DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
10903 New Hampshire Avenue
Document Control Center – WO66-G609
Silver Spring, MD 20993-0002

September 26, 2017

Hologic, Inc.
Ron Domingo
Regulatory Affairs Manager
10210 Genetic Center Drive
San Diego CA 92121

Re: K171963
Trade/Device Name: Panther Fusion Flu A/B/RSV Assay
Regulation Number: 21 CFR 866.3980
Regulation Name: Respiratory viral panel multiplex nucleic acid assay
Regulatory Class: II
Product Code: OCC, OOI
Dated: June 29, 2017
Received: June 30, 2017

Dear Mr. Domingo:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you, however, that device labeling must be truthful and not misleading.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Parts 801 and 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR 803); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820); and if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR 1000-1050.

If you desire specific advice for your device on our labeling regulations (21 CFR Parts 801 and 809), please contact the Division of Industry and Consumer Education at its toll-free number (800) 638 2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm. Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to http://www.fda.gov/MedicalDevices/Safety/ReportaProblem/default.htm for the CDRH's Office of Surveillance and Biometrics/Division of Postmarket Surveillance.

You may obtain other general information on your responsibilities under the Act from the Division of Industry and Consumer Education at its toll-free number (800) 638-2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm.

Sincerely yours,

Steven R. Gitterman -S for

Uwe Scherf, M.Sc., Ph.D.
Director
Division of Microbiology Devices
Office of In Vitro Diagnostics
and Radiological Health
Center for Devices and Radiological Health

Enclosure

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: January 31, 2017
See PRA Statement below.

510(k) Number *(if known)*
K171963

Device Name
Panther Fusion Flu A/B/RSV Assay

Indications for Use *(Describe)*
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 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 any culture specimens.

Type of Use *(Select one or both, as applicable)*

☒ Prescription Use (Part 21 CFR 801 Subpart D) ☐ Over-The-Counter Use (21 CFR 801 Subpart C)

CONTINUE ON A SEPARATE PAGE IF NEEDED.

This section applies only to requirements of the Paperwork Reduction Act of 1995.

DO NOT SEND YOUR COMPLETED FORM TO THE PRA STAFF EMAIL ADDRESS BELOW.

The burden time for this collection of information is estimated to average 79 hours per response, including the time to review instructions, search existing data sources, gather and maintain the data needed and complete and review the collection of information. Send comments regarding this burden estimate or any other aspect of this information collection, including suggestions for reducing this burden, to:

Department of Health and Human Services
Food and Drug Administration
Office of Chief Information Officer
Paperwork Reduction Act (PRA) Staff
PRAStaff@fda.hhs.gov

"An agency may not conduct or sponsor, and a person is not required to respond to, a collection of information unless it displays a currently valid OMB number."

510(k) SUMMARY
Panther Fusion Flu A/B/RSV Assay

I. SUBMITTER

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

Contact Person: Ron Domingo, MS, RAC
Regulatory Affairs Manager
Ron.Domingo@Hologic.com
Phone: (858) 410-8167
Fax: (858) 410-5557

Date Prepared: June 28, 2017

II. DEVICE

Proprietary Name of Device:	Panther Fusion Flu A/B/RSV Assay
Classification Name:	Respiratory viral panel multiplex nucleic acid assay
Regulation Number:	21 CFR 866.3980 and 862.2570
Regulatory Class:	Class II
Product Code:	OCC and OOI

III. PREDICATE DEVICE

The predicate device is the Prodesse ProFlu+ Assay (K153219; cleared November 20, 2015, Hologic, San Diego, CA).

IV. 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: Sample lysis, nucleic acid capture and elution, and multiplex RT-PCR where analytes (when present) are simultaneously amplified, detected and differentiated. Nucleic acid capture and elution takes place in a single tube on the Panther Fusion system. The eluate is transferred to the Panther Fusion system reaction tube containing the assay reagents. Multiplex RT-PCR is then performed for the eluted nucleic acid on the Panther Fusion system.

Nucleic acid capture and elution: Prior to processing and testing on the Panther Fusion system, specimens are transferred to a tube containing specimen transport media (STM) that lyses the cells, releases target nucleic acid and protects them from degradation during storage. The Internal Control-S (IC-S) is added to each test specimen and controls via the working Panther Fusion Capture Reagent-S (wFCR-S). The IC-S in the reagent 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.

Elution transfer and RT-PCR: During the elution transfer step, eluted nucleic acid is transferred to a Panther Fusion reaction tube already containing oil and reconstituted master mix. Target amplification occurs via RT-PCR. 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. The analytes and the channel used for their detection on the Panther Fusion system is summarized in the table below.

