SARS-CoV-2/Flu A/B/RSV Assay (Panther Fusion™ System)

For *in vitro* diagnostic use.

For U.S. Export only.

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General Information

Intended Use

The Panther Fusion™ SARS-CoV-2/Flu A/B/RSV assay is a fully automated multiplexed real-time RT-PCR test intended for the qualitative detection and differentiation of RNA from SARS-CoV-2 virus, influenza A virus (Flu A), influenza B virus (Flu B) and respiratory syncytial virus (RSV) isolated and purified from nasopharyngeal (NP) swab specimens obtained from individuals exhibiting signs and symptoms of a respiratory tract infection. Clinical signs and symptoms of respiratory viral infection due to SARS-CoV-2, influenza and RSV can be similar. This assay is intended to aid in the differential diagnosis of SARS-CoV-2, 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 SARS-CoV-2, 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.

Summary and Explanation of the Test

Respiratory viruses are responsible for a wide range of acute respiratory tract infections including the common cold, influenza (flu), RSV infection, COVID-19 and croup and represent the most common cause of acute illness in the United States. Some symptoms of COVID-19, flu and RSV are similar making diagnosis based on symptoms alone virtually impossible.[1,2]

Disease severity of flu and RSV 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),[3] decreasing the overall cost of care, reducing the potential for further development of antimicrobial resistance due to excessive and inappropriate use of antibiotics,[4] 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.[5]

Influenza is an acute respiratory illness caused by infection with the influenza virus, primarily types A and B.[6] Influenza A viruses are further categorized into subtypes based on the two major surface protein antigens: hemagglutinin (H) and neuraminidase (N).[7] Influenza B viruses are not categorized into subtypes.[7] 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.[8]

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 58,000 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.[9]

Coronaviruses are a large family of viruses which may cause illness in animals or humans. In humans, several coronaviruses are known to cause respiratory infections ranging from the common cold to more severe diseases such as Middle East Respiratory Syndrome (MERS) and Severe Acute Respiratory Syndrome (SARS). The most recently discovered coronavirus, SARS-CoV-2, causes the associated coronavirus disease COVID-19. This new virus and disease were unknown before outbreak in Wuhan, China, in December 2019.[9]

People with COVID-19 have had a wide range of symptoms reported, ranging from mild symptoms to severe illness. Symptoms may appear 2-14 days after exposure to the virus. People with COVID-19 may exhibit fever or chills, cough, shortness of breath or difficulty breathing, fatigue, muscle or body aches, headache, new loss of taste or smell, sore throat, congestion or runny nose, nausea or vomiting, and/or diarrhea.[10] On March 11, 2020, the COVID-19 outbreak was characterized as a pandemic by the World Health Organization (WHO).[11]

Principles of the Procedure

The Panther Fusion SARS-CoV-2/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 collected in universal transport media (UTM) and viral transport medium (VTM) are transferred to a Specimen Lysis Tube containing specimen transport media (STM). Alternatively, samples can be collected with the RespDirect Collection Kit which contains enhanced specimen transport media (eSTM). STM and eSTM lyse 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
SARS-CoV-2	ORF1ab	ROX
Influenza B Virus	Matrix	RED647
Internal Control	Not applicable	RED677

Warnings and Precautions

A. For *in vitro* diagnostic use. Carefully read this entire package insert and the *Panther/Panther Fusion System Operator's Manual*.

B. For professional use.

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. Handle all specimens as if infectious using safe laboratory procedures. Refer to Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with 2019-nCoV. https://www.cdc.gov/coronavirus/2019-ncov/lab/lab-biosafety-guidelines.html.

F. 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.*

G. If infection with SARS-CoV-2 is suspected based on current clinical screening criteria recommended by public health authorities, specimens should be collected with appropriate infection control precautions.

H. Use appropriate personal protective equipment when collecting and handling specimens from individuals suspected of being infected with SARS-CoV-2 as outlined in the CDC Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with 2019 Novel Coronavirus (SARS-CoV-2).

I. Use only supplied or specified disposable laboratory ware.

J. Wear disposable, powderless gloves, protective eye wear, and laboratory coats when handling specimens and reagents. Wash hands thoroughly after handling specimens and reagents. Dispose of all material that has come into contact with specimens and reagents in accordance with applicable national, international, and regional regulations.

K. Expiration dates listed on the RespDirect Collection Kit and the Panther Fusion Specimen Lysis Tubes pertain 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.

