00E+07	CFU/ml
	Streptococcus pneumoniae	1.00E+06	CFU/ml
	Streptococcus pyogenes	1.00E+07	CFU/ml
	Streptococcus salivarius	1.00E+06	CFU/ml
16	No microorganisms (SCM only)	N/A	N/A

Panel member 8 was composed of HPIV-1, HPIV-2, HPIV-3 and HPIV-4, because these are also the viruses being spiked into each panel member, panel member 8 was not spiked with virus. Competitive interference was tested in a separate study (Table 17) and was therefore not repeated here.

No cross-reactivity was seen with organisms in any of the panels except panel member 14. When this panel was spiked with HPIV-2, one out of nine replicates (from reagent Lot B testing) was positive for HPIV-2, which is not expected. Six additional replicates, three from same samples and three from newly prepared samples, were tested with the reagent lot B. All additional replicates tested were negative for HPIV-2, for a final HPIV-2 positivity of 6.7% (1/15). The one HPIV-2 positive reaction is likely caused by a rare cross-carryover event (carryover has been document with this instrument previously), and not due to cross-reactivity. For all other panel members, when tested spiked with intended organisms were 100.0% positive for intended targets, and when tested un-spiked were 0.0% positive for all targets. The final results for all conditions tested are detailed in the table below.

Table 19. Cross-reactivity study results

Panel Member	**Spiked organism***	**Detection Channel (Target), % Positive (reactive n/valid n)****				
		FAM (HPIV-1)	**HEX (HPIV-2)**	**ROX (HPIV-3)**	**RED647 (HPIV-4)**	**RED677 (IC)**
1	HPIV-1	100.0% (12/12)	0.0% (0/12)	0.0% (0/12)	0.0% (0/12)	100.0% (12/12)
	HPIV-2	0.0% (0/9)	100.0% (9/9)	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)
	HPIV-3	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)	0.0% (0/9)	100.0% (9/9)
	HPIV-4	0.0% (0/9)	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)	100.0% (9/9)
	Unspiked	0.0% (0/33)	0.0% (0/33)	0.0% (0/33)	0.0% (0/33)	100.0% (33/33)
2	HPIV-1	100.0% (11/11)	0.0% (0/11)	0.0% (0/11)	0.0% (0/11)	100.0% (11/11)
	HPIV-2	0.0% (0/9)	100.0% (9/9)	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)
	HPIV-3	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)	0.0% (0/9)	100.0% (9/9)
	HPIV-4	0.0% (0/9)	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)	100.0% (9/9)
	Unspiked	0.0% (0/30)	0.0% (0/30)	0.0% (0/30)	0.0% (0/30)	100.0% (30/30)
3	HPIV-1	100.0% (11/11)	0.0% (0/11)	0.0% (0/11)	0.0% (0/11)	100.0% (11/11)
	HPIV-2	0.0% (0/9)	100.0% (9/9)	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)
	HPIV-3	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)	0.0% (0/9)	100.0% (9/9)
	HPIV-4	0.0% (0/9)	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)	100.0% (9/9)
	Unspiked	0.0% (0/30)	0.0% (0/30)	0.0% (0/30)	0.0% (0/30)	100.0% (30/30)