Analyte	Gene Targeted	Instrument Channel
Influenza A Virus	Matrix	FAM
Respiratory Syncytial Virus A/B	Matrix	ROX
Influenza B Virus	Matrix	HEX
Internal Control	Not applicable	RED677

Assay Components

The reagents required to perform the Panther Fusion Flu A/B/RSV assay are packaged and sold separately. There are 7 boxes containing 9 reagents which are required for sample processing. A description of the components that are required to perform the Panther Fusion Flu A/B/RSV assay are detailed in **Table 1.** There is one ancillary kit, Panther Fusion Specimen Lysis Tubes, which is required for processing of specimens prior to testing on the Panther Fusion system.

Table 1: Reagents Required to Perform the Panther Fusion Flu A/B/RSV Assay

Box	Components Description
1	Panther Fusion Flu A/B/RSV Assay Cartridges
2	Panther Fusion Extraction Reagent-S • Box Contains: o Panther Fusion Capture Reagent-S o Panther Fusion Enhancer Reagent-S
3	Panther Fusion Internal Control-S
4	Panther Fusion Reconstitution Buffer I
5	Panther Fusion Elution Buffer
6	Panther Fusion Oil Reagent
7	Panther Fusion Flu A/B/RSV Assay Controls • Box Contains: o Panther Fusion Flu A/B/RSV Positive Control (Non- infectious nucleic acids with targeted sequences for the Flu A, Flu B and RSV in a buffered detergent solution) o Panther Fusion Negative Control (Buffered detergent solution)

In addition, select components can also be ordered in the following bundles:

- Panther Fusion Universal Fluids Kit: (contains Panther Fusion Oil and Panther Fusion Elution Buffer).
- Panther Fusion Assay Fluids Kit I-S: (contains Panther Fusion Extraction Reagents-S, Panther Fusion Internal Control-S, and Panther Fusion Reconstitution Buffer I).

Instrumentation

The Panther Fusion Flu A/B/RSV assay has been designed for and validated on the Panther Fusion system. The Panther Fusion system is an integrated hardware and software system that together with the Panther Fusion Flu A/B/RSV assay fully automates all the steps necessary to perform the assay.

The Panther Fusion system integrates Hologic's commercialized Panther instrument system with an add-on sidecar, the Panther Fusion module, which extends the functionality of the Panther system by increasing the assay processing capabilities to include real-time RT-PCR. The Panther Fusion module includes instrument hardware and software and can be installed on existing Panther instruments or ordered with new Panther instruments.

The Panther Fusion system employs non-specific target capture (NSTC) for the purification of RNA and DNA from the sample, followed by nucleic acid amplification and real-time fluorescent detection. The process involves sample loading and preparation (i.e. nucleic acid extraction) on the Panther instrument using the same workflow and processing steps as for other commercialized Hologic Aptima TMA assays. The extracted nucleic acid for each sample is transferred to the Panther Fusion module where PCR amplification and detection occurs.

V. INDICATIONS FOR USE

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 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 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 any culture specimens.

VI. COMPARISON OF TECHNOLOGICAL CHARACTERISTICS WITH THE PREDICATE DEVICE

A comparison of the Panther Fusion Flu A/B/RSV assay to the predicate Prodesse ProFlu+ (K153219) is summarized in **Table 2** (similarities) and **Table 3** (differences).

Table 2: Comparison of Similarities Between Predicate Device and Subject Device

Item	**Prodesse ProFlu+ Assay (Predicate Device) K153219**	**Panther Fusion Flu A/B/RSV Assay (Subject Device)**
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 3: Comparison of Differences Between Predicate Device and Subject Device

Item	Prodesse ProFlu+ Assay (Predicate Device) K153219	Panther Fusion Flu A/B/RSV Assay (Subject Device)
Platform	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	Automated real-time RT-PCR platform. Uses Panther Fusion system for all steps including nucleic acid extraction, amplification, detection and result processing.
Time to Obtain Test Results	Approximately 4 hours	Approximately 2.5 hours
User Complexity	High	Moderate

VII. PERFORMANCE DATA

The following performance data (analytical and clinical) were provided in support of the substantial equivalence determination.

Brief Description of Analytical (Non-Clinical) Studies

The following analytical studies (non-clinical) were conducted to support the clearance of the Panther Fusion Flu A/B/RSV Assay on the Panther Fusion system.

