510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:
K171963

B. Purpose for Submission:
The purpose of this submission is to show that the Panther Fusion Flu A/B/RSV Assay (for use on the Panther Fusion system) is substantially equivalent to the Prodesse ProFlu+ Assay (K153219) and to obtain clearance for the Panther Fusion Flu A/B/RSV Assay.

C. Measurand:
The Panther Fusion Flu A/B/RSV Assay detects influenza A, influenza B, and respiratory syncytial virus matrix 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 influenza A, influenza B, and RSV 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 Flu A/B/RSV

G. Regulatory Information:

1. Regulation section:
   21 CFR §866.3890 – Respiratory Viral Panel Multiplex Nucleic Acid Assay
   21 CFR §866.2570 – Instrumentation for Clinical Multiplex Test Systems

2. Classification:
   Class II

3. Product code:
   OCC – Respiratory Virus Panel Nucleic Acid Assay System
   OOI – Real Time Nucleic Acid Amplification System

4. Panel:
   Microbiology (83)

H. Intended Use:

1. Intended use(s):
   The Panther Fusion Flu A/B/RSV assay is a multiplex real-time PCR (RT-PCR) *in vitro* diagnostic test for the rapid and qualitative detection and differentiation of influenza A virus, influenza B virus, and respiratory syncytial virus (RSV). 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 influenza A virus, influenza B virus and RSV infections in humans and is not intended to detect influenza C virus infections. Negative results do not preclude influenza A virus, influenza B virus or RSV 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.

   Performance characteristics for influenza A Virus were established when Influenza A(H3N2) and A(H1N1)pdm09 were the predominant influenza A viruses in circulation. When other influenza A viruses are emerging, performance characteristics may vary. If infection with a novel influenza A virus is suspected based on current clinical and epidemiological screening criteria recommended by public health authorities, specimens should be collected with appropriate infection control precautions for novel virulent influenza viruses and sent to state or local health department for testing. Viral culture should not be attempted in these cases unless a BSL 3+ facility is available to receive and culture specimens.

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 Flu A/B/RSV 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 influenza A, influenza B, and respiratory syncytial virus (RSV) directly from the nasopharyngeal swab specimens.

The Panther Fusion Flu A/B/RSV 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 PRoFlu+ Assay

2. Predicate 510(k) number(s):
   K153219

3. Comparison with predicate:

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

Item	Panther Fusion Flu A/B/RSV Assay (Subject Device)	Prodesse ProFlu+ Assay (Predicate Device) K153219
Technology Principle of Operation	Multiplex Real Time RT-PCR	Same
Organisms Detected	Influenza A, Influenza B, and Respiratory Syncytial 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 Flu A/B/RSV Assay (Subject Device)	Prodesse ProFlu+ Assay (Predicate Device) K153219
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 Flu A/B/RSV assay is a multiplex real-time PCR (RT-PCR) *in vitro* diagnostic test for the rapid and qualitative detection and differentiation of influenza A virus, influenza B virus, and respiratory syncytial virus (RSV). 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 influenza A virus, influenza B virus and RSV	The Prodesse ProFlu+ Assay is a multiplex Real-Time PCR (RT-PCR) *in vitro* diagnostic test for the rapid and qualitative detection and discrimination of Influenza A Virus, Influenza B Virus, and Respiratory Syncytial Virus (RSV) nucleic acids isolated and purified from nasopharyngeal (NP) swab specimens obtained from symptomatic patients. This test is intended for use to aid in the differential diagnosis of Influenza A, Influenza B and RSV viral infections in humans and is not intended to detect Influenza C.

Item	Panther Fusion Flu A/B/RSV Assay (Subject Device)	Prodesse ProFlu+ Assay (Predicate Device) K153219
	infections in humans and is not intended to detect influenza C virus infections. Negative results do not preclude influenza A virus, influenza B virus or RSV 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. Performance characteristics for Influenza A Virus were established when Influenza A(H3N2) and A(H1N1)pdm09 were the predominant Influenza A viruses in circulation. When other Influenza A viruses are emerging, performance characteristics may vary. If infection with a novel Influenza A virus is suspected based on current clinical and epidemiological screening criteria recommended by public health authorities, specimens should be collected with appropriate infection control precautions for novel virulent Influenza viruses and sent to state or local health department for testing. Viral culture should not be attempted in these cases unless a BSL 3+ facility is available to receive and culture specimens.	Negative results do not preclude influenza or RSV virus infection and should not be used as the sole basis for treatment or other management decisions. Conversely, positive results do not rule-out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease. The use of additional laboratory testing and clinical presentation must be considered in order to obtain the final diagnosis of respiratory viral infection. Performance characteristics for Influenza A Virus were established when Influenza A/H3 and A/H1 were the predominant Influenza A viruses in circulation (2006 – 2007 respiratory season). Performance characteristics for Influenza A were confirmed when Influenza A/H1, Influenza A/H3, and Influenza A/2009 H1N1 were the predominant Influenza A viruses in circulation (2008 and 2009). When other Influenza A viruses are emerging, performance characteristics may vary. If infection with a novel Influenza A virus is suspected based on current clinical and epidemiological screening criteria recommended by public health authorities, specimens should be collected with appropriate infection control precautions for novel virulent Influenza viruses and sent to state or local health department for testing. Viral culture should not be attempted in these cases unless a BSL 3+ facility is available to receive and culture specimens.
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 influenza A, influenza B, and RSV. 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 influenza A, influenza B, RSV, 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
Influenza A Virus	Matrix	FAM
Respiratory Syncytial Virus A/B	Matrix	HEX
Influenza B Virus	Matrix	ROX
Internal Control-S	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	Flu A (1X LoD)
2	RSV (3X LoD) /Flu A (0.01X LoD)
3	RSV (1X LoD)
4	Flu B (1X LoD)
5	Flu B(3X LoD) /RSV (0.01X LoD)
6	Negative (unspiked)
7	Flu A(3X LoD) /Flu B(0.01X LoD)

Out of 63 total runs, there were 9 invalid runs: 2 due to hardware failure, 1 due to liquid level sensor failure, 4 due to operator error, and 2 due to one of the days' runs being invalid. Of the 54 valid runs, there were no invalid results.