4	HPIV-1	100.0% (9/9)	0.0% (0/9)	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)
	HPIV-2	0.0% (0/9)	100.0% (9/9)	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)
	HPIV-3	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)	0.0% (0/9)	100.0% (9/9)
	HPIV-4	0.0% (0/9)	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)	100.0% (9/9)
	Unspiked	0.0% (0/33)	0.0% (0/33)	0.0% (0/33)	0.0% (0/33)	100.0% (33/33)
5	HPIV-1	100.0% (9/9)	0.0% (0/9)	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)
	HPIV-2	0.0% (0/9)	100.0% (9/9)	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)
	HPIV-3	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)	0.0% (0/9)	100.0% (9/9)
	HPIV-4	0.0% (0/9)	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)	100.0% (9/9)
	Unspiked	0.0% (0/30)	0.0% (0/30)	0.0% (0/30)	0.0% (0/30)	100.0% (30/30)
6	HPIV-1	100.0% (12/12)	0.0% (0/12)	0.0% (0/12)	0.0% (0/12)	100.0% (12/12)
	HPIV-2	0.0% (0/9)	100.0% (9/9)	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)
	HPIV-3	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)	0.0% (0/9)	100.0% (9/9)
	HPIV-4	0.0% (0/9)	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)	100.0% (9/9)
	Unspiked	0.0% (0/30)	0.0% (0/30)	0.0% (0/30)	0.0% (0/30)	100.0% (30/30)
7	HPIV-1	100.0% (12/12)	0.0% (0/12)	0.0% (0/12)	0.0% (0/12)	100.0% (12/12)
	HPIV-2	0.0% (0/9)	100.0% (9/9)	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)
	HPIV-3	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)	0.0% (0/9)	100.0% (9/9)
	HPIV-4	0.0% (0/9)	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)	100.0% (9/9)
	Unspiked	0.0% (0/27)	0.0% (0/27)	0.0% (0/27)	0.0% (0/27)	100.0% (27/27)
8	Unspiked composed of HPIV-1, HPIV-2, HPIV-3, HPIV- 4	100.0% (27/27)	100.0% (27/27)	100.0% (27/27)	100.0% (27/27)	0.0% (0/27)
9	HPIV-1	100.0% (12/12)	0.0% (0/12)	0.0% (0/12)	0.0% (0/12)	100.0% (12/12)
	HPIV-2	0.0% (0/9)	100.0% (9/9)	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)
	HPIV-3	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)	0.0% (0/9)	100.0% (9/9)
	HPIV-4	0.0% (0/9)	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)	100.0% (9/9)
	Unspiked	0.0% (0/27)	0.0% (0/27)	0.0% (0/27)	0.0% (0/27)	100.0% (27/27)
10	HPIV-1	100.0% (12/12)	0.0% (0/12)	0.0% (0/12)	0.0% (0/12)	100.0% (12/12)
	HPIV-2	0.0% (0/9)	100.0% (9/9)	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)
	HPIV-3	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)	0.0% (0/9)	100.0% (9/9)
	HPIV-4	0.0% (0/9)	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)	100.0% (9/9)
	Unspiked	0.0% (0/27)	0.0% (0/27)	0.0% (0/27)	0.0% (0/27)	100.0% (27/27)
11	HPIV-1	100.0% (12/12)	0.0% (0/12)	0.0% (0/12)	0.0% (0/12)	100.0% (12/12)
	HPIV-2	0.0% (0/9)	100.0% (9/9)	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)
	HPIV-3	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)	0.0% (0/9)	100.0% (9/9)
	HPIV-4	0.0% (0/9)	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)	100.0% (9/9)
	Unspiked	0.0% (0/27)	0.0% (0/27)	0.0% (0/27)	0.0% (0/27)	100.0% (27/27)
12	HPIV-1	100.0% (12/12)	0.0% (0/12)	0.0% (0/12)	0.0% (0/12)	100.0% (12/12)
	HPIV-2	0.0% (0/9)	100.0% (9/9)	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)
	HPIV-3	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)	0.0% (0/9)	100.0% (9/9)