Analytical Sensitivity and Limit of Detection (LoD) of Nasopharyngeal Swab Specimens

The LoD was determined by testing dilution panels for two strains of Flu A H1, two strains of Flu A H3, two strains of Flu B and one strain of RSV A and RSV B, in pooled negative clinical matrix. At least 36 replicates were tested using three reagent lots on three instruments per test concentration, per each virus types. The LoD was based on results from the lowest concentration with $\geq$ 95% positive rate. The LoD values for each virus type were verified by testing newly prepared panel for at least 20 replicates. Panel descriptions are shown in **Table 4.**

Table 4: LoD Determination Panel Description

Viral Strain	LoD Concentration
Influenza A/California/07/2009 (H1N1)	$1x10^{-1.0}$ TCID50/mL
Influenza A/Massachusetts/15/13 (H1N1)	$1x10^{-1.5}$ TCID50/mL
Influenza A/Switzerland/9715293/2013 (H3N2)	$1x10^{-1.5}$ TCID50/mL
Influenza A/Victoria/361/2011 (H3N2)	$1x10^{-1.5}$ TCID50/mL
Influenza B/Brisbane/33/08	$1x10^{-0.5}$ TCID50/mL
Influenza B/Massachusetts/02/2012	$1x10^{-2.0}$ TCID50/mL
RSV A	$1x10^{0.5}$ TCID50/mL
RSV B	$1x10^{0.0}$ TCID50/mL

Analytical Reactivity (Inclusivity)

The reactivity of the Panther Fusion Flu A/B/RSV assay was evaluated against multiple strains of Influenza A, Influenza B, and Respiratory Syncytial Viruses. Test concentration on and detection results are shown in **Table 5** below.

Table 5: Analytical Reactivity (Inclusivity) Result Summary

Description	Type	Concentration	Flu A	Flu B	RSV
A/Aichi/2/1968	Influenza A/H3N2	$1x10^{2}$ CEID50/mL	+	-	-
A/Brazil/02/1999	Influenza A/H3N2	$1x10^{2}$ TCID50/mL	+	-	-
A/Brazil/1137/1999	Influenza A/H3N2	$1x10^{2}$ TCID50/mL	+	-	-
A/Brisbane/59/2007	Influenza A/H1N1	$1x10^{2}$ TCID50/mL	+	-	-
A/California/07/2009	Influenza A/H1N1	$1x10^{-1}$ TCID50/mL	+	-	-
A/Costa Rica/07/1999	Influenza A/H3N2	$1x10^{2}$ TCID50/mL	+	-	-
A/Denver/1/57	Influenza A/H1N1	$1x10^{2}$ CEID50/mL	+	-	-
A/Dominican Republic/7293/13	Influenza A/H1N1	$1x10^{2}$ TCID50/mL	+	-	-
A/Fujian/156/2000	Influenza A/H1N1	$1x10^{2}$ TCID50/mL	+	-	-
A/Georgia/F32551/12 2009	Influenza A/H1N1	$1x10^{2}$ TCID50/mL	+	-	-
A/Hawaii/15/2001	Influenza A/H1N1	$1x10^{2}$ TCID50/mL	+	-	-
A/Henan/8/2005	Influenza A/H1N1	$1x10^{2}$ TCID50/mL	+	-	-
A/Hiroshima/52/2005	Influenza A/H3N2	$1x10^{2}$ TCID50/mL	+	-	-
A/Hong Kong/218/2006	Influenza A/H3N2	$1x10^{2}$ TCID50/mL	+	-	-
A/Hong Kong/4801/2014	Influenza A/H3N2	$1x10^{2}$ TCID50/mL	+	-	-
A/Hong Kong/486/97 RNA	Influenza A/H5N1	16.4 ng/mL	+	-	-
A/Hong Kong/8/1968	Influenza A/H3N2	$1x10^{2}$ CEID50/mL	+	-	-
A/Indiana/08/2011	Influenza A/H3N2	$1x10^{2}$ TCID50/mL	+	-	-
A/Japan/305/1957	Influenza A/H2N2	0.003 ug/mL	+	-	-

