Flu A/B/RSV Assay (Panther Fusion® System)

For *in vitro* diagnostic use.

Rx Only

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General Information

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 predominate 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.

Summary and Explanation of the Test

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

Influenza is an acute respiratory illness caused by infection with the influenza virus, primarily types A and B.[3] Influenza A viruses are further categorized into subtypes based on the two major surface protein antigens: hemagglutinin (H) and neuraminidase (N).[4] Influenza B viruses are not categorized into subtypes.[4] Influenza viruses continuously undergo genetic changes including drift (random mutation) and variation (genomic reassortment), generating new strains of virus each year, leaving the human population vulnerable to these seasonal changes. Epidemics occur yearly (typically in winter) and while both types A and B circulate in the population, type A is usually dominant. Transmission of influenza is primarily via airborne droplet (coughing or sneezing). Symptoms arise on average 1 to 2 days post-exposure and include fever, chills, headache, malaise, cough, and coryza.

Complications due to influenza include pneumonia causing increased morbidity and mortality in pediatric, elderly and immunocompromised populations. Influenza occurs globally with an annual attack rate estimated at 5%–10% in adults and 20%–30% in children. Illnesses can result in hospitalization and death mainly among high-risk groups (the very young, elderly or chronically ill). Worldwide, these annual epidemics are estimated to result in about 3 to 5 million cases of severe illness, and about 250,000 to 500,000 deaths.[5]

Respiratory syncytial virus (RSV) is a leading cause of respiratory infections in infants and children. There are 2 types of RSV (A and B) based on antigenic and surface protein variations.

Most yearly epidemics (typically during winter) contain a mix of type A and B viruses, but one subgroup can dominate during a season. RSV infection can cause severe respiratory illness among all ages but is more prevalent in pediatric, elderly and immunocompromised populations. Annually in the United States, RSV infection has been associated with an estimated 57,527 hospitalizations and 2.1 million outpatient visits among children younger than 5 years, and 177,000 hospitalizations and 14,000 deaths among adults older than 65 years.[6]

Principles of the Procedure

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

Nucleic acid capture and elution: Prior to processing and testing on the Panther Fusion system, specimens are transferred to a Specimen Lysis Tube containing specimen transport media (STM) that lyses the 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 monitors specimen processing, amplification and detection.

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

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

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

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 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	HEX
Influenza B Virus	Matrix	ROX
Internal Control	Not applicable	RED677

Warnings and Precautions

A. For *in vitro* diagnostic use.

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

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

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

E. Specimens may be infectious. Use Universal Precautions when performing this assay. Proper handling and disposal methods should be established by the laboratory director. Only personnel adequately trained in handling infectious materials should be permitted to perform this diagnostic procedure.[7]

Note: *If infection with a novel influenza A virus is suspected based on current clinical and epidemiological screening criteria recommended by public health authorities, collect specimens with appropriate infection control precautions for novel virulent influenza viruses and send to state or local health department for testing. Do not attempt viral culture in these cases unless a BSL 3+ facility is available to receive and culture specimens.*

F. Use only supplied or specified disposable laboratory ware.

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

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

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

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

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

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

M. Store assay components at the recommended storage condition. See *Reagent Storage and Handling Requirements (*page 7), and *Panther Fusion System Test Procedure* (page 12) for more information.

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

O. Avoid microbial and ribonuclease contamination of reagents.

P. Quality control requirements must be performed in conformance with local, state, and/or federal regulations or accreditation requirements and your laboratory's standard quality control procedures.