	HPIV-4	0.0% (0/9)	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)	100.0% (9/9)
	Unspiked	0.0% (0/27)	0.0% (0/27)	0.0% (0/27)	0.0% (0/27)	100.0% (27/27)
13	HPIV-1	100.0% (11/11)	0.0% (0/11)	0.0% (0/11)	0.0% (0/11)	100.0% (11/11)
	HPIV-2	0.0% (0/9)	100.0% (9/9)	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)
	HPIV-3	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)	0.0% (0/9)	100.0% (9/9)
	HPIV-4	0.0% (0/9)	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)	100.0% (9/9)
	Unspiked	0.0% (0/27)	0.0% (0/27)	0.0% (0/27)	0.0% (0/27)	100.0% (27/27)
14	HPIV-1	100.0% (15/15)	6.7% (1/15)	0.0% (0/15)	0.0% (0/15)	100.0% (15/15)
	HPIV-2	0.0% (0/9)	100.0% (9/9)	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)
	HPIV-3	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)	0.0% (0/9)	100.0% (9/9)
	HPIV-4	0.0% (0/9)	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)	100.0% (9/9)
	Unspiked	0.0% (0/27)	0.0% (0/27)	0.0% (0/27)	0.0% (0/27)	100.0% (27/27)
15	HPIV-1	100.0% (12/12)	0.0% (0/12)	0.0% (0/12)	0.0% (0/12)	100.0% (12/12)
	HPIV-2	0.0% (0/9)	100.0% (9/9)	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)
	HPIV-3	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)	0.0% (0/9)	100.0% (9/9)
	HPIV-4	0.0% (0/9)	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)	100.0% (9/9)
	Unspiked	0.0% (0/27)	0.0% (0/27)	0.0% (0/27)	0.0% (0/27)	100.0% (27/27)
16	HPIV-1	100.0% (12/12)	0.0% (0/12)	0.0% (0/12)	0.0% (0/12)	100.0% (12/12)
	HPIV-2	0.0% (0/9)	100.0% (9/9)	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)
	HPIV-3	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)	0.0% (0/9)	100.0% (9/9)
	HPIV-4	0.0% (0/9)	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)	100.0% (9/9)
	Unspiked	0.0% (0/27)	0.0% (0/27)	0.0% (0/27)	0.0% (0/27)	100.0% (27/27)

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 Paraflu 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 Paraflu 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 for HPIV-1, HPIV-2, HPIV-3, and 350 for HPIV-4 for determination of positivity in their specific channels (FAM, HEX, ROX, RED647 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. <u>Comparison studies:</u>

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 HPIV-1, HPIV-2, and HPIV-3 and PCR

followed by sequencing for HPIV-4. 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 HPIV-1, HPIV-2, HPIV-3, and HPIV-4 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.

One aberrant result was detected for HPIV-3 samples, where one sample was positive for HPIV-1, HPIV-2, and HPIV-4 in addition to HIPIV-3. Additional vials of the same panel were tested again and all 8 replicates showed detection only of HIPV-3. The aberrant result appears to be an anomaly with an unknown cause.
The testing results support use of all 5 Viral Transport Media types evaluated.

Table 20. Matrix Comparison Results for HPIV-1 Positivity

Conc. and Sample Type	Time-point	VTM Type	Valid N	HPIV-1		HPIV-2		HPIV-3		HPIV-4		IC	
				# pos	% Positive	# pos	% Positive	# pos	% Positive	# pos	% Positive	# pos	% valid
+1.0 log LoD HPIV-1	T=0	M4	6	6	100.0	0	0.0	0	0.0	0	0.0	6	100.0
		M4RT	6	6	100.0	0	0.0	0	0.0	0	0.0	6	100.0
		M5	6	6	100.0	0	0.0	0	0.0	0	0.0	6	100.0
		M6	6	6	100.0	0	0.0	0	0.0	0	0.0	6	100.0
		BD	6	6	100.0	0	0.0	0	0.0	0	0.0	6	100.0
	T>72	M4	6	6	100.0	0	0.0	0	0.0	0	0.0	6	100.0
		M4RT	6	6	100.0	0	0.0	0	0.0	0	0.0	6	100.0
		M5	6	6	100.0	0	0.0	0	0.0	0	0.0	6	100.0
		M6	6	6	100.0	0	0.0	0	0.0	0	0.0	6	100.0
		BD	6	6	100.0	0	0.0	0	0.0	0	0.0	6	100.0
+0.5 log LoD HPIV-1	T=0	M4	24	24	100.0	0	0.0	0	0.0	0	0.0	24	100.0
		M4RT	24	24	100.0	0	0.0	0	0.0	0	0.0	24	100.0
		M5	24	24	100.0	0	0.0	0	0.0	0	0.0	24	100.0
		M6	24	24	100.0	0	0.0	0	0.0	0	0.0	24	100.0
		BD	24*	24	100.0	0	0.0	0	0.0	0	0.0	24	100.0
	T>72	M4	24	24	100.0	0	0.0	0	0.0	0	0.0	24	100.0
		M4RT	24	24	100.0	0	0.0	0	0.0	0	0.0	24	100.0
		M5	24	24	100.0	0	0.0	0	0.0	0	0.0	24	100.0
		M6	24	24	100.0	0	0.0	0	0.0	0	0.0	24	100.0
		BD	24	24	100.0	0	0.0	0	0.0	0	0.0	24	100.0
		M4	6	5	83.3	0	0.0	0	0.0	0	0.0	6	100.0