Description	Type	Concentration	Flu A	Flu B	RSV
A/Jiangxi/160/2005	Influenza A/H1N1	$1x10^2$ TCID50/mL	+	-	-
A/Kentucky/2/2006	Influenza A/H1N1	$1x10^2$ TCID50/mL	+	-	-
A/Malaya/302/54	Influenza A/H1N1	$1x10^2$ CEID50/mL	+	-	-
A/Mexico/4108/2009	Influenza A/H1N1	$1x10^2$ TCID50/mL	+	-	-
A/Minnesota/11/2010	Influenza A/H3N2	36 ng/mL	+	-	-
A/New Jersey/8/1976	Influenza A/H1N1	$1x10^3$ TCID50/mL	+	-	-
A/Ohio/09SW1477/2009	Influenza A/H1N2	$1x10^2$ TCID50/mL	+	-	-
A/Perth/16/2009	Influenza A/H3N2	$1x10^2$ TCID50/mL	+	-	-
A/Port Chalmers/1/1973	Influenza A/ H3N2	$1x10^2$ TCID50/mL	+	-	-
A/Puerto Rico/8/34	Influenza A/H1N1	$1x10^2$ TCID50/mL	+	-	-
A/Solomon Islands/03/2009	Influenza A/H1N1	$1x10^2$ TCID50/mL	+	-	-
A/Switzerland/9715293/2013	Influenza A/H3N2	$1x10^{-1.5}$ TCID50/mL	+	-	-
A/Taiwan/42/2006	Influenza A/H1N1	$1x10^2$ TCID50/mL	+	-	-
A/Victoria/3/1975	Influenza A/ H3N2	$1x10^2$ CEID50/mL	+	-	-
A/Vietnam/1203 RNA	Influenza A/H5N1	0.27 ug/mL	+	-	-
A/WS/33	Influenza A/H1N1	$1x10^2$ TCID50/mL	+	-	-
B/Brisbane/60/2008	Influenza B	$1x10^2$ TCID50/mL	-	+	-
B/Florida/2/2006 (Yamagata lineage)	Influenza B	$1x10^2$ TCID50/mL	-	+	-
B/Florida/7/2004	Influenza B	$1x10^2$ TCID50/mL	-	+	-
B/Hawaii/11/2005	Influenza B	$1x10^2$ TCID50/mL	-	+	-
B/Hawaii/33/2004	Influenza B	$1x10^2$ TCID50/mL	-	+	-
B/Lee/40	Influenza B	$1x10^2$ CEID50/mL	-	+	-
B/Michigan/2/2006	Influenza B	$1x10^2$ TCID50/mL	-	+	-
B/Ohio/1/2005	Influenza B	$1x10^2$ TCID50/mL	-	+	-
B/Panama/45/90	Influenza B	$1x10^2$ TCID50/mL	-	+	-
B/Phuket/3073/2013 (Victoria Lineage)	Influenza B	$1x10^2$ TCID50/mL	-	+	-
B/St. Petersburg/04/2006	Influenza B	$1x10^2$ TCID50/mL	-	+	-
RSV A/A2	RSV	$1x10^2$ TCID50/mL	-	-	+
RSV A/Long	RSV	$1x10^2$ TCID50/mL	-	-	+
RSV A/Vero	RSV	$1x10^2$ CEID50/mL	-	-	+
RSV B/9320	RSV	$1x10^2$TCID50/mL	-	-	+
RSV B/Wash/18537/62	RSV	$2x10^2$ 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	+	-	-

Description	Type	Concentration	Flu A	Flu B	RSV
A/gyrfalcon/Washington/41088-6/2014	Influenza A/H5N8	$1x10^3$ TCID50/mL	+	-	-
A/Northern pintail/Washington/40964/2014	Influenza A/H5N2	$1x10^3$ TCID50/mL	+	-	-
A/Swine/ NY/01/2009	Influenza A/H1N1	$1x10^2$ TCID50/mL	+	-	-
A/Swine/Iowa/2006	Influenza A/H1N1	$1x10^2$ 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	+	-	-

Interfering Substances

Mucin, whole blood and other potentially interfering substances (medications and over-the-counter or OTC products) that may be present in the samples were evaluated in the Panther Fusion Flu A/B/RSV assay. Clinically relevant amount of the potentially interfering substances were added to simulated clinical matrix and tested unspiked or spiked with cultured Flu A, Flu B and RSV at their respective 3X LoD concentrations. The substances consisted of nasal sprays (liquid and powder), ingestible pills, lozenges, injectable and endogenous substances. No interference in performance of the Panther Fusion Flu A/B/RSV assay was observed in the presence of a representative brand of the following potentially interfering substances at the concentrations stated in **Table 6**.