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

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

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

Panther Fusion Oil

Polydimethylsiloxane 95-100%

WARNING

H315 - Causes skin irritation
H319 - Causes serious eye irritation
P264 - Wash face, hands and any exposed skin thoroughly after handling
P280 - Wear protective gloves/protective clothing/eye protection/face protection
P305 + P351 + P338 - IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses if present and easy to do. Continue rinsing
P337 + P313 - If eye irritation persists: Get medical advice/attention
P302 + P352 - IF ON SKIN: Wash with plenty of soap and water
P332 + P313 - If skin irritation occurs: Get medical advice/attention
P362 - Take off contaminated clothing and wash before reuse

Panther Fusion Enhancer Reagent-S

Lithium Hydroxide, Monohydrate 5-10%

DANGER

H302 - Harmful if swallowed
H314 - Causes severe skin burns and eye damage
P260 - Do not breathe dust/fume/gas/mist/vapors/spray
P264 - Wash face, hands and any exposed skin thoroughly after handling
P270 - Do not eat, drink or smoke when using this product
P280 - Wear protective gloves/protective clothing/eye protection/face protection
P301 + P312 - IF SWALLOWED: Call a POISON CENTER or doctor/physician if you feel unwell
P301 + P330 + P331 - IF SWALLOWED: rinse mouth. Do NOT induce vomiting
P303 + P361 + P353 - IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower
P304 + P340 - IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing
P305 + P351 + P338 - IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing
P310 - Immediately call a POISON CENTER or doctor/physician
P330 - Rinse mouth
P363 - Wash contaminated clothing before reuse

Note: *For information on any hazard and precautionary statements that may be associated with reagents, refer to the Safety Data Sheet Library at www.hologicsds.com.*

Reagent Storage and Handling Requirements

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

Reagent	Unopened Storage	On Board/ Open Stability[1]	Opened Storage
Panther Fusion Flu A/B/RSV Assay Cartridge	2°C to 8°C	60 days	2°C to 8°C [2]
Panther Fusion Capture Reagent-S (FCR-S)	15°C to 30°C	30 days	15°C to 30°C
Panther Fusion Enhancer Reagent-S (FER-S)	15°C to 30°C	30 days	15°C to 30°C
Panther Fusion Internal Control-S (IC-S)	2°C to 8°C	(In wFCR-S)	Not applicable
Panther Fusion Elution Buffer	15°C to 30°C	60 days	15°C to 30°C
Panther Fusion Oil	15°C to 30°C	60 days	15°C to 30°C
Panther Fusion Reconstitution Buffer I	15°C to 30°C	60 days	15°C to 30°C
Panther Fusion 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

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

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

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

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

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

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

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

F. **Do not freeze reagents.**

Specimen Collection and Storage

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

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

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

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

A. Specimen collection

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

The following types of VTM were verified for use.

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

B. Specimen processing

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

     ***Note:** When testing frozen specimen, allow specimen to reach room temperature prior to processing.*
2. Storing specimens before testing
   a. After collection, specimens can be stored at 2°C to 8°C up to 96 hours before transferred to the Panther Fusion Specimen Lysis Tube. Remaining specimen volumes can be stored at ≤-70°C.
   b. Specimen in the Panther Fusion Specimen Lysis Tube may be stored under one of the following conditions:
      - 15°C to 30°C up to 6 days or
      - 2°C to 8°C up to 3 months.

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

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

D. Storing samples after testing

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

Specimen Transport

Maintain specimen storage conditions as described in the *Specimen Collection and Storage section on* page 8.

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

Panther Fusion System

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

Reagents and Materials Provided for the Panther Fusion Flu A/B/RSV Assay

Assay Packaging

Components[1]	Part No.	Storage
Panther Fusion Flu A/B/RSV Assay Cartridges 96 Tests Panther Fusion Flu A/B/RSV assay cartridge, 12 tests, 8 per box	PRD-04328	2°C to 8°C
Panther Fusion Internal Control-S 960 Tests Panther Fusion Internal Control-S tube, 4 per box	PRD-04332	2°C to 8°C
Panther Fusion Flu A/B/RSV Assay Controls Panther Fusion Flu A/B/RSV Positive Control tube, 5 per box Panther Fusion Negative Control tube, 5 per box	PRD-04336	2°C to 8°C
Panther Fusion Extraction Reagent-S 960 Tests Panther Fusion Capture Reagent-S bottle, 240 tests, 4 per box Panther Fusion Enhancer Reagent-S bottle, 240 tests, 4 per box	PRD-04331	15°C to 30°C
Panther Fusion Elution Buffer 2400 Tests Panther Fusion Elution Buffer pack, 1200 tests, 2 per box	PRD-04334	15°C to 30°C
Panther Fusion Reconstitution Buffer I 1920 Tests Panther Fusion Reconstitution Buffer I pack, 960 tests, 2 per box	PRD-04333	15°C to 30°C
Panther Fusion Oil 1920 Tests Panther Fusion Oil pack, 960 tests, 2 per box	PRD-04335	15°C to 30°C