-1.0 log LoD HPIV-1	T=0	M4RT	6	3	50.0	0	0.0	0	0.0	0	0.0	6	100.0
		M5	6	5	83.3	0	0.0	0	0.0	0	0.0	6	100.0
		M6	6	4	66.7	0	0.0	0	0.0	0	0.0	6	100.0
		BD	6*	5	83.3	0	0.0	0	0.0	0	0.0	6	100.0
	T>72	M4	6	6	100.0	0	0.0	0	0.0	0	0.0	6	100.0
		M4RT	6	4	66.7	0	0.0	0	0.0	0	0.0	6	100.0
		M5	6	5	83.3	0	0.0	0	0.0	0	0.0	6	100.0
		M6	6	5	83.3	0	0.0	0	0.0	0	0.0	6	100.0
		BD	6	3	50.0	0	0.0	0	0.0	0	0.0	6	100.0

* Seven reactions were invalid due to instrument error. Samples were retested and were valid. Results from the combined valid reactions are shown.

Table 21. Matrix Comparison Results for HPIV-2 Positivity

Conc. and Sample Type	Time-point	VTM Type	Valid N	HPIV-1		HPIV-2		HPIV-3		HPIV-4		IC	
				# pos	% Positive	# pos	% Positive	# pos	% Positive	# pos	% Positive	pos n	% valid
+1.0 log LoD HPIV-2	T=0	M4	6	0	0.0	6	100.0	0	0.0	0	0.0	6	100.0
		M4RT	6	0	0.0	6	100.0	0	0.0	0	0.0	6	100.0
		M5	6	0	0.0	6	100.0	0	0.0	0	0.0	6	100.0
		M6	6	0	0.0	6	100.0	0	0.0	0	0.0	6	100.0
		BD	6	0	0.0	6	100.0	0	0.0	0	0.0	6	100.0
	T>72	M4	6	0	0.0	6	100.0	0	0.0	0	0.0	6	100.0
		M4RT	6	0	0.0	6	100.0	0	0.0	0	0.0	6	100.0
		M5	6	0	0.0	6	100.0	0	0.0	0	0.0	6	100.0
		M6	6	0	0.0	6	100.0	0	0.0	0	0.0	6	100.0
		BD	6	0	0.0	6	100.0	0	0.0	0	0.0	6	100.0
+0.5 log LoD HPIV-2	T=0	M4	24	0	0.0	24	100.0	0	0.0	0	0.0	24	100.0
		M4RT	24	0	0.0	24	100.0	0	0.0	0	0.0	24	100.0
		M5	24	0	0.0	24	100.0	0	0.0	0	0.0	24	100.0
		M6	24	0	0.0	24	100.0	0	0.0	0	0.0	24	100.0
		BD	24	0	0.0	24	100.0	0	0.0	0	0.0	24	100.0
	T>72	M4	24	0	0.0	24	100.0	0	0.0	0	0.0	24	100.0
		M4RT	24*	0	0.0	24	100.0	0	0.0	0	0.0	24	100.0
		M5	24	0	0.0	24	100.0	0	0.0	0	0.0	24	100.0
		M6	24	0	0.0	24	100.0	0	0.0	0	0.0	24	100.0
		BD	24	0	0.0	24	100.0	0	0.0	0	0.0	24	100.0
-1.0 log LoD	T=0	M4	6	0	0.0	6	100.0	0	0.0	0	0.0	6	100.0
		M4RT	6	0	0.0	6	100.0	0	0.0	0	0.0	6	100.0
		M5	6	0	0.0	6	100.0	0	0.0	0	0.0	6	100.0
		M6	6	0	0.0	6	100.0	0	0.0	0	0.0	6	100.0
		BD	6	0	0.0	6	100.0	0	0.0	0	0.0	6	100.0
		M4	6	0	0.0	6	100.0	0	0.0	0	0.0	6	100.0