L. 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.

M. 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.

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

O. 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.

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

Q. Avoid microbial and ribonuclease contamination of reagents.

R. 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.

S. 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.

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

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

V. Do not use material that may contain Guanidinium thiocyanate or any guanidine-containing materials on the instrument. Highly reactive and/or toxic compounds may form if combined with sodium hypochlorite.

W. Some reagents in the kit are labeled with hazard information.

Note: *Hazard communication reflects the EU Safety Data Sheet (SDS) classifications. For hazard communication information specific to your region, refer to the region specific SDS on the Safety Data Sheet Library at www.hologicsds.com. For more information on the symbols, refer to the symbol legend on www.hologic.com/package-inserts.*

EU Hazard Information	
	**Panther Fusion Oil** ***Polydimethylsiloxane 100%*** **WARNING** H315 - Causes skin irritation H319 - Causes serious eye irritation
 	**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 P280 - Wear protective gloves protective clothing/eye protection/face protection P303 + P361 + P353 - IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/showerP305 + 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 P280 - Wear eye protection/ face protection

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 SARS-CoV-2/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 SARS-CoV-2/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 SARS-CoV-2/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 SARS-CoV-2/Flu A/B/RSV assay, this includes NP swab specimens in viral transport medium (VTM), universal transport medium (UTM), or collected in eSTM with the RespDirect Collection kit.

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.*

Specimen Collection

Note: *Collect NP swab specimens according to standard technique using a polyester-, rayon, or nylon-tipped swab. Immediately place the swab specimen into 3 mL of VTM or UTM. The Hologic RespDirect Collection Kit may be used for the collection of NP swab samples.*

Specimen Processing

Specimen Processing with the Panther Fusion Specimen Lysis Tube

A. Prior to testing on the Panther Fusion system, transfer 500 µL of the specimen collected in UTM or VTM into a Panther Fusion Specimen Lysis Tube.

   Note: *When testing frozen specimen, allow specimen to reach room temperature prior to processing.*

Specimen Processing with the Enhanced Direct Load Tube (RespDirect Collection Kit)

A. After collecting the specimen into the Enhanced Direct Load Tube (RespDirect Collection Kit), the specimen may be loaded on the Panther Fusion system.

   Note: *If clots are observed, samples may be vortexed for 5–10 minutes at 1,800 rpm on a multi-tube vortex (or setting 5 on Cat. No. 102160G).*

   Alternatively, individual tubes may be vortexed by hand for 15 seconds on max. speed on a standard bench top vortex.

   If previously pierced, recap tubes with a new penetrable cap before vortexing.

   If a CLT result is obtained upon retesting, collect a new sample.

   Note: *When testing frozen specimen, allow specimen to reach room temperature prior to loading on the Panther Fusion system.*

   Note: *If the lab receives an Enhanced Direct Load Tube (RespDirect Collection Kit) with no swab or two swabs, the specimen must be rejected.*

Specimen Storage

A. Storing Specimens with the Panther Fusion Specimen Lysis Tube

1. 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.
2. Samples (in the Panther Fusion Specimen Lysis Tube) can be stored under the following conditions:
    - 15°C to 30°C up to 6 days or
    - 2°C to 8°C, -20°C, and -70°C for up to 3 months
3. Previously tested samples should be covered with a new, clean plastic film or foil barrier.
4. 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 previously tested and recapped samples, 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.

B. Storing Specimens with the Enhanced Direct Load Tube (RespDirect Collection Kit)

1. Samples can be stored under the following conditions:
    - 2°C to 30°C up to 6 days or
    - 2°C to 8°C, –20°C, and –70°C for up to 3 months.
2. Previously tested 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 previously tested and recapped samples, specimen tubes may be centrifuged for 5 minutes at 420 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 Panther Fusion SARS-CoV-2/Flu A/B/RSV Assay

Assay Packaging

Components[1]	Part No.	Storage
Panther Fusion SARS-CoV-2/Flu A/B/RSV Assay Cartridges 96 Tests Panther Fusion SARS-CoV-2/Flu A/B/RSV assay cartridge, 12 tests, 8 per box	PRD-07400	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 SARS-CoV-2/Flu A/B/RSV Controls Panther Fusion SARS-CoV-2/Flu A/B/RSV Positive Controls tube, 5 per box Panther Fusion Negative Control tube, 5 per box	PRD-07401	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
Hologic RespDirect Collection Kit, 50 per box	PRD-07403