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

Individually Packaged Items

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

Materials Required and Available Separately

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

Material	Cat. No.
Panther System	303095
Panther Fusion Module	ASY-09600
Aptima Assay Fluids Kit (Aptima Wash Solution, Aptima Buffer for Deactivation Fluid, and Aptima Oil Reagent)	303014 (1000 tests)
Multi-tube units (MTUs)	104772-02
Panther Waste Bag Kit	902731
Panther Waste Bin Cover	504405
Or Panther System Run Kit for Real Time Assays contains MTUs, waste bags, waste bin covers, and assay fluids	PRD-03455 (5000 tests)
Or Panther System Run Kit (when running TMA assays in parallel with real time-TMA assays) contains MTUs, waste bags, waste bin covers, auto detect*, and assay fluids	303096 (5000 tests)
Panther Fusion Tube Trays, 1008 tests, 18 trays per box	PRD-04000
Liquid Handling (LiHa) Disposable Tips, 1000 µL	10612513 (Tecan)
Aptima penetrable caps (optional)	105668
Replacement non-penetrable caps (optional)	103036A
Replacement extraction reagent bottle caps	CL0040
P1000 pipettor and tips with hydrophobic plugs	-
Bleach, 5% to 7% (0.7 M to 1.0 M) sodium hypochlorite solution **Note**: Mix one part bleach with one part deionized water to make diluted working bleach solution 2.5% to 3.5% (0.35 M to 0.5 M) sodium hypochlorite solution.	-
Disposable powderless gloves	-

*Needed only for Panther Aptima TMA assays.

Panther Fusion System Test Procedure

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

A. Work Area Preparation

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

B. Reagent Preparation

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

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

C. Specimen Handling

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

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

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

D. System Preparation

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

Procedural Notes

A. Controls

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

Quality Control

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

Negative and Positive Controls

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

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

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

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

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

Internal Control

An internal control is added to each sample during the extraction process. During processing, the internal control acceptance criteria is automatically verified by the Panther Fusion system software. Detection of the internal control is not required for samples that are positive for Flu A, Flu B and/or RSV. The internal control must be detected in all samples that are negative for

Flu A, Flu B and RSV targets; samples that fail to meet that criteria will be reported as Invalid. Each sample with an Invalid result must be retested.

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

Interpretation of Results

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

Table1 shows the possible results reported in a valid run with result interpretations.

Table 1: Result Interpretation

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

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

Note: Detection of internal control is not required for samples that are positive for Flu A, Flu B, and/or RSV.

Limitations

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

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

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

D. Negative results do not preclude influenza A virus, influenza B virus, or RSV infections and should not be used as the sole basis for treatment or other management decisions.

E. This test does not differentiate influenza A subtypes (i.e. H1N1, H3N2) or RSV subgroups (i.e., A or B); additional testing is required to differentiate any specific influenza A subtypes or strains or specific RSV subgroups, in consultation with local public health departments.

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

Panther Fusion System Assay Performance

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 as shown in Table 2.

Table 2: 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	Composition	Conc. (TCID 50/mL)	Flu A	Flu B	RSV	N[1]	(%) 95% CI	N[1]	(%) 95% CI	N[1]	(%) 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)

[1]A total of 11 samples had final invalid results and were not included in the calculation of overall agreement.

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. For the sources of variation except the 'within run' factor, %CV values were ≤1.29% as shown in Table 3.

Table 3: 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	34.7	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	33.4	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	37.2	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	36.4	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	38.3	0.37	0.98	0.0	0.0	0.49	1.29	<0.1	<0.1	1.32	3.45	1.46	3.81
RSVMod Pos	89	36.1	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 Flu A, Flu B, and RSV assay positive control (see Table 4).