Table 5. Precision study results

Target	Member ID	Valid N	% Positive (pos n/valid n)	% Agreement (95% CI)
Flu A	7	162	100.0% (162/162)	100.0% (97.7 - 100%)
	1	162	100.0% (162/162)	100.0% (97.7 - 100%)
	2	162	8.6% (14/162)	91.4% (86.0 - 94.8%)
	6	162	0.0% (0/162)	100.0% (97.7 - 100%)
Flu B	5	162	100.0% 162/162)	100.0% (97.7 - 100%)
	4	162	94.4% (153/162)	94.4% (89.8 - 97.0%)
	7	162	4.3% (7/162)	95.7% (91.4 - 97.9%)
	6	162	0.6% (1/162)	99.4% (96.6 - 99.9%)
RSV	2	162	100.0% (162/162)	100.0% (97.7 - 100%)
	3	162	99.4% (161/162)	99.4% (96.6 - 99.9%)
	5	162	4.9% (8/162)	95.1% (90.6 - 97.5%)
	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, except for the Flu B 1x LoD panel member which was 94.4% positive for Flu B (95% CI of 89.8-97.0%) and 0.0% positive for Flu A and for RSV. Negative panel member was 0.0% positive for Flu A and RSV but 0.6% positive for Flu B (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 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 (%)
Flu A	7	35.0	0.1	0.3	0.2	0.5	0.0	0.0	0.0	0.0	0.2	0.6	0.7	2.1	0.8	2.4
	1	35.3	0.0	0.1	0.1	0.5	0.0	0.0	0.0	0.0	0.2	0.6	0.8	2.4	0.9	2.5
	2	38.1	0.3	0.9	0.2	0.6	0.3	0.9	0.0	0.0	0.0	0.0	0.9	2.3	1.0	2.8
Flu B	5	36.5	0.0	0.1	0.1	0.5	0.0	0.0	0.0	0.0	0.1	0.3	0.7	1.9	0.7	2.0
	4	38.0	0.2	0.5	0.0	0.0	0.0	0.1	0.0	0.0	0.1	0.4	0.8	2.1	0.8	2.2
	7	39.4	0.3	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.9	0.5	1.3
RSV	2	36.2	0.2	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.3	3.5	1.3	3.6
	3	38.2	0.3	0.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.6	4.2	1.6	4.3
	5	40.7	0.0	0.0	0.0	0.0	0.2	0.6	0.4	1.0	0.0	0.0	0.2	0.5	0.5	1.3
IC	6	33.1	0.1	0.3	0.2	0.6	0.0	0.0	0.1	0.3	0.2	0.6	0.3	1.1	0.5	1.5

*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 $\leq$ 4.3%. The greatest source of variability was from the RSV 1X LoD panel member (within-run % CV of 4.2%). 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

Sample #	Analyte	Panel Member	Target Concentration
1	Influenza A	Low Positive	1 X LoD
2		Moderate Positive	3 X LoD
3	Influenza B	Low Positive	1 X LoD
4		Moderate Positive	3 X LoD
5	RSV	Low Positive	1 X LoD
6		Moderate Positive	3 X 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 630 samples were tested yielding 619 valid results. Six samples had invalid results due to a hardware error and five samples were not tested due to a hardware error.

Table 8. Reproducibility study results

Panel Member			Flu A		Flu B		RSV	
			Agreement with Expected	% Agreement (95% CI)	Agreement with Expected	% Agreement (95% CI)	Agreement with Expected	% Agreement (95% CI)
1	Flu A	Low Positive	86/86	100 (95.7-100)	86/86	100 (95.7-100)	86/86	100 (95.7-100)
2		Moderate Positive	88/88	100 (95.8-100)	88/88	100 (95.8-100)	88/88	100 (95.8-100)
3	Flu B	Low Positive	89/89	100 (95.9-100)	89/89	100 (95.9-100)	89/89	100 (95.9-100)
4		Moderate Positive	89/89	100 (95.9-100)	89/89	100 (95.9-100)	89/89	100 (95.9-100)
5	RSV	Low Positive	89/89	100 (95.9-100)	89/89	100 (95.9-100)	87/89	97.8 (92.2-99.4)
6		Moderate Positive	89/89	100 (95.9-100)	89/89	100 (95.9-100)	89/89	100 (95.9-100)
7	Neg.	Negative	89/89	100 (95.9-100)	89/89	100 (95.9-100)	89/89	100 (95.9-100)

Agreement was 100% for Flu A, Flu B and RSV moderate positive panel members and for the negative panel member tested with the Panther Fusion Flu A/B/RSV assay.

Agreement was also 100% for Flu A and Flu B low positive panel members tested with the Panther Fusion Flu A/B/RSV assay. However, agreement for the RSV low positive panel member was 97.8% (87/89; 95% CI: 92.2-99.4). 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. Precision study Ct signal variability analysis results

			Between Sites		Between Operators		Between Days		Between Runs		Within Runs		Total	
Panel Member	Analyte	Average Ct	Std. Dev	CV (%)	Std. Dev	CV (%)	Std. Dev	CV (%)	Std. Dev	CV (%)	Std. Dev	CV (%)	Std. Dev	CV (%)
1	Flu A	34.69	0	0	0.1	0.39	<0.	<0.1	<0.1	0.11	1.11	3.2	1.12	3.23
2		33.42	0	0	0.17	0.51	0.12	0.36	<0.1	<0.1	0.75	2.25	0.78	2.34
3	Flu B	37.17	0	0	0	0	0	0	0	0	0.98	2.65	0.98	2.65
4		36.43	0.18	0.5	0	0	0	0	0	0	0.8	2.19	0.82	2.24
5	RSV	38.34	0.37	0.98	0	0	0.49	1.29	<0.1	<0.1	1.32	3.45	1.46	3.81
6		36.1	0.31	0.85	0	0	0.31	0.86	<0.1	<0.1	1.1	3.05	1.18	3.28

The table above shows the variability of the Panther Fusion Flu A/B/RSV assay between sites, operator, days, and runs. The largest source of variation was the 'within runs' factor with %CV values ranging from 2.19% to 3.45%. All other sources of variation had %CV values less than 1.30%. The total performance variability (%CV) across all assay results was <4%.