Table 6: Interfering Substances Description

Type	Substance Name	Active Ingredient(s)	Concentration
Endogenous	Mucin	Purified mucin protein	60 µg/mL
	Human blood	Blood	2% v/v
Nasal sprays or drops	Neo-Synephrine®	Phenylephrine	15% v/v
	Anefrin	Oxymetazoline	15% v/v
	Saline	Sodium chloride	15% v/v
	Ventolin® HFA	Albuterol	15% v/v
Nasal corticosteroids	QVAR®, Beconase AQ	Beclomethasone	5% v/v
	Dexacort	Dexamethasone	5% v/v
	AEROSPAN®	Flunisolide	5% v/v
	Nasacort	Triamcinolone	5% v/v
	Rhinocort	Budesonide	5% v/v
	Nasonex	Mometasone	5% v/v
	Flonase	Fluticasone	5% v/v

Type	Substance Name	Active Ingredient(s)	Concentration
Nasal gel	Zicam® (Allergy Relief)	Luffa opperculata, Galphimia, Glauca, Histaminum hydrochloricum, Sulfur	5% v/v
Throat lozenges	Chloraseptic Throat Lozenges	Benzocaine	0.63 mg/mL
		Menthol	
Anti-viral drugs	Relenza®	Zanamivir	3.3 mg/mL
	TamiFlu	Oseltamivir	25 mg/mL
	Rebitol	Ribavirin	20 mg/mL
Antibiotic, nasal ointment	Bactroban cream	Mupirocin	10 mg/mL
Antibiotic, systemic	Tobramycin	Tobramycin	4.0 µg/mL

Competitive Interference

Competitive Interference of the Panther Fusion Flu A/B/RSV assay was evaluated using a simulated clinical matrix with pairs of target viruses at two different concentrations. One of the concentrations was near the Limit of Detection (3 - 5X LoD) while the other concentration was high (1000X LoD). The presence of two viruses at varying concentrations in a single sample had no effect on the analytical sensitivity (100% detection for both targets) at the concentration tested (**Table 7**).

Table 7: Co-Infection Concentrations and Results

Condition	Target 1		Target 2		Flu A	Flu B	RSV
	Description	Concentration	Description	Concentration			
1	FLU A	3X LoD	RSV	1000X LoD	+	-	+
2	FLU A	3X LoD	FLU B	1000X LoD	+	+	-
3	FLU B	5X LoD	FLU A	1000X LoD	+	+	-
4	FLU B	3X LoD	RSV	1000X LoD	-	+	+
5	RSV	3X LoD	FLU A	1000X LoD	+	-	+
6	RSV	3X LoD	FLU B	1000X LoD	-	+	+

Analytical Specificity The analytical specificity of the Panther Fusion Flu A/B/RSV assay was evaluated by testing a panel of 52 organisms, consisting of 25 viral, 26 bacterial, and 1 yeast

strains representing common respiratory pathogens or flora commonly present in respiratory tract. Bacteria and yeast were tested at concentrations of 10^5 to10^8 CFU/mL or IFU/mL, except where noted. Viruses were tested at concentrations of 10^3 to 10^7 TCID50/mL. Analytical specificity of the Panther Fusion Flu A/B/RSV assay was 100% for Flu A, Flu B, and RSV.

Table 8: Specificity Results

Organism	Concentration	Flu A	Flu B	RSV
Adenovirus 1	$1x10^5$ $TCID_{50}$/mL	-	-	-
Adenovirus 7a	$1x10^5$ $TCID_{50}$/mL	-	-	-
Bordetella bronchiseptica	$1x10^7$ CFU/ml	-	-	-
Bordetella pertussis	$1x10^8$ CFU/mL	-	-	-
Candida albicans	$1x10^7$ CFU/mL	-	-	-
Chlamydia trachomatis	$1x10^5$ CFU/mL	-	-	-
Chlamydophila pneumoniae (formerly Chlamydia pneumoniae)	$1x10^5$ IFU/mL	-	-	-
CMV Strain AD 169	$1x10^4$ $TCID_{50}$/mL	-	-	-
Coronavirus 229E	$1x10^4$ $TCID_{50}$/mL	-	-	-
Corynebacterium diphtheria	$1x10^7$ CFU/mL	-	-	-
Coxsackie B4	$1x10^6$ $TCID_{50}$/mL	-	-	-
Coxsackie B5/10/2006	$1x10^5$ $TCID_{50}$/mL	-	-	-
E. coli	$1x10^7$ CFU/mL	-	-	-
EBV	$1x10^7$ $TCID_{50}$/mL	-	-	-
Echovirus 2	$1x10^4$ $TCID_{50}$/mL	-	-	-
Echovirus 3	$1x10^5$ $TCID_{50}$/mL	-	-	-
Echovirus 6	$1x10^4$ $TCID_{50}$/mL	-	-	-
Echovirus 11	$1x10^5$ $TCID_{50}$/mL	-	-	-
Enterovirus 68	$1x10^5$ $TCID_{50}$/mL	-	-	-
Enterovirus 70	$1x10^4$ $TCID_{50}$/mL	-	-	-
Haemophilus Influenzae	$1x10^7$ CFU/mL	-	-	-
hMPV Subtype A2	$1x10^6$ $TCID_{50}$/mL	-	-	-
HPIV-1	$1x10^4$ $TCID_{50}$/mL	-	-	-
HPIV-2	$1x10^5$ $TCID_{50}$/mL	-	-	-
HPIV-3	$1x10^5$ $TCID_{50}$/mL	-	-	-
HPIV-4	$1x10^4$ $TCID_{50}$/mL	-	-	-
HSV-1 Macinytre Strain	$1x10^5$ $TCID_{50}$/mL	-	-	-