Table 4: 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	30.9	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	33.7	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	33.4	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 from some factors may be numerically negative, SD and CV are shown as 0.0.

Clinical Performance

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 and with reference viral culture followed by direct fluorescent antibody (DFA) identification. A validated PCR assay was used for discordant resolution testing, if applicable. 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 (due to incomplete reference testing results, insufficient volumes for testing, expiration prior to testing, or mishandling) and 2930 samples were processed in valid Panther Fusion Flu A/B/RSV runs, 2876 (98.2%) had final valid results (including 7 samples with invalid reference results), and 54 (1.8%) had final invalid results. Of the 2876 samples with valid Panther results, 2869 samples from females (1354) and males (1515) were evaluable for analyses (see Table 5).

Table 5: Summary of Subject Demographics for Prospective 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)

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. Table 6 shows the positivity for each analyte by age group.

Table 6: Panther Fusion Flu A/B/RSV Assay Positivity by Analyte and Age Group

	% Positivity (n/N)		
Analyte	**Flu A**	**Flu B**	**RSV**
All	6.6% (189/2869)	1.9% (55/2869)	12.7% (365/2869)
0 to 28 days	0.0% (0/82)	0.0% (0/82)	18.3% (15/82)
29 days to < 2 years	4.4% (33/758)	0.3% (2/758)	26.6% (202/758)
2 to 5 years	3.9% (16/407)	2.5% (10/407)	19.9% (81/407)
6 to 11 years	11.6% (30/258)	3.9% (10/258)	4.7% (12/258)
12 to 17 years	12.7% (23/181)	2.2% (4/181)	4.4% (8/181)
18 to 21 years	5.5% (4/73)	2.7% (2/73)	2.7% (2/73)
22 to 64 years	9.4% (65/691)	3.2% (22/691)	4.1% (28/691)
≥ 65 years	4.3% (18/419)	1.2% (5/419)	4.1% (17/419)

Performance characteristics for detection of Flu A, Flu B, and RSV in prospective NP samples were calculated (see Table 7).

Table 7: Panther Fusion Flu A/B/RSV Assay Performance Relative to Culture/DFA

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	129[5]	2501	3[5]	8.3 (7.4-9.4)	98.7 (96.4-99.6)	95.1 (94.2-95.9)

FN=false negative, FP=false positive, 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

Analytical Sensitivity

The analytical sensitivity (limit of detection or LoD) of the Panther Fusion Flu A/B/RSV assay was determined by testing pooled Flu A/B/RSV negative clinical specimens spiked with the following virus cultures at various concentrations: 4 Flu A strains, 2 Flu B strains, 1 strain each for RSV A and RSV B. Twelve replicates were tested with each of the three reagent lots for a combined total of 36 replicates. Target specific LoD concentrations were verified by testing an additional 20 replicates with one reagent lot. Analytical sensitivity (LoD) is defined as the lowest concentration at which ≥95% of all replicates tested positive, as summarized in Table 8.

Table 8: NP Swab Sensitivity

Viral Strain	LoD Concentration
Influenza A/California/07/2009 (H1N1)	$1x10^{-1.0}$ $TCID_{50}$/mL
Influenza A/Massachusetts/15/13 (H1N1)	$1x10^{-1.5}$ $TCID_{50}$/mL
Influenza A/Switzerland/9715293/2013 (H3N2)	$1x10^{-1.5}$ $TCID_{50}$/mL
Influenza A/Victoria/361/2011 (H3N2)	$1x10^{-1.5}$ $TCID_{50}$/mL
Influenza B/Brisbane/33/08 (Victoria lineage)	$1x10^{-0.5}$ $TCID_{50}$/mL
Influenza B/Massachusetts/02/2012 (Yamagata lineage)	$1x10^{-2.0}$ $TCID_{50}$/mL
RSV A	$1x10^{0.5}$ $TCID_{50}$/mL
RSV B	$1x10^{0.0}$ $TCID_{50}$/mL

Reactivity

The reactivity of the Panther Fusion assay was evaluated against multiple strains of Influenza A, Influenza B, and Respiratory Syncytial Viruses. Viral strains were tested in triplicates with each of the three reagent lots for a combined total of 9 replicates. Viruses present at concentrations below those tested for Reactivity may not be detected by the Panther Fusion Flu A/B/RSV assay.