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 Flu A/B/RSV 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 Flu A/B/RSV 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 Flu A/B/RSV 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 Flu A/B/RSV 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 Flu A/B/RSV 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 Flu A/B/RSV assay components during shipment would not impact the performance of the assay. One lot of components and controls for the Panther Fusion Flu A/B/RSV 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 Flu A/B/RSV assay were exposed to the extreme conditions.

The Panther Fusion A/B/RSV 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 and 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 A/B/RSV 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 Flu A/B/RSV assay when high titer Flu A/B/RSV 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 Flu A at 10^4 TCID50/mL (over 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 (Flu A)	HEX (RSV)	ROX (Flu B)	RED677 (IC)
Baseline	Negative	300	0.0% (0/300)	0.0% (0/300)	0.0% (0/300)	100.0% (300/300)
Runs 2-4	Negative	449*	0.4% (2/449)	0.0% (0/449)	0.0% (0/449)	100.0% (449/449)
Runs 2-4	Positive	449**	100% (449/449)	0.0% (0/449)	0.0% (0/449)	100.0% (449/449)***

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 Flu A positive. Two negative samples from the checkerboard runs were Flu A positive, for 0.4% overall carryover contamination rate (2/449).

d. Detection limit:

The purpose of this study was to evaluate and verify the analytical sensitivity and Limit of Detection (LoD) of Flu A, Flu B, and RSV viruses in pooled negative clinical nasopharyngeal swab specimens when tested with the Panther Fusion Flu A/B/RSV assay. Target-specific LoD values were obtained using multiple strains for each targeted virus. Dilutions of two strains of Flu A H1, two strains of Flu A H3, two strains of Flu B, one strain of RSV A, and one strain of RSV B 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 values 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 Type	Strain	Confirmed LOD Concentration (TCID50/ml)	Detection Channel (Target)			
			FAM (Flu A)	ROX (Flu B)	HEX (RSV)	RED677 (IC)
Flu A, H1N1	Influenza A /California/07/2009	10^ -1.0	100.0% (20/20)	0.0% (0/20)	0.0% (0/20)	100.0% (20/20)
	Influenza A /Massachusetts/15/13	10^ -1.5	100.0% (20/20)	0.0% (0/20)	0.0% (0/20)	100.0% (20/20)
Flu A,H3N2	Influenza A /Switzerland/9715293/2013	10^ -1.5	100.0% (20/20)	0.0% (0/20)	0.0% (0/20)	100.0% (20/20)
	Influenza A /Victoria/361/2011	10^ -1.5	100.0% (20/20)	0.0% (0/20)	0.0% (0/20)	100.0% (20/20)
Flu B	Influenza B /Brisbane/33/08	10^ -0.5	0.0% (0/20)	95.0% (19/20)	0.0% (0/20)	100.0% (20/20)
	Influenza B /Massachusetts/02/2012*	10^-2.0	1.7% (1/60)	95.0% (57/60)	1.7% (1/60)	100.0% (60/60)
RSV	RSV A	10^ 0.5	0.0% (0/20)	10.0% (2/20)	100.0% (20/20)	100.0% (20/20)
	RSV B	10^0.0	0.0% (0/20)	0.0% (0/20)	100.0% (20/20)	100.0% (20/20)

e. Analytical specificity:

Analytical Reactivity:

This study was performed to determine the analytical reactivity of the Panther Fusion Flu A/B/RSV assay with clinically relevant and non-seasonal strains of Flu A, Flu B and RSV. Inclusivity panel was prepared by spiking various Flu A/B/ RSV virus serotypes/subtypes/strains encompassing temporal and geographical diversity into simulated clinical matrix at a concentration of at least 10^2 $TCID_{50}$/ml or equivalent, and tested at least three replicates. Testing utilized one lot of reagents and three Panther Fusion systems. The strains tested and the assay results are shown in the table below.

Table 13. Analytical reactivity results

Inclusivity Strains*	Type	Concentration	Result		
			Flu A	Flu B	RSV
A/Aichi/2/1968	Influenza A/H3N2	10^2 CEID50 /mL	+	-	-
A/Brazil/02/1999	Influenza A/H3N2	10^2 TCID50 /mL	+	-	-

A/Brazil/1137/1999	Influenza A/H3N2	10^2 TCID50 /mL	+	-	-
A/Brisbane/59/2007	Influenza A/H1N1	10^2 TCID50 /mL	+	-	-
A/California/07/2009^	Influenza A/H1N1	10^-1.0 TCID50 /mL	+	-	-
A/Costa Rica/07/1999	Influenza A/H3N2	10^2 TCID50 /mL	+	-	-
A/Denver/1/57	Influenza A/H1N1	10^2 CEID50 /mL	+	-	-
A/Dominican Republic/7293/13	Influenza A/H1N1	10^2 TCID50 /mL	+	-	-
A/Fujian/156/2000	Influenza A/H1N1	10^2 TCID50 /mL	+	-	-
A/Georgia/F32551/12 2009	Influenza A/H1N1	10^2 TCID50 /mL	+	-	-
A/Hawaii/15/2001	Influenza A/H1N1	10^2 TCID50 /mL	+	-	-
A/Henan/8/2005	Influenza A/H1N1	10^2 TCID50 /mL	+	-	-
A/Hiroshima/52/2005	Influenza A/ H3N2	10^2 TCID50 /mL	+	-	-
A/Hong Kong/218/2006	Influenza A/ H3N2	10^2 TCID50 /mL	+	-	-
A/Hong Kong/4801/2014^	Influenza A/H3N2	10^2 TCID50 /mL	+	-	-
A/Hong Kong/486/97 RNA	Influenza A/H5N1	16.4 ng/mL	+	-	-
A/Hong Kong/8/1968	Influenza A/H3N2	10^2 CEID50 /mL	+	-	-
A/Indiana/08/2011	Influenza A/ H3N2	10^2 TCID50 /mL	+	-	-
A/Japan/305/1957	Influenza A/ H2N2	0.003 ug/mL	+	-	-
A/Jiangxi/160/2005	Influenza A/H1N1	10^2 TCID50 /mL	+	-	-
A/Kentucky/2/2006	Influenza A/H1N1	10^2 TCID50 /mL	+	-	-
A/Malaya/302/54	Influenza A/H1N1	10^2 CEID50 /mL	+	-	-
A/Mexico/4108/2009	Influenza A/H1N1	10^2 TCID50 /mL	+	-	-
A/Minnesota/11/2010	Influenza A/H3N2	36 ng/mL	+	-	-
A/New Jersey/8/1976	Influenza A/H1N1	10^3 TCID50 /mL	+	-	-