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 System	PRD-04172
Panther Fusion Module	PRD-04173
Panther System Continuous Fluid and Waste (Panther Plus)	PRD-06067
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
Tips, 1000 µL, filtered, liquid-sensing, conductive, and disposable. *Not all products are available in all regions. Contact your representative for region-specific information*.	901121 (10612513 Tecan) 903031 (10612513 Tecan) MME-04134 (30180117 Tecan) MME-04128
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 8.25% (0.7 M to 1.16 M) sodium hypochlorite solution **Note**: Refer to the *Panther/Panther Fusion System Operator's Manual* for instructions on preparing diluted sodium hypochlorite solution.	-
Disposable powderless gloves	-

*Needed only for Panther Aptima TMA assays.

Optional Materials

Material	Cat. No.
Multitube Vortex	102160G
Benchtop Vortex	-

Panther Fusion System Test Procedure

Note: *Refer to the Panther/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.*

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.*

Note: *For the Enhanced Direct Load Tube (RespDirect Collection Kit), there is sufficient volume to perform 4 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/Panther Fusion System Operator's Manual.*

Procedural Notes

A. Controls

1. The Panther Fusion SARS-CoV-2/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 SARS-CoV-2/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 SARS-CoV-2, Flu A, Flu B and/or RSV. The internal control must be detected in all samples that are negative for SARS-CoV-2, Flu A, Flu B, and RSV; 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*/*Panther Fusion System Operator's Manual.*

Interpretation of Results

The Panther Fusion system automatically determines the test results for samples and controls. Results for SARS-CoV-2, Flu A, Flu B, and RSV detection are reported separately. A test result may be negative, positive, or invalid.

Table 1 shows the possible results reported in a valid run with result interpretations.

Table 1: Result Interpretation

SARS-CoV-2 Result	Flu A Result	Flu B Result	RSV Result	IC Result	Interpretation
Neg	Neg	Neg	Neg	Valid	SARS-CoV-2, Flu A, Flu B, and RSV not detected.
Neg	POS	Neg	Neg	Valid	Flu A detected. SARS-CoV-2, Flu B, and RSV not detected.
Neg	Neg	POS	Neg	Valid	Flu B detected. SARS-CoV-2, Flu A, and RSV not detected.
Neg	Neg	Neg	POS	Valid	RSV detected. SARS-CoV-2, Flu A, and Flu B not detected.
POS	Neg	Neg	Neg	Valid	SARS-CoV-2 detected. Flu A, Flu B, and RSV not detected.
Neg	POS	POS	Neg	Valid	Flu A and Flu B detected. SARS-CoV-2 and RSV not detected.
Neg	Neg	POS	POS	Valid	Flu B and RSV detected. SARS-CoV-2 and Flu A not detected.
Neg	POS	Neg	POS	Valid	Flu A and RSV detected. SARS-CoV-2 and Flu B not detected.
POS	POS	Neg	Neg	Valid	SARS-CoV-2 and Flu A detected. Flu B and RSV not deteted
POS	Neg	POS	Neg	Valid	SARS-CoV-2 and Flu B detected. Flu A and RSV not detected.
POS	Neg	Neg	POS	Valid	SARS-CoV-2 and RSV detected. Flu A and Flu B not detected.
Neg	POS	POS	POS	Valid	Flu A, Flu B, and RSV detected. SARS-CoV-2 not detected Triple infections are rare. Retest to confirm result.
POS	Neg	POS	POS	Valid	SARS-CoV-2, Flu B, and RSV detected. Flu A not detected. Triple infections are rare. Retest to confirm result.
POS	POS	Neg	POS	Valid	SARS-CoV-2, Flu A, and RSV detected. Flu B not detected. Triple infections are rare. Retest to confirm result.
POS	POS	POS	Neg	Valid	SARS-CoV-2, Flu A, and Flu B detected. RSV not detected. Triple infections are rare. Retest to confirm result.
POS	POS	POS	POS	Valid	SARS-CoV-2, Flu A, Flu B, and RSV detected. Quadruple infections are rare. Retest to confirm result.
Invalid	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 SARS-CoV-2, Flu A, Flu B, and/or RSV.

Limitations

A. This product can be used only with the Panther Fusion System.

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

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

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

E. Negative results do not preclude SARS-CoV-2, influenza A virus, influenza B virus, or RSV infections and should not be used as the sole basis for treatment or other management decisions.