Table 9: Analytical Reactivity (inclusivity) Test Summary

Description	Type	Concentration	Flu A	Flu B	RSV
A/Aichi/2/1968	Influenza A/H3N2	$1x10^{2}$ $CEID_{50}$/mL	+	-	-
A/Brazil/02/1999	Influenza A/H3N2	$1x10^{2}$ $TCID_{50}$/mL	+	-	-
A/Brazil/1137/1999	Influenza A/H3N2	$1x10^{2}$ $TCID_{50}$/mL	+	-	-
A/Brisbane/59/2007	Influenza A/H1N1	$1x10^{2}$ $TCID_{50}$/mL	+	-	-
A/California/07/2009	Influenza A/H1N1	$1x10^{-1}$ $TCID_{50}$/mL	+	-	-
A/Costa Rica/07/1999	Influenza A/H3N2	$1x10^{2}$ $TCID_{50}$/mL	+	-	-
A/Denver/1/57	Influenza A/H1N1	$1x10^{2}$ $CEID_{50}$/mL	+	-	-
A/Dominican Republic/7293/13	Influenza A/H1N1	$1x10^{2}$ $TCID_{50}$/mL	+	-	-
A/Fujian/156/2000	Influenza A/H1N1	$1x10^{2}$ $TCID_{50}$/mL	+	-	-
A/Georgia/F32551/12 2009	Influenza A/H1N1	$1x10^{2}$ $TCID_{50}$/mL	+	-	-
A/Hawaii/15/2001	Influenza A/H1N1	$1x10^{2}$ $TCID_{50}$/mL	+	-	-
A/Henan/8/2005	Influenza A/H1N1	$1x10^{2}$ $TCID_{50}$/mL	+	-	-
A/Hiroshima/52/2005	Influenza A/H3N2	$1x10^{2}$ $TCID_{50}$/mL	+	-	-
A/Hong Kong/218/2006	Influenza A/H3N2	$1x10^{2}$ $TCID_{50}$/mL	+	-	-
A/Hong Kong/4801/2014	Influenza A/H3N2	$1x10^{2}$ $TCID_{50}$/mL	+	-	-
A/Hong Kong/486/97 RNA	Influenza A/H5N1	16.4 ng/mL	+	-	-
A/Hong Kong/8/1968	Influenza A/H3N2	$1x10^{2}$ $CEID_{50}$/mL	+	-	-
A/Indiana/08/2011	Influenza A/H3N2	$1x10^{2}$ $TCID_{50}$/mL	+	-	-
A/Japan/305/1957	Influenza A/H2N2	0.003 ug/mL	+	-	-
A/Jiangxi/160/2005	Influenza A/H1N1	$1x10^{2}$ $TCID_{50}$/mL	+	-	-
A/Kentucky/2/2006	Influenza A/H1N1	$1x10^{2}$ $TCID_{50}$/mL	+	-	-
A/Malaya/302/54	Influenza A/H1N1	$1x10^{2}$ $CEID_{50}$/mL	+	-	-
A/Mexico/4108/2009	Influenza A/H1N1	$1x10^{2}$ $TCID_{50}$/mL	+	-	-
A/Minnesota/11/2010	Influenza A/H3N2	36 ng/mL	+	-	-
A/New Jersey/8/1976	Influenza A/H1N1	$1x10^{3}$ $TCID_{50}$/mL	+	-	-
A/Ohio/09SW1477/2009	Influenza A/H1N2	$1x10^{2}$ $TCID_{50}$/mL	+	-	-
A/Perth/16/2009	Influenza A/H3N2	$1x10^{2}$ $TCID_{50}$/mL	+	-	-
A/Port Chalmers/1/1973	Influenza A/-H3N2	$1x10^{2}$ $TCID_{50}$/mL	+	-	-
A/Puerto Rico/8/34	Influenza A/H1N1	$1x10^{2}$ $TCID_{50}$/mL	+	-	-
A/Solomon Islands/03/2009	Influenza A/H1N1	$1x10^{2}$ $TCID_{50}$/mL	+	-	-
A/Switzerland/9715293/2013	Influenza A/H3N2	$1x10^{-1.5}$ $TCID_{50}$/mL	+	-	-
A/Taiwan/42/2006	Influenza A/H1N1	$1x10^{2}$ $TCID_{50}$/mL	+	-	-
A/Victoria/3/1975	Influenza A/-H3N2	$1x10^{2}$ $CEID_{50}$/mL	+	-	-
A/Vietnam/1203 RNA	Influenza A/H5N1	0.27 ug/mL	+	-	-
A/WS/33	Influenza A/H1N1	$1x10^{2}$ $TCID_{50}$/mL	+	-	-
B/Brisbane/60/2008	Influenza B	$1x10^{2}$ $TCID_{50}$/mL	-	+	-
B/Florida/2/2006 (Yamagata lineage)	Influenza B	$1x10^{2}$ $TCID_{50}$/mL	-	+	-
B/Florida/7/2004	Influenza B	$1x10^{2}$ $TCID_{50}$/mL	-	+	-
B/Hawaii/11/2005	Influenza B	$1x10^{2}$ $TCID_{50}$/mL	-	+	-
B/Hawaii/33/2004	Influenza B	$1x10^{2}$ $TCID_{50}$/mL	-	+	-