A/Ohio/09SW1477/2009	Influenza A/H1N2	10^2 TCID50 /mL	+	-	-
A/Perth/16/2009	Influenza A/H3N2	10^2 TCID50 /mL	+	-	-
A/Port Chalmers/1/1973	Influenza A/ H3N2	10^2 TCID50 /mL	+	-	-
A/Puerto Rico/8/34	Influenza A/H1N1	10^2 TCID50 /mL	+	-	-
A/Solomon Islands/03/2009	Influenza A/H1N1	10^2 TCID50 /mL	+	-	-
A/Switzerland/9715293/2013 ^	Influenza A/H3N2	10^-1.5 TCID50 /mL	+	-	-
A/Taiwan/42/2006	Influenza A/H1N1	10^2 TCID50 /mL	+	-	-
A/Victoria/3/1975	Influenza A/ H3N2	10^2 CEID50 /mL	+	-	-
A/Vietnam/1203 RNA	Influenza A/H5N1	0.27 ug/mL	+	-	-
A/WS/33	Influenza A/H1N1	10^2 TCID50 /mL	+	-	-
B/Brisbane/60/2008^	Influenza	10^2 TCID50 /mL	-	+	-
B/Florida/2/2006	Influenza	10^2 TCID50 /mL	-	+	-
B/Florida/7/2004	Influenza	10^2 TCID50 /mL	-	+	-
B/Hawaii/11/2005	Influenza	10^2 TCID50 /mL	-	+	-
B/Hawaii/33/2004	Influenza	10^2 TCID50 /mL	-	+	-
B/Lee/40	Influenza	10^2 CEID50 /mL	-	+	-
B/Michigan/2/2006	Influenza	10^2 TCID50 /mL	-	+	-
B/Ohio/1/2005	Influenza	10^2 TCID50 /mL	-	+	-
B/Panama/45/90	Influenza	10^2 TCID50 /mL	-	+	-
B/Phuket/3073/2013^	Influenza	10^2 TCID50 /mL	-	+	-
B/St. Petersburg/04/2006	Influenza	10^2 TCID50 /mL	-	+	-
RSV A/A2	RSV	10^2 TCID50 /mL	-	-	+
RSV A/Long Lot 020207	RSV	10^2 TCID50 /mL	-	-	+
RSV A/Long Lot 303012	RSV	10^2 TCID50 /mL	-	-	-
RSV A/Vero	RSV	10^2 CEID50 /mL	-	-	+
RSV B/9320	RSV	10^2 TCID50 /mL	-	-	+
RSV B/Wash/18537/62	RSV	10^2 TCID50 /mL	-	-	+
A/Chicken/Germany/N/49	Influenza A/H10N7	68 ng/mL	+	-	-
A/Duck/Alberta/35/76	Influenza A/H1N1	1 ng/mL	+	-	-
A/Duck/Chabarovsk/1610/1972	Influenza A/H3N8	1 ng/mL	+	-	-
A/Duck/Czechoslovakia/1956^^	Influenza A/H4N6	2.6 ng/mL	+	-	-

A/Duck/Memphis/546/1974	Influenza A/H11N9	8 ng/mL	+	-	-
A/Duck/Pennsylvania/10218/1984	Influenza A/H5N2	3 ng/mL	+	-	-
A/Duck/Singapore/645/97	Influenza A/H5N3	2 ng/mL	+	-	-
A/Duck/Ukraine/1963	Influenza A/H3N8	3 ng/mL	+	-	-
A/gyrfalcon/Washington/41088-6/2014	Influenza A/H5N8	10^3 TCID50 /mL	+	-	-
A/Northern pintail/Washington/ 40964/2014	Influenza A/H5N2	10^3 TCID50 /mL	+	-	-
A/Swine/ NY/01/2009	Influenza A/H1N1	10^2 TCID50 /mL	+	-	-
A/Swine/Iowa/2006	Influenza A/H1N1	10^2 CEID50 /mL	+	-	-
A/Turkey/Massachusetts /3740/1965	Influenza A/H6N2	1 ng/mL	+	-	-
A/Turkey/Ontario/6118/1968	Influenza A/H8N4	2 ng/mL	+	-	-
A/Turkey/Wisconsin/1/1966	Influenza A/H9N2	23 ng/mL	+	-	-

*Multiple concentrations were tested for some of the strains. Lowest concentration that produced 100% reactivity for the intended target is shown, unless noted otherwise.
^Strains recommended by FDA to be included in vaccine 2015-2016 and 2016-2017. California/07/2009 and Switzerland/9715293/2013 were tested as part of the LOD study (VAR-05327).
^^When tested at higher concentration, low level of cross reactivity for the RSV has been observed.

The results from this study demonstrate that the Panther Fusion Flu A/B/RSV assay is capable of detecting multiple clinically relevant strains of Flu A, Flu B, and RSV, including the strains targeted by the Flu vaccine 2015-2017.

Interfering Substances

This study evaluated the performance of the Panther Fusion Flu A/B/RSV 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 (Fu A, Flu B and RSV) 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 (Dipropionate))	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 hydrochloricum, Sulfur	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)			
		Flu A	**RSV**	**Flu B**	**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, Flonase)	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)
Flu A	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)	100.0% (9/9)	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)	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)	100.0% (9/9)	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)
RSV	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, Aerospan)	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)	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)
Flu B	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, Aerospan)	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)	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% (10/10)	100.0% (10/10)
	Panel 11 (SCM)	0.0% (0/10)	0.0% (0/10)	100.0% (10/10)	100.0% (10/10)

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 Flu A/B/RSV Assay at the concentrations tested.