Organism	Concentration	Flu A	Flu B	RSV
HSV-2 Type 2G Strain	$1x10^5$ $TCID_{50}$/mL	-	-	-
Klebsiella pneumonia	$1x10^7$ CFU/mL	-	-	-
Lactobacillus plantarum	$1x10^7$ CFU/mL	-	-	-
Legionella pneumophila	$1x10^7$ CFU/mL	-	-	-
Measles/7/2000	$1x10^5$ $TCID_{50}$/mL	-	-	-
Moraxella catarrhalis	$1x10^6$ CFU/mL	-	-	-

Carry-Over/Contamination

The carry-over/cross-contamination study was performed with negative samples alternately placed between high positive samples and tested. High positive samples were prepared by spiking (over 10,000X LoD). A total of nine separate runs with negative samples and positive samples placed in a checkerboard pattern were tested over three different instruments for a combined total of 449 positive and 449 negative samples. The carry-over rate was 0.4%.

Assay Precision

Panther Fusion Flu A/B/RSV assay precision was evaluated with a 7-member panel. The panel was tested by three operators on two separate runs per day, using three reagent lots on three Panther Fusion systems over 45 days. The panel members, along with a summary of the agreement with expected results for each target is presented in **Table 9**. The mean and variability analysis between instruments, between reagent lots, between operators, between days, between runs and within runs, and overall (total) for Ct are also presented in **Table 10**.

Table 9: Percent Positive and Agreement

Target	Panel Member	% Positive	% Agreement (95% CI)
Flu A	Flu A 3x LoD	100.0% (162/162)	100.0% (97.7 - 100%)
	Flu A 1x LoD	100.0% (162/162)	100.0% (97.7 - 100%)
	Flu A 0.01x LoD	8.6% (14/162)	91.4% (86.0 - 94.8%)
	Negative	0.0% (0/162)	100.0% (97.7 - 100%)
	Flu B 3x LoD	100.0% (162/162)	100.0% (97.7 - 100%)

Target	Panel Member	% Positive	% Agreement (95% CI)
Flu B	Flu B	94.4%	94.4%
	1x LoD	(153/162)	(89.8 – 97.0%)
	Flu B	4.3%	95.7%
	0.01x LoD	(7/162)	(91.4 - 97.9%)
	Negative	0.6% (1/162)	99.4% (96.6 - 99.9%)
RSV	RSV	100.0%	100.0%
	3x LoD	(162/162)	(97.7 - 100%)
	RSV	99.4%	99.4%
	1x LoD	(161/162)	(96.6 - 99.9%)
	RSV	4.9%	95.1%
	0.01x LoD	(8/162)	(90.6 - 97.5%)
	Negative	0.0% (0/162)	100.0% (97.7 - 100%)

Table 10: Signal Variability Analysis Results

Target	Panel Member	Mean Ct	Between Instrument		Between Reagent Lots		Between Operators		Between Days		Between Runs		Within Runs		Total	
			SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
Flu A	Flu A 3x LoD	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
	Flu A 1x LoD	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
	Flu A 0.01x LoD	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	Flu B 3x LoD	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
	Flu B 1x LoD	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
	Flu B 0.01x LoD	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	RSV 3x	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
	RSV 1x	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
	RSV 0.01x LoD	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	Negative	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

Brief Description of Clinical Studies

Clinical testing of the Panther Fusion Flu A/B/RSV assay on the Panther Fusion system included performance and reproducibility testing. Substantial equivalence is based in part on the performance study.