HPIV-2	T>72	M4RT	6	0	0.0	6	100.0	0	0.0	0	0.0	6	100.0
		M5	6	0	0.0	6	100.0	0	0.0	0	0.0	6	100.0
		M6	6	0	0.0	6	100.0	0	0.0	0	0.0	6	100.0
		BD	6	0	0.0	6	100.0	0	0.0	0	0.0	6	100.0

* One reaction was invalid due to instrument error. Samples were retested and were valid. Results from the combined valid reactions are shown here.

Table 22. Matrix Comparison Results for HPIV-3 Positivity

Conc. and Sample Type	Time-point	VTM Type	Valid N	HPIV-1		HPIV-2		HPIV-3		HPIV-4		IC	
				# pos	% Positive	# pos	% Positive	# pos	% Positive	# pos	% Positive	pos n	% valid
+1.0 log LoD HPIV-3	T=0	M4	6	0	0.0	0	0.0	6	100.0	0	0.0	6	100.0
		M4RT	6	0	0.0	0	0.0	6	100.0	0	0.0	6	100.0
		M5	6	0	0.0	0	0.0	6	100.0	0	0.0	6	100.0
		M6	6	0	0.0	0	0.0	6	100.0	0	0.0	6	100.0
		BD	6	0	0.0	0	0.0	6	100.0	0	0.0	6	100.0
	T>72	M4	6	0	0.0	0	0.0	6	100.0	0	0.0	6	100.0
		M4RT	6	0	0.0	0	0.0	6	100.0	0	0.0	6	100.0
		M5	6	0	0.0	0	0.0	6	100.0	0	0.0	6	100.0
		M6	6	0	0.0	0	0.0	6	100.0	0	0.0	6	100.0
		BD	6	0	0.0	0	0.0	6	100.0	0	0.0	6	100.0
+0.5 log LoD HPIV-3	T=0	M4	24	0	0.0	0	0.0	24	100.0	0	0.0	24	100.0
		M4RT	24	0	0.0	0	0.0	24	100.0	0	0.0	24	100.0
		M5	24	0	0.0	0	0.0	24	100.0	0	0.0	24	100.0
		M6	24	0	0.0	0	0.0	24	100.0	0	0.0	24	100.0
		BD	24	0	0.0	0	0.0	24	100.0	0	0.0	24	100.0
	T>72	M4	24	0	0.0	0	0.0	24	100.0	0	0.0	24	100.0
		M4RT	24	0	0.0	0	0.0	24	100.0	0	0.0	24	100.0
		M5	24	0	0.0	0	0.0	24	100.0	0	0.0	24	100.0
		M6	24	0	0.0	0	0.0	24	100.0	0	0.0	24	100.0
		BD	32**	1	3.1	1	3.1	32	100.0	1	3.1	32	100.0
-1.0 log LoD HPIV-3	T=0	M4	6	0	0.0	0	0.0	4	66.7	0	0.0	6	100.0
		M4RT	6	0	0.0	0	0.0	2	33.3	0	0.0	6	100.0
		M5	6	0	0.0	0	0.0	4	66.7	0	0.0	6	100.0
		M6	6	0	0.0	0	0.0	3	50.0	0	0.0	6	100.0
		BD	6	0	0.0	0	0.0	5	83.3	0	0.0	6	100.0
	T>72	M4	6	0	0.0	0	0.0	2	33.3	0	0.0	6	100.0
		M4RT	6	0	0.0	0	0.0	2	33.3	0	0.0	6	100.0
		M5	6	0	0.0	0	0.0	6	100.0	0	0.0	6	100.0
		M6	6	0	0.0	0	0.0	1	16.7	0	0.0	6	100.0
		BD	6*	0	0.0	0	0.0	4	66.7	0	0.0	6	100.0

* One reaction was invalid due to instrument error. Samples were retested and were valid. Results from the combined valid reactions are shown.