Competitive Interference:

The purpose of this study was to demonstrate that samples tested with the Panther Fusion Flu A/B/RSV assay that are co-infected with multiple types of targeted organisms do not inhibit the detection of either one (competitive interference). One

representative strain of each targeted organism (Flu A, Flu B and RSV) 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
1	Flu A 3X LoD	RSV 1,000X LoD
2	Flu A 3X LoD	Flu B 1,000X LoD
3	Flu B 3X LoD	Flu A 1,000X LoD
4	Flu B 3X LoD	RSV 1,000X LoD
5	RSV 3X LoD	Flu A 1,000X LoD
6	RSV 3X LoD	Flu B 1,000X LoD
7	Flu B 10X LoD	Flu A 1,000X LoD
8	Flu B 5X LoD	Flu A 1,000X LoD

Interference was observed for Flu B in Panel member 3 with Flu B spiked at 3X LoD (Low) and Flu A spiked at 1000X LoD (high). Panel member 3 was 100% positive for Flu A (6/6) but 83.3% positive for Flu B (5/6). A second lot of the Panel member 3 was made and tested for additional 20 replicates. Initial and combined test result was less than 95% for Flu B (92.3%, 24/26). Therefore, two new panel members were made and tested where Flu B was spiked at a slightly higher concentrations: Panel member 7 (Flu B at 10X LoD and Flu A at 1000X LoD) and Panel member 8 (Flu B at 5X LoD and Flu A at 1000X LoD). Both produced 100% positive for Flu B and for Flu A (6/6), demonstrating that the competitive interference for the Flu B by Flu A is not observed when Flu B is present at least at 5X LoD in the presence of Flu A at 1000X LoD. This is acceptable as 5X LoD is a titer that is much lower than what is commonly found in clinical samples. All the other 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)	Flu A	RSV	Flu B	IC
1	Flu A	RSV	100.0% (6/6)	100.0% (6/6)	0.0% (0/6)	100.0% (6/6)
2	Flu A	Flu B	100.0% (6/6)	0.0% (0/6)	100.0% (6/6)	100.0% (6/6)
3, Lot 1	Flu B	Flu A	100.0% (6/6)	0.0% (0/6)	**83.3% (5/6)**	100.0% (6/6)
3, Lot 2	Flu B	Flu A	100.0% (20/20)	0.0% (0/20)	**95.0% (19/20)**	100.0% (20/20)
3, Lot 1 and 2 Combined	Flu B	Flu A	100.0% (26/26)	0.0% (0/26)	**92.3% (24/26)**	100.0% (26/26)
4	Flu B	RSV	0.0% (0/6)	100.0% (6/6)	100.0% (6/6)	100.0% (6/6)
5	RSV	Flu A	100.0% (6/6)	100.0% (6/6)	0.0% (0/6)	100.0% (6/6)
6	RSV	Flu B	0.0% (0/6)	100.0% (6/6)	100.0% (6/6)	100.0% (6/6)
7	Flu B (10X LoD)	Flu A	100.0% (6/6)	0.0% (0/6)	100.0% (6/6)	100.0% (6/6)
8	Flu B (5X LoD)	Flu A	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 Flu A/B/RSV 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 (Flu A, Flu B, RSV) 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
3	Coronavirus 229E	1.00E+04	$TCID_{50}$ /ml
	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	*Staphlycoccus aureus*	1.00E+07	CFU/ml
	Staphlycoccus 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

For panel member 9 composed of HSV-1, HSV-2 and hMPV subtype A2, one replicate was positive for Flu B when tested unspiked with one reagent lot, when 0% Flu B positivity is expected. Panel member was tested for additional 6 replicates all of which were negative for Flu B, for a final Flu B % positivity of 3.0% (1/33). This one Flu B positive reaction was likely caused by a rare carryover event, and not due to cross-reactivity (there is no sequence alignment of Flu B primers with HSV-1, HSV-2 or hMPV). Panel member 9 was 0% positive for Flu A and RSV, as expected. All other panel members were 100.0% positive for spiked samples, and were 0.0% positive for un-spiked samples. The final results for all conditions tested are detailed in the table below.

Table 19. Cross-reactivity study results

Panel Member	Spiked organism*	Target % Positive (reactive n/valid n)**			
		Flu A	RSV	Flu B	IC
1	Unspiked	0.0% (0/27)	0.0% (0/27)	0.0% (0/27)	100.0% (27/27)
	Flu A	100.0% (9/9)	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)
	Flu B	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)	100.0% (9/9)
	RSV A	0.0% (0/9)	100.0% (9/9)	0.0% (0/9)	100.0% (9/9)
2	Unspiked	0.0% (0/33)	0.0% (0/33)	0.0% (0/33)	100.0% (33/33)
	Flu A	100.0% (9/9)	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)
	Flu B	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)	100.0% (9/9)
	RSV A	0.0% (0/10)	100.0% (10/10)	0.0% (0/10)	100.0% (10/10)
3	Unspiked	0.0% (0/33)	0.0% (0/33)	0.0% (0/33)	100.0% (33/33)
	Flu A	100.0% (9/9)	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)
	Flu B	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)	100.0% (9/9)
	RSV A	0.0% (0/9)	100.0% (9/9)	0.0% (0/9)	100.0% (9/9)
4	Unspiked	0.0% (0/30)	0.0% (0/30)	0.0% (0/30)	100.0% (30/30)
	Flu A	100.0% (9/9)	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)
	Flu B	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)	100.0% (9/9)
	RSV A	0.0% (0/9)	100.0% (9/9)	0.0% (0/9)	100.0% (9/9)
5	Unspiked	0.0% (0/36)	0.0% (0/36)	0.0% (0/36)	100.0% (36/36)
	Flu A	100.0% (9/9)	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)
	Flu B	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)	100.0% (9/9)
	RSV A	0.0% (0/9)	100.0% (9/9)	0.0% (0/9)	100.0% (9/9)
6	Unspiked	0.0% (0/27)	0.0% (0/27)	0.0% (0/27)	100.0% (27/27)
	Flu A	100.0% (9/9)	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)
	Flu B	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)	100.0% (9/9)
	RSV A	0.0% (0/11)	100.0% (11/11)	0.0% (0/11)	100.0% (11/11)
7	Unspiked composed of Flu A, Flu B and RSV A	100.0% (27/27)	100.0% (27/27)	100.0% (27/27)	100.0% (27/27)
8	Unspiked	0.0% (0/27)	0.0% (0/27)	0.0% (0/27)	100.0% (27/27)
	Flu A	100.0% (9/9)	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)
	Flu B	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)	100.0% (9/9)
	RSV A	0.0% (0/9)	100.0% (9/9)	0.0% (0/9)	100.0% (9/9)
9	Unspiked	0.0% (0/33)	0.0% (0/33)	3.0% (1/33)	100.0% (33/33)
	Flu A	100.0% (9/9)	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)
	Flu B	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)	100.0% (9/9)
	RSV A	0.0% (0/9)	100.0% (9/9)	0.0% (0/9)	100.0% (9/9)
10	Unspiked	0.0% (0/27)	0.0% (0/27)	0.0% (0/27)	100.0% (27/27)
	Flu A	100.0% (9/9)	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)
	Flu B	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)	100.0% (9/9)