Table 9: Analytical Reactivity (inclusivity) Test Summary (continued)

Description	Type	Concentration	Flu A	Flu B	RSV
B/Lee/40	Influenza B	$1x10^2$ $CEID_{50}$/mL	-	+	-
B/Michigan/2/2006	Influenza B	$1x10^2$ $TCID_{50}$/mL	-	+	-
B/Ohio/1/2005	Influenza B	$1x10^2$ $TCID_{50}$/mL	-	+	-
B/Panama/45/90	Influenza B	$1x10^2$ $TCID_{50}$/mL	-	+	-
B/Phuket/3073/2013 (Victoria Lineage)	Influenza B	$1x10^2$ $TCID_{50}$/mL	-	+	-
B/St. Petersburg/04/2006	Influenza B	$1x10^2$ $TCID_{50}$/mL	-	+	-
RSV A/A2	RSV	$1x10^2$ $TCID_{50}$/mL	-	-	+
RSV A/Long	RSV	$1x10^2$ $TCID_{50}$/mL	-	-	+
RSV A/Vero	RSV	$1x10^2$ $CEID_{50}$/mL	-	-	+
RSV B/9320	RSV	$1x10^2$ $TCID_{50}$/mL	-	-	+
RSV B/Wash/18537/62	RSV	$2x10^2$ $TCID_{50}$/mL	-	-	+

Table 10: Additional Analytical Reactivity (inclusivity) Test Summary

Description	Type	Concentration	Flu A	Flu B	RSV
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	$1x10^3$ $TCID_{50}$/mL	+	-	-
A/Northern pintail/Washington/40964/2014	Influenza A/H5N2	$1x10^3$ $TCID_{50}$/mL	+	-	-
A/Swine/ NY/01/2009	Influenza A/H1N1	$1x10^2$ $TCID_{50}$/mL	+	-	-
A/Swine/Iowa/2006	Influenza A/H1N1	$1x10^2$ $CEID_{50}$/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	+	-	-

Note: These are avian and swine strains that have not been shown to circulate in humans.

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 strain 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 $TCID_{50}$/mL.

Analytical specificity of the Panther Fusion Flu A/B/RSV assay was 100% for Flu A, Flu B, and RSV.