** One reaction was positive for HPIV-1, HPIV-2 and HPIV-4. Sample was re-tested (n=8). Result from the combined valid reactions (n=32) are shown.

Table 23. Matrix Comparison Results for HPIV-4 Positivity

Conc. and Sample Type	Time-point	VTM Type	Valid N	HPIV-1		HPIV-2		HPIV-3		HPIV-4		IC	
				# pos	% Positive	# pos	% Positive	# pos	% Positive	# pos	% Positive	pos n	% valid
+1.0 log LoD HPIV-4	T=0	M4	6	0	0.0	0	0.0	0	0.0	6	100.0	6	100.0
		M4RT	6	0	0.0	0	0.0	0	0.0	6	100.0	6	100.0
		M5	6	0	0.0	0	0.0	0	0.0	6	100.0	6	100.0
		M6	6	0	0.0	0	0.0	0	0.0	6	100.0	6	100.0
		BD	6	0	0.0	0	0.0	0	0.0	6	100.0	6	100.0
	T>72	M4	6	0	0.0	0	0.0	0	0.0	6	100.0	6	100.0
		M4RT	6	0	0.0	0	0.0	0	0.0	6	100.0	6	100.0
		M5	6	0	0.0	0	0.0	0	0.0	6	100.0	6	100.0
		M6	6	0	0.0	0	0.0	0	0.0	6	100.0	6	100.0
		BD	6	0	0.0	0	0.0	0	0.0	6	100.0	6	100.0
+0.5 log LoD HPIV-4	T=0	M4	24	0	0.0	0	0.0	0	0.0	24	100.0	24	100.0
		M4RT	24	0	0.0	0	0.0	0	0.0	24	100.0	24	100.0
		M5	24	0	0.0	0	0.0	0	0.0	24	100.0	24	100.0
		M6	24	0	0.0	0	0.0	0	0.0	24	100.0	24	100.0
		BD	24	0	0.0	0	0.0	0	0.0	24	100.0	24	100.0
	T>72	M4	24	0	0.0	0	0.0	0	0.0	24	100.0	24	100.0
		M4RT	24	0	0.0	0	0.0	0	0.0	24	100.0	24	100.0
		M5	24	0	0.0	0	0.0	0	0.0	24	100.0	24	100.0
		M6	24	0	0.0	0	0.0	0	0.0	24	100.0	24	100.0
		BD	24	0	0.0	0	0.0	0	0.0	24	100.0	24	100.0
-1.0 log LoD HPIV-4	T=0	M4	6	0	0.0	0	0.0	0	0.0	0	0.0	6	100.0
		M4RT	6	0	0.0	0	0.0	0	0.0	4	66.7	6	100.0
		M5	6	0	0.0	0	0.0	0	0.0	2	33.3	6	100.0
		M6	6	0	0.0	0	0.0	0	0.0	2	33.3	6	100.0
		BD	6	0	0.0	0	0.0	0	0.0	4	66.7	6	100.0
	T>72	M4	6	0	0.0	0	0.0	0	0.0	4	66.7	6	100.0
		M4RT	6	0	0.0	0	0.0	0	0.0	3	50.0	6	100.0
		M5	6	0	0.0	0	0.0	0	0.0	4	66.7	6	100.0
		M6	6	0	0.0	0	0.0	0	0.0	3	50.0	6	100.0
		BD	6	0	0.0	0	0.0	0	0.0	3	50.0	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 Paraflu Assay as well as performed the comparator shell vial culture/DFA testing (HPIV-1, HPIV-2, HPIV-3) and

dual reverse transcriptase-PCR (RT-PCR) followed by bi-directional sequencing (HPIV-4). 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 67 specimens had invalid results; 53 invalid results on the Panther Fusion Paraflu Assay and 14 invalid results from the reference testing. The overall invalid rate for the Panther Fusion Paraflu Assay was 1.8% (53/2930).

The tables below show the performance of the Panther Fusion Paraflu Assay, the data are stratified by analyte: HPIV-1, HPIV-2, HPIV-3, and HPIV-4. All data below represent performance for prospectively collected specimens. The data includes 1112 freshly collected and tested specimens and 1757 samples prospectively collected and stored frozen until testing.