	RSV A	0.0% (0/10)	100.0% (10/10)	0.0% (0/10)	100.0% (10/10)
11	Unspiked	0.0% (0/27)	0.0% (0/27)	0.0% (0/27)	100.0% (27/27)
	Flu A	100.0% (9/9)	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)
	Flu B	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)	100.0% (9/9)
	RSV A	0.0% (0/9)	100.0% (9/9)	0.0% (0/9)	100.0% (9/9)
12	Unspiked	0.0% (0/27)	0.0% (0/27)	0.0% (0/27)	100.0% (27/27)
	Flu A	100.0% (9/9)	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)
	Flu B	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)	100.0% (9/9)
	RSV A	0.0% (0/9)	100.0% (9/9)	0.0% (0/9)	100.0% (9/9)
13	Unspiked	0.0% (0/27)	0.0% (0/27)	0.0% (0/27)	100.0% (27/27)
	Flu A	100.0% (9/9)	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)
	Flu B	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)	100.0% (9/9)
	RSV A	0.0% (0/9)	100.0% (9/9)	0.0% (0/9)	100.0% (9/9)
14	Unspiked	0.0% (0/27)	0.0% (0/27)	0.0% (0/27)	100.0% (27/27)
	Flu A	100.0% (9/9)	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)
	Flu B	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)	100.0% (9/9)
	RSV A	0.0% (0/9)	100.0% (9/9)	0.0% (0/9)	100.0% (9/9)
15	Unspiked	0.0% (0/27)	0.0% (0/27)	0.0% (0/27)	100.0% (27/27)
	Flu A	100.0% (9/9)	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)
	Flu B	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)	100.0% (9/9)
	RSV A	0.0% (0/9)	100.0% (9/9)	0.0% (0/9)	100.0% (9/9)
16	Unspiked	0.0% (0/27)	0.0% (0/27)	0.0% (0/27)	100.0% (27/27)
	Flu A	100.0% (9/9)	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)
	Flu B	0.0% (0/9)	0.0% (0/9)	100.0% (9/9)	100.0% (9/9)
	RSV A	0.0% (0/9)	100.0% (9/9)	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 Flu A/B/RSV 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 Flu A/B/RSV 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 1,000 for Flu A, 500 for Flu B, 1000 for RSV for determination of positivity in their specific channels (FAM, ROX, HEX, respectively) and 500 RFU range for IC 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 additional details on the composite 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 Flu A, Flu B, and RSV were then spiked into each VTM type at concentrations 1log 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.

Table 20. Matrix comparison results for Flu A positivity

Sample Conc.	Time point	VTM Type	Valid N	Flu A		RSV		Flu B	
				Pos n	% Positive	Pos n	% Positive	Pos n	% Positive
+1.0 log LoD	T=0	M4	6	6	100.0	0	0.0	0	0.0
		M4RT	6	6	100.0				
		M5	6	6	100.0				
		M6	6	6	100.0				
		BD	6	6	100.0				
	T>72	M4	6	6	100.0	0	0.0	0	0.0
		M4RT	6	6	100.0				
		M5	6	6	100.0				
		M6	6	6	100.0				
		BD	6	6	100.0				
+0.5 log LoD	T=0	M4	24	24	100.0	0	0.0	0	0.0
		M4RT	24	24	100.0				
		M5	24	24	100.0				
		M6	24	24	100.0				
		BD	24	24	100.0				
	T>72	M4	24	24	100.0	0	0.0	0	0.0
		M4RT	24	24	100.0				
		M5	24	24	100.0				
		M6	24	24	100.0				
		BD	24	24	100.0				
-1.0 log LoD	T=0	M4	6	4	66.7	0	0.0	0	0.0
		M4RT	6	5	83.3				
		M5	6	4	66.7				
		M6	6	4	66.7				
		BD	6	4	66.7				
	T>72	M4	6	4	66.7	0	0.0	0	0.0
		M4RT	6	4	66.7				
		M5	6	6	100.0				
		M6	6*	3	50.0				
		BD	6	3	50.0				

* One reaction was invalid. Sample (same vial) was re-tested and was valid. Result from the combined valid reactions (n=6) is shown here.

Table 21. Matrix comparison results for Flu B positivity

Conc. and Sample Type	Time-point	VTM Type	Valid N	Flu A		RSV		Flu B	
				pos n	% Positive	pos n	% Positive	pos n	% Positive
+1.0 log LoD	T=0	M4	6	0	0.0	0	0.0	6	100.0
		M4RT	6					6	100.0
		M5	6					6	100.0
		M6	6					6	100.0
		BD	6					6	100.0
	T>72	M4	6	0	0.0	0	0.0	6	100.0
		M4RT	6					6	100.0
		M5	6					6	100.0
		M6	6					6	100.0
		BD	6					6	100.0
+0.5 log LoD	T=0	M4	24	0	0.0	0	0.0	24	100.0
		M4RT	24					24	100.0
		M5	24					24	100.0
		M6	24					24	100.0
		BD	24					24	100.0
	T>72	M4	24	0	0.0	0	0.0	24	100.0
		M4RT	24					24	100.0
		M5	24					24	100.0
		M6	24					24	100.0
		BD	24					24	100.0
-1.0 log LoD	T=0	M4	6	0	0.0	0	0.0	2	33.3
		M4RT	6					3	50.0
		M5	6					2	33.3
		M6	6					4	66.7
		BD	6					2	33.3
	T>72	M4	6	0	0.0	0	0.0	2	33.3
		M4RT	6					2	33.3
		M5	6					1	16.7
		M6	6					2	33.3
		BD	6					2	33.3