F. 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.

G. 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.

SARS-CoV-2/Flu A/B/RSV Assay Performance

Analytical Sensitivity

The analytical sensitivity (limit of detection or LoD) of the Panther Fusion SARS-CoV-2/Flu A/B/RSV assay was determined by testing dilutions of processed negative clinical nasopharyngeal (NP) swab VTM/UTM matrix spiked with the WHO International Standard for SARS-CoV-2, NIBSC (20/146) or viral cultures of SARS-CoV-2 (1 strain), Influenza A (2 strains), Influenza B (2 strains), RSV A and RSV B (1 strain each). A minimum of 24 replicates were tested with each of three reagent lots. The LoD for each target was determined by Probit analysis for each reagent lot and was confirmed with an additional 24 replicates using a single reagent lot. Analytical sensitivity is defined as the lowest concentration at which ≥95% of all replicates tested positive, as summarized in Table 2.

LoD testing was also performed with the RespDirect Collection Kit. Negative clinical eSTM matrix was spiked with the WHO International Standard for SARS-CoV-2 and 1 strain each for Flu A, Flu B, RSV A, and RSV B. Thirty replicates were tested with a single reagent lot. The lowest concentration that observed ≥95% detection was 98.6 IU/mL for the WHO International Standard for SARS-CoV-2, 0.11 $TCID_{50}$/mL for Influenza A/Kansas/14/17 (H3N2), 0.03 $TCID_{50}$/mL for Influenza B/Washington/02/19 (Victoria lineage), 0.03 $TCID_{50}$/mL for RSV A and 0.05 $TCID_{50}$/mL for RSV B.

Note: *The stated LoDs pertain to the concentrations in the tubes loaded onto the instrument. For samples collected in VTM/UTM, this is the concentration in the processed sample in an SLT. For samples collected using the RespDirect Collection kit, this is the concentration in the Enhanced Direct Load tube (RespDirect Collection Kit).*

Table 2: Analytical Sensitivity

Viral Strain/Standard	LoD concentration in the processed sample*	Units
WHO International Standard SARS-CoV-2, NIBSC (20/146)	47.20	IU/mL
SARS-CoV-2 USA-WA1/2020	0.03	$TCID_{50}$/mL
Influenza A/Brisbane/02/18 (H1N1)	0.06	$TCID_{50}$/mL
Influenza A/Kansas/14/17 (H3N2)	0.10	$TCID_{50}$/mL
Influenza B/Washington/02/19 (Victoria lineage)	0.03	$TCID_{50}$/mL
Influenza B/Phuket/3073/13 (Yamagata lineage)	0.003	$TCID_{50}$/mL
RSV A	0.03	$TCID_{50}$/mL
RSV B	0.03	$TCID_{50}$/mL

*Processed sample: 0.50 mL VTM/UTM primary clinical sample + 0.71 mL STM in an SLT

Reactivity-Wet Testing

The reactivity of the Panther Fusion SARS-CoV-2/Flu A/B/RSV assay was determined by testing virus strains in the processed negative clinical NP swab VTM/UTM matrix. Each strain was tested in triplicate at ~3x LoD with one reagent lot. For strains not detected at 3x LoD, additional testing at higher concentrations was performed until 100% positivity was observed. Table 3 shows the lowest concentration of each strain in which 100% positivity was observed.

Table 3: Analytical Reactivity Summary for SARS-CoV-2, Flu A and Flu B and RSV Strains