Table 11: 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	-	-	-
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	-	-	-

Table 11: Specificity Results (continued)

Organism	Concentration	Flu A	Flu B	RSV
Mumps virus	$1x10^4$ $TCID_{50}$/mL	-	-	-
Mycobacterium intracellulare	$1x10^{10}$ rRNA copies/mL	-	-	-
Mycobacterium tuberculosis	$1x10^{10}$ rRNA copies/mL	-	-	-
Mycoplasma pneumoniae	$1x10^6$ CFU/mL	-	-	-
Neisseria gonorrhea	$1x10^7$ CFU/mL	-	-	-
Neisseria meningitides	$1x10^7$ CFU/mL	-	-	-
Neisseria mucosa	$1x10^7$ CFU/mL	-	-	-
Polio virus	$1x10^6$ $TCID_{50}$/mL	-	-	-
Proteus mirabilis	$1x10^7$ CFU/mL	-	-	-
Proteus vulgaris	$1x10^7$ CFU/mL	-	-	-
Pseudomonas aeruginosa	$1x10^7$ CFU/mL	-	-	-
Rhinovirus 1A	$1x10^5$ $TCID_{50}$/mL	-	-	-
Staphlycoccus aureus	$1x10^7$ CFU/mL	-	-	-
Staphlycoccus epidermidis	$1x10^7$ CFU/mL	-	-	-
Streptococcus pneumoniae	$1x10^6$ CFU/mL	-	-	-
Streptococcus pyogenes	$1x10^7$CFU/mL	-	-	-
Streptococcus salivarius	$1x10^6$ CFU/mL	-	-	-
Tatlockia micdadei (formerly Legionella micdadei)	$1x10^7$ CFU/mL	-	-	-
Varicella Zoster Virus	$1x10^3$ $TCID_{50}$/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 noted in Table 12.

Table 12: Competitive Interference

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	-	+	+

*When this combination was tested with Flu B at 3X LoD, Flu B detection rate was 92.3%.

Interference

Mucin, whole blood and other potentially interfering substances (medications and over-the-counter or OTC products) that may be present in the samples were evaluated in the Panther Fusion 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, as shown in Table 13.

All of the substances tested were found to have no impact on the performance of the Panther Fusion Flu A/B/RSV assay, at the concentrations tested.

Table 13: Potentially Interfering Substances

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

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 are described in Table 14, along with a summary of the agreement with expected results for each targets. Table 15 presents the mean and variability analysis between instruments, between reagent lots, between operators, between days, between runs and within runs, and overall (total) for Ct.

Table 14: Percent Agreement to the Expected Result

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

Table 15: Signal Variability

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 LoD	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 LoD	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

Bibliography

1. Centers for Disease Control and Prevention. National Respiratory and Enteric Virus Surveillance System. Centers for Disease Control and Prevention Web site. http://www.cdc.gov/surveillance/nrevss/. Accessed October, 2015.
2. Kahn, J.S. 2006. Epidemiology of human metapneumovirus. Clin. Microbiol. Rev. 19:546-557.
3. Couch, R.B.and Kasel, J.A. 1995. Influenza *in Diagnostic Procedures for Viral, Rickettsial, and Chlamydial Infections*. 7th Edition. 431-446.
4. Harper, S.A., Fukida, K., Uyeki, T.M., Cox, N.J., and Bridges, C.B. 2005. Prevention and Control of Influenza. MMWR. 54(RR08):1-40.
5. World Health Organization. Influenza (Seasonal) Fact Sheet N° 211 March 2014. Centers for Disease Control and Prevention Web site. http://www.who.int/mediacentre/factsheets/fs211/en/. Accessed October 2015.
6. Centers for Disease Control and Prevention. Respiratory Syncytial Virus Circulation in the United States, July 2012-June 2014 MMWR 2014;62:141-4 Centers for Disease Control and Prevention Web site.www.cdc.gov/rsv/research/us-serveillance.html. Accessed October 2015.
7. Clinical & Laboratory Standards Institute. Document M29 Protection of Laboratory Workers from Occupationally Acquired Infections. CLSI Web site https://clsi.org/standards/products/microbiology/documents/m29/. Accessed September 2017.

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