Table 22. Matrix comparison results for RSV positivity

Conc. and Sample Type	Time-point	VTM Type	Valid N	Flu A		RSV		Flu B	
				posn	% Positive	posn	% Positive	posn	% Positive
+1.0 log LoD	T=0	M4	6	0	0.0	6	100.0	0	0.0
		M4RT	6			6	100.0		
		M5	6			6	100.0		
		M6	6			6	100.0		
		BD	6			6	100.0		
	T>72	M4	6	0	0.0	6	100.0	0	0.0
		M4RT	6			6	100.0		
		M5	6			6	100.0		
		M6	6			6	100.0		
		BD	6			6	100.0		
+0.5 log LoD	T=0	M4	24	0	0.0	24	100.0	0	0.0
		M4RT	24			24	100.0		
		M5	24			24	100.0		
		M6	24			24	100.0		
		BD	24			24	100.0		
	T>72	M4	24	0	0.0	24	100.0	0	0.0
		M4RT	24			24	100.0		
		M5	24			24	100.0		
		M6	24			24	100.0		
		BD	24			24	100.0		
-1.0 log LoD	T=0	M4	6*	0	0.0	5	83.3	0	0.0
		M4RT	6			3	50.0		
		M5	6			2	33.3		
		M6	6			3	50.0		
		BD	6			6	100.0		
	T>72	M4	6	0	0.0	5	83.3	0	0.0
		M4RT	6			3	50.0		
		M5	6			4	66.7		
		M6	6			1	16.7		
		BD	6			5	83.3		

***** Five reactions were invalid due to instrument error. Same sample vials were retested; all were valid. Result from the combined valid reactions (n=6) is shown here.

The study demonstrated the performance equivalency of the Panther Fusion Flu A/B/RSV assay across multiple types of VTM: Remel Micro Test M4, M4RT, M5, M6 Viral Transport Medium, Copan Universal Transport Medium, and BD Universal Viral Transport Medium.

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 Flu A/B/RSV assay as well as performed the comparator shell vial culture/DFA testing. 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 61 specimens had invalid results; 54 invalid results on the Panther Fusion Flu A/B/RSV and 7 invalid results from the reference testing. The overall invalid rate for the Panther Fusion Flu A/B/RSV was 1.8% (54/2930).

The tables below show the performance of the Panther Fusion Flu A/B/RSV assay, the data are stratified by analyte: Flu A, Flu B, and RSV. 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 23. Panther Fusion Flu A/B/RSV Assay Performance Relative to Culture/DFA for Prospective NP Samples

Analyte	N	TP	FP	TN	FN	Prevalence (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Flu A	2869	131	58[b]	2679	1[a]	4.6 (3.9-5.4)	99.2 (95.8-99.9)	97.9 (97.3-98.4)
Flu B	2869	46	9[c]	2813	1[a]	1.6 (1.2-2.2)	97.9 (88.9-99.6)	99.7 (99.4-99.8)
RSV	2869	236	129[d]	2501	3[a]	8.3 (7.4-9.4)	98.7 (96.4-99.6)	95.1 (94.2-95.9)

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

[a]Confirmed negative by PCR

[b]55/58 samples were confirmed positive by PCR

[c]6/9 samples were confirmed positive by PCR

[d]114/129 samples were confirmed positive by PCR

Fresh vs. frozen performance was analyzed by comparing the sensitivity and specificity of fresh vs. frozen samples for each analyte. The data below demonstrates that there is no significant difference between fresh and frozen samples for any analyte using the Panther Fusion Flu A/B/RSV assay.

Table 24. Fresh vs. frozen study samples

	Fresh					Frozen				
Analyte	**N**	**TP**	**FP**	**TN**	**FN**	**N**	**TP**	**FP**	**TN**	**FN**
Flu A	1112	64	32	1016	0	1757	67	26	1663	1
Flu B	1112	14	2	1095	1	1757	32	7	1718	0
RSV	1112	93	83	933	3	1757	143	46	1568	0

Table 25. Fresh vs. frozen study performance

	Sensitivity (95% CI)		Specificity (95%)	
Analyte	**Fresh**	**Frozen**	**Fresh**	**Frozen**
Flu A	100 (94.3-100)	98.5 (92.1-99.7)	96.9 (95.7-97.8)	98.5 (97.8-98.9)
Flu B	93.3 (70.2-98.8)	100 (89.3-100)	99.8 (99.3->99.9)	99.6 (99.2-99.8)
RSV	96.9 (91.2-98.9)	100 (97.4-100)	91.8 (90.0-93.4)	97.1 (96.2-97.9)

4. Expected values/Reference range:

The Panther Fusion Flu A/B/RSV 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 influenza A, influenza B, and RSV, as determined by the Panther Fusion Flu A/B/RSV Assay are shown by age category below.

Table 26. Observed prevalence for Flu A, Flu B and RSV during the clinical study

		Flu A		Flu B		RSV	
Age Group	Number of Patients	Number of Positives	% Positive	Number of Positives	% Positive	Number of Positives	% Positive
0 to 28 days	82	0	0.0%	0	0.0%	15	18.3%
29 days to < 2 years	758	33	4.4%	2	0.3%	202	26.6%
2 to 5 years	407	16	3.9%	10	2.5%	81	19.9%
6 to 11 years	258	30	11.6%	10	3.9%	12	4.7%
12 to 17 years	181	23	12.7%	4	2.2%	8	4.4%
18 to 21 years	73	4	5.5%	2	2.7%	2	2.7%
22 to 64 years	691	65	9.4%	22	3.2%	28	4.1%
≥ 65 years	419	18	4.3%	5	1.2%	17	4.1%
Total	2869	189	6.6%	55	1.9%	365	12.7%

N. Instrument Name:

Panther System and Panther Fusion System, software version 6.1

O. System Descriptions:

1. Modes of Operation:

Does the applicant's device contain the ability to transmit data to a computer, webserver, or mobile device?