Clinical Performance Study

This study was performed to demonstrate clinical performance characteristics for the Panther Fusion Flu A/B/RSV assay. A prospective multicenter study was conducted with leftover, remnant nasopharyngeal (NP) swab specimens from male and female individuals of all ages exhibiting signs and/or symptoms of a respiratory tract infection. Four participating US pediatric/adolescent, private and/or university hospitals obtained 2961 leftover, remnant NP swab specimens. The samples were tested with the Panther Fusion Flu A/B/RSV assay, with reference viral culture followed by direct fluorescent antibody (DFA) identification. A validated PCR assay was used for discordant resolution testing. Performance characteristics were estimated relative to valid culture/DFA results for each sample. Sensitivity and specificity were estimated with corresponding 2 sided 95% Score CIs. Analyses were performed separately for each target analyte (Flu A, Flu B and RSV).

Of the 2961 specimens, 31 specimens/samples were withdrawn and 2930 samples were processed in valid Panther Fusion Flu A/B/RSV runs, 2876 (98.2%) had final valid results and 54 (1.8%) had final invalid results. Of the 2876 samples with valid Panther results, 2869 specimens from females (1354) and males (1515) were evaluable for analyses (see **Table 11**).

Table 11: Summary of Subject Demographics for Prospective NP Samples in the Panther Fusion Flu A/B/RSV Assay Evaluation

	N (%)
Total	2869 (100)
Sex	
Female	1354 (47.2)
Male	1515 (52.8)
Age Group	
0 to 28 days	82 (2.9)
29 days to < 2 years	758 (26.4)
2 to 5 years	407 (14.2)
6 to 11 years	258 (9.0)
12 to 17 years	181 (6.3)
18 to 21 years	73 (2.5)
22 to 64 years	691 (24.1)
≥ 65 years	419 (14.6)

NP=nasopharyngeal

Of the 2869 samples tested using the Panther Fusion Flu A/B/RSV assay, 6.6% (189/2869) were positive for Flu A, 1.9% (55/2869) were positive for Flu B, and 12.7% (365/2869) were positive for RSV.

Performance characteristics for detection of Flu A, Flu B, and RSV in prospective NP samples were calculated (see **Table 12**). For Flu A, sensitivity was 99.2% (131/132, 95% CI: 95.8% to 99.9%) and specificity was 97.9% (2679/2737, 95% CI: 97.3% to 98.4%). For Flu B, sensitivity was 97.9% (46/47, 95% CI: 88.9% to 99.6%) and specificity was 99.7% (2813/2822, 95% CI: 99.4% to 99.8%). For RSV, sensitivity was 98.7% (236/239, 95% CI: 96.4% to 99.6%) and specificity was 95.1% (2501/2630, 95% CI: 94.2% to 95.9%).

Table 12: Panther Fusion Flu A/B/RSV Assay Performance Relative to Culture/DFA for Prospective NP Samples

Analyte	N	TP	FP	TN	FN	Prevalence[1] (95% CI)[2]	Sensitivity (95% CI)[2]	Specificity (95% CI)[2]
Flu A	2869	131	58[3]	2679	1[3]	4.6 (3.9-5.4)	99.2 (95.8-99.9)	97.9 (97.3-98.4)
Flu B	2869	46	9[4]	2813	1[4]	1.6 (1.2-2.2)	97.9 (88.9-99.6)	99.7 (99.4-99.8)
RSV	2869	236	1295	2501	35	8.3 (7.4-9.4)	98.7 (96.4-99.6)	95.1 (94.2-95.9)

FN=false negative, FP=false positive, NP=nasopharyngeal, TP=true positive, TN=true negative

[1]Study prevalence reported, [2]Score Confidence Interval

[3] 55/58 false positive results were confirmed positive and 1/1 false negative result was confirmed negative for Flu A by PCR

[4] 6/9 false positive results were confirmed positive and 1/1 false negative result was confirmed negative for Flu B by PCR

[5] 114/129 false positive results were confirmed positive and 3/3 false negative results were confirmed negative for RSV by PCR

Reproducibility

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

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

The agreement with expected results was 100% in the negative and moderate positive panel members and ≥97.8% in low-positive panel members for Flu A, Flu B and RSV (**Table 13**). A lower agreement for low positive panel members was expected, since the analyte concentration of these panel members ranged between 1-2X LoD, which is expected to yield approximately 95% to 100% detection rate.