Table 24. Panther Fusion Paraflu Assay Performance Relative to Culture/DFA or RT-PCR and sequencing for Prospective Nasopharyngeal (NP) Samples

Analyte	N	TP	FP	TN	FN	Prevalence (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
HPIV-1	2870	33	10[b]	2826	1[a]	1.2 (0.8-1.7)	97.1 (85.1-99.5)	99.6 (99.4-99.8)
HPIV-2	2870	22	15[c]	2831	2[a]	0.8 (0.6-1.2)	91.7 (74.2-97.7)	99.5 (99.1-99.7)
HPIV-3	2870	52	28[d]	2788	2[a]	1.9 (1.4-2.4)	96.3 (87.5-99.0)	99.0 (98.6-99.3)
HPIV-4	2870	29	5[e]	2835	1	1.0 (0.7-1.5)	96.7 (83.3-99.4)	99.8 (99.6->99.9)

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

[a] Confirmed negative by PCR

[b] 8/10 samples were confirmed positive by PCR

[c] 14/15 samples were confirmed positive by PCR

[d] 26/28 samples were confirmed positive by PCR

[e] No additional testing was available for HPIV-4 FP results

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 between fresh and frozen samples for any analyte using the Panther Fusion Paraflu Assay.

Table 25. Fresh vs. frozen study samples

	Fresh					Frozen				
Analyte	**N**	**TP**	**FP**	**TN**	**FN**	**N**	**TP**	**FP**	**TN**	**FN**
HPIV-1	1113	1	0	1112	0	1757	32	10	1714	1
HPIV-2	1113	20	15	1078	0	1757	2	0	1753	2
HPIV-3	1113	21	10	1081	1	1757	31	18	1707	1
HPIV-4	1113	9	1	1110	0	1757	20	4	1725	1

Table 26. Fresh vs. frozen study performance

	Sensitivity (95% CI)		Specificity (95%)	
Analyte	**Fresh**	**Frozen**	**Fresh**	**Frozen**
HPIV-1	100 (20.7-100)	97.0 (84.7-99.5)	100 (99.7-100)	99.4 (98.9-99.7)
HPIV-2	100 (83.9-100)	50.0 (15.0-85.0)	98.6 (97.7-99.2)	100 (99.8-100)
HPIV-3	95.5 (78.2-99.2)	96.9 (84.3-99.4)	99.1 (98.3-99.5)	99.0 (98.4-99.3)
HPIV-4	100 (70.1-100)	95.2 (77.3-99.2)	>99.9 (99.5->99.9)	99.8 (99.4->99.9)

4. Expected values/Reference range:

The Panther Fusion Paraflu 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 of HPIV-1, HPIV-2, HPIV-3 and HPIV-4, as determined by the Panther Fusion Paraflu Assay are shown by age category below.

Table 27. Observed prevalence for HPIV-1, HPIV-2, HPIV-3 and HPIV-4 during the clinical study

		HPIV-1		HPIV-2		HPIV-3		HPIV-4	
Age Group	Number of Patients	Number of Positives	% Positive	Number of Positives	% Positive	Number of Positives	% Positive	Number of Positives	% Positive
0 to 28 days	82	0	0.0%	0	0.0%	1	1.2%	0	0.0%
29 days to < 2 years	758	16	2.1%	18	2.4%	33	4.4%	13	1.7%
2 to 5 years	407	10	2.5%	9	2.2%	14	3.4%	9	2.2%
6 to 11 years	258	4	1.6%	2	0.8%	1	0.4%	6	2.3%
12 to 17 years	181	3	1.7%	6	3.3%	2	1.1%	1	0.6%
18 to 21 years	73	0	0.0%	0	0.0%	2	2.7%	0	0.0%
22 to 64 years	691	5	0.7%	0	0.0%	15	2.2%	3	0.4%
≥ 65 years	419	5	1.2%	2	0.5%	12	2.9%	2	0.5%
Total	2869	43	1.5%	37	1.3%	80	2.8%	34	1.2%

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.