Yes X or No _____

Does the applicant's device transmit data to a computer, webserver, or mobile device using wireless transmission?

Yes _____ or No X

2. Changes to the Panther System:

Several changes were made to the Panther instrument to enable the addition of the Panther Fusion module as described below:

- A replacement of the barcode reader with a slimmer design for the sample compartment.

- A redesign of the TCR Carousel module to include additional TCR/TER bottle positions and positions for Internal Control tubes, as well as a change in the mixing profile for the TCR Carousel.
- Sample dispense slot increased to a 3 slot dispense station to accommodate up to three MTUs
- Removal of one unused slot of the Chiller ramp module to create space for the additional sample dispense slots
- Existing fans upgraded for Panther left chassis and added to the Panther power supply area
- Updates to the system software to implement the hardware changes and functionality required for the Panther Fusion System configuration

For a description of system components see Device Description (Section I.1 above).

3. Software:

FDA has reviewed applicant's Hazard Analysis and software development processes for this line of product types:

Yes ___X_____ or No _____

Hologic has provided sufficient documentation in K171963 for review of software. A summary of software documentation is described below.

Level of Concern
Moderate

Software Description
There are two software components of the Panther Fusion System that are required to perform Panther Fusion assays:

- System Software—Panther Fusion System Software includes the Master Controller Software and instrument firmware. This software is assay independent and does not include assay specific parameters.
- Assay Software—Assay software contains all the information that is specific to a given assay (reagent volumes, incubation times/temperatures, sequence of steps, etc.).

Additionally, the system contains Off-the-Shelf (OTS) software obtained from third parties.

Device Hazard Analysis
A device risk analysis was performed that accounted for known and foreseeable device hazards associated with the device's Intended Use, hardware hazards, software hazards (including OTS), potential user-induced hazards due to intentional or inadvertent misuse, and cybersecurity hazards. Hazards associated with the system were placed into two categories: Instrument Specific Hazards and Assay Specific Hazards. In addition, a risk analysis was performed to evaluate new consumable items used by the system in order to

process Panther Fusion assays. A few examples of hazards evaluated for the system included:

Table 27. Hazards evaluated in risk analysis

Electric Shock	Potential manufacturing, shipping, and storage	Usability
Fire	Contamination	Cybersecurity
Exposure to barcode scanner light source	Defective ancillaries and accessories	Electro-mechanical
Unintended contact between operator and moving parts	Software	Environmental

Hologic also reported that a risk assessment was performed to ensure updates implemented within the system software v6.1 (including the related hardware updates) did not introduce new safety hazards or adversely impact assay performance of approved TMA assays. The changes assessed in this separate analysis included the software updates to move from v5.3 to v6.1 for the Panther System and the hardware and software updates to support the addition of the Panther Fusion module onto the Panther System.

After all potential hazards associated with the system were evaluated for risk, and the various risk control measures were implemented and verified, an assessment of the overall risk management activities and the residual risk for the Aptima and Panther Fusion assays on the system was made. There were no hazards that fell within the *"Undesirable"* or *"Unacceptable"* residual risk regions. Potential hazards were mitigated to a residual risk rating level of *"Broadly Acceptable"* or *"As Far As Possible (AFAP)."*

Software Requirement Specifications (SRS) and Software Design Specifications (SDS)
SRS documents were provided that described the requirements for various components of the system software relevant for processing of the Aptima and Panther Fusion assays and the trace matrix of the consolidated SRS. The SRS covered the following (not an exhaustive list):

Table 28. Software Requirement Specifications provided

Assay	Reports
Barcode Definition	Security
Database	User Interface
Inventory	Event and Error Handling
Maintenance	System Workflow

It was reported that the process of capturing and analyzing software requirements was an iterative process covering risk analysis and software architectural design and that SDS were developed for key software components.

Architecture Design Chart
Detailed information was provided to describe the system architecture, including flow charts for each of the system components for running the Aptima and Panther Fusion

assays.

Traceability Analysis
A traceability matrix for the system was provided that tracked activities from system requirements, through instrument and software design inputs, up to verification and validation of outputs. Risk management traceability matrices were also provided.

Software Development Environment Description
The software development environment description summarized the software development life cycle, configuration/change management, and comparability assessments.

Verification and Validation (V&V)
V&V testing was performed with traceability to specific design inputs. System validation included the collective body of all V&V activities associated with the assay, software, firmware, hardware, and instrument in order to demonstrate that the device conformed to defined user needs and Intended Use. According to Hologic, system software encompassed the following V&V activities: instrument validation, assay verification, instrument verification, and software verification and validation.

It was stated that the system for use with the Aptima and Panther Fusion assays (with software v6.1) met user needs, Intended Use, and safety requirements as demonstrated by the validation testing.

Revision Level History
A list was provided of the software versions generated for the system software as well as the Aptima and Panther Fusion assay software versions. The revision level history included the release date and most current version of software-system software version 6.1 and Panther Fusion Flu A/B/RSV Assay version 2.4.7. Clinical and non-clinical (analytical studies) for K171963 were conducted with system software v6.0 and Panther Fusion Flu/A/B/RSV v1.116.

Unresolved Anomalies
A summary of unresolved anomalies in the system software up to the current release version (6.1) was documented in the Summary Anomaly Report for Panther and Panther Fusion System Software. The report contained a complete list of open anomalies in the Panther/Panther Fusion system software, v6.1 that were reported up through completion of software V&V testing. Each software anomaly was assessed for impact to patient and user safety, and it was determined that there were no known software anomalies that exceeded the severity level of *"Minor."*

Cybersecurity and OTS
A cybersecurity risk analysis was performed as part of the risk management process to ensure cybersecurity hazards were minimized and controlled. Hazard analysis for OTS software was conducted as part of the system hazard analysis.

3. Calibration:
Real Time Fluorometers (RTF) undergo a single calibration during manufacturing. No additional calibration is performed by the end user.

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.