Table 13: Agreement of Panther Fusion Flu A/B/RSV Assay Results with Expected Results

Panels			Expected Results			Agreement with Expected Results					
						Flu A		Flu B		RSV	
Description	Compo-sition	Conc. ($TCID_{50}$/ mL)	Flu A	Flu B	RSV	N	(%) 95% CI	N	(%) 95% CI	N	(%) 95% CI
Flu A Low Pos	1-2X LoD	3.16E-02	+	-	-	86/86	100 (95.7-100)	86/86	100 (95.7-100)	86/86	100 (95.7-100)
Flu A Mod Pos	2-3X LoD	9.49E-02	+	-	-	88/88	100 (95.8-100)	88/88	100 (95.8-100)	88/88	100 (95.8-100)

Flu B Low Pos	1-2X LoD	1.90E-02	-	+	-	89/89	100 (95.9-100)	89/89	100 (95.9-100)	89/89	100 (95.9-100)
Flu B Mod Pos	2-3X LoD	3.00E-02	-	+	-	89/89	100 (95.9-100)	89/89	100 (95.9-100)	89/89	100 (95.9-100)
RSV Low Pos	1-2X LoD	3.16E+00	-	-	+	89/89	100 (95.9-100)	89/89	100 (95.9-100)	87/89	97.8 (92.2-99.4)
RSV Mod Pos	2-3X LoD	9.49E+00	-	-	+	89/89	100 (95.9-100)	89/89	100 (95.9-100)	89/89	100 (95.9-100)
Neg	N/A	N/A	-	-	-	89/89	100 (95.9-100)	89/89	100 (95.9-100)	89/89	100 (95.9-100)

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

The total Flu A, Flu B, and RSV signal variability measured as %CV ranged from 2.24% to 3.81% in low and moderate positive panel members between sites, between lots, between operators, between runs, within runs, and overall in panel members (**Table 14**).

Table 14: Signal Variability of the Panther Fusion Flu A/B/RSV Assay by Panel Member

			Between Sites		Between Operators		Between Days		Between Runs		Within Runs		Total	
Panel Description	**N**	**Mean Ct**	**SD**	**CV (%)**	**SD**	**CV (%)**	**SD**	**CV (%)**	**SD**	**CV (%)**	**SD**	**CV (%)**	**SD**	**CV (%)**
Flu A Low Pos	86	34.69	0.0	0.0	0.13	0.39	<0.1	<0.1	<0.1	0.11	1.11	3.20	1.12	3.23
Flu A Mod Pos	88	33.42	0.0	0.0	0.17	0.51	0.12	0.36	<0.1	<0.1	0.75	2.25	0.78	2.34
Flu B Low Pos	89	37.17	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.98	2.65	0.98	2.65
Flu B Mod Pos	89	36.43	0.18	0.50	0.0	0.0	0.0	0.0	0.0	0.0	0.80	2.19	0.82	2.24
RSV Low Pos	87	38.34	0.37	0.98	0.0	0.0	0.49	1.29	<0.1	<0.1	1.32	3.45	1.46	3.81
RSV Mod Pos	89	36.10	0.31	0.85	0.0	0.0	0.31	0.86	<0.1	<0.1	1.10	3.05	1.18	3.28

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

The signal variability as measured as %CV was ≤1.45% between sites, between operators, between days, or overall for the Panther Fusion FluA/FluB/RSV assay positive control (**Table 15**).

Table 15: Signal Variability of the Panther Fusion Flu A/B/RSV Assay Controls

				Between Sites		Between Operators		Between Days		Between Runs		Within Runs		Total	
Control	**Analyte**	**N**	**Mean Ct**	**SD**	**CV(%)**	**SD**	**CV(%)**	**SD**	**CV(%)**	**SD**	**CV(%)**	**SD**	**CV(%)**	**SD**	**CV(%)**
Pos	Flu A	30	30.93	0.0	0.0	0.20	0.63	0.0	0.0	0.0	0.0	0.30	0.97	0.36	1.16
	Flu B	30	33.74	0.0	0.0	0.31	0.93	0.0	0.0	0.0	0.0	0.38	1.12	0.49	1.45
	RSV	30	33.40	0.0	0.0	0.20	0.60	0.0	0.0	0.0	0.0	0.32	0.96	0.38	1.13

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

VIII. CONCLUSIONS

The analytical and clinical study results demonstrate that the Panther Fusion Flu A/B/RSV assay on the Panther Fusion system performs comparably to the predicate device that is currently marketed for the same intended use. Hardware and software verification and validation demonstrate that the Panther Fusion Flu A/B/RSV assay on the Panther Fusion system will perform as intended.