Description	Subtype	Concentration	SARS-CoV-2	Flu A	Flu B	RSV
USA-WA1/2020*	SARS-CoV-2	0.09 $TCID_{50}$/mL	+	-	-	-
USA-CA1/2020	SARS-CoV-2	0.09 $TCID_{50}$/mL	+	-	-	-
USA-AZ1/2020	SARS-CoV-2	0.09 $TCID_{50}$/mL	+	-	-	-
USA-WI1/2020	SARS-CoV-2	0.09 $TCID_{50}$/mL	+	-	-	-
USA/OR-OHSU-PHL00037/ 2021|B.1.1.7	SARS-CoV-2	0.09 $TCID_{50}$/mL	+	-	-	-
Uganda/MUWRP-20200195568/ 2020|A.23.1	SARS-CoV-2	0.09 $TCID_{50}$/mL	+	-	-	-
USA/PHC658/2021|B.1.617.2	SARS-CoV-2	0.09 $TCID_{50}$/mL	+	-	-	-
USA/MD-HP05285/2021|B.1.617.2	SARS-CoV-2	0.09 $TCID_{50}$/mL	+	-	-	-
USA/CA/VRLC009/2021|B.1.427	SARS-CoV-2	0.09 $TCID_{50}$/mL	+	-	-	-
USA/CA/VRLC012/2021|P.2	SARS-CoV-2	0.30 $TCID_{50}$/mL	+	-	-	-
USA/MD-HP03056/2021|B.1.525	SARS-CoV-2	0.30 $TCID_{50}$/mL	+	-	-	-
USA/CA-Stanford-16_S02/ 2021|B.1.617.1	SARS-CoV-2	0.09 $TCID_{50}$/mL	+	-	-	-
Peru/un-CDC-2-4069945/ 2021|C.37	SARS-CoV-2	0.09 TCID50/mL	+	-	-	-
USA/MD-HP20874/2021|B.1.1.529	SARS-CoV-2	0.09 $TCID_{50}$/mL	+	-	-	-
USA/GA-EHC-2811C/ 2021|B.1.1.529	SARS-CoV-2	0.09 $TCID_{50}$/mL	+	-	-	-
A/Brisbane/02/18*	Flu A (H1N1)	0.18 $TCID_{50}$/mL	-	+	-	-
A/Michigan/45/2015	Flu A (H1N1)	0.18 $TCID_{50}$/mL	-	+	-	-
A/Christ Church/16/2010	Flu A (H1N1)	180^{1} $TCID_{50}$/mL	-	+	-	-
A/Kentucky/2/06	Flu A (H1N1)	0.60 $TCID_{50}$/mL	-	+	-	-
A/Solomon Islands/03/06	Flu A (H1N1)	0.60 $TCID_{50}$/mL	-	+	-	-
A/Guangdong-maonan/1536/2019	Flu A (H1N1)	180^{1} $TCID_{50}$/mL	-	+	-	-
A/Taiwan/42/2006	Flu A (H1N1)	0.60 $TCID_{50}$/mL	-	+	-	-
A/Henan/8/05	Flu A (H1N1)	0.60 $TCID_{50}$/mL	-	+	-	-
A/Hawaii/15/01	Flu A (H1N1)	18^{3} $TCID_{50}$/mL	-	+	-	-

Table 3: Analytical Reactivity Summary for SARS-CoV-2, Flu A and Flu B and RSV Strains (Continued)

Description	Subtype	Concentration	SARS-CoV-2	Flu A	Flu B	RSV
A/California/07/2009	Flu A (H1N1)	0.18 $TCID_{50}$/mL	-	+	-	-
A/Hawaii/66/2019	Flu A (H1N1)	180 $CEID_{50}$/mL	-	+	-	-
A/Indiana/02/2020	Flu A (H1N1)	60 $CEID_{50}$/mL	-	+	-	-
A/Michigan/45/2015 pdm09-like virus	Flu A (H1N1)	0.60 $TCID_{50}$/mL	-	+	-	-
A/Kansas/14/17*	Flu A (H3N2)	0.33 $TCID_{50}$/mL	-	+	-	-
A/Arizona/45/2018	Flu A (H3N2)	3.3 FFU/mL	-	+	-	-
A/New York/21/2020	Flu A (H3N2)	3.3 FFU/mL	-	+	-	-
A/Hong Kong/45/2019	Flu A (H3N2)	3.3 FFU/mL	-	+	-	-
A/Singapore/INFIMH-16-0019/ 2016	Flu A (H3N2)	110 $CEID_{50}$/mL	-	+	-	-
A/Hong Kong/2671/2019	Flu A (H3N2)	11^2 $TCID_{50}$/mL	-	+	-	-
A/Hiroshima/52/05	Flu A (H3N2)	1.1 $TCID_{50}$/mL	-	+	-	-
A/Costa Rica/07/99	Flu A (H3N2)	11^3 $TCID_{50}$/mL	-	+	-	-
A/Port Chalmers/1/73	Flu A (H3N2)	1.1 $TCID_{50}$/mL	-	+	-	-
A/Brazil/113/99	Flu A (H3N2)	1.1 $TCID_{50}$/mL	-	+	-	-
A/Perth/16/2009	Flu A (H3N2)	0.33 $TCID_{50}$/mL	-	+	-	-
A/Texas/50/2012	Flu A (H3N2)	0.33 $TCID_{50}$/mL	-	+	-	-
A/Hong Kong/4801/2014	Flu A (H3N2)	1.1 $TCID_{50}$/mL	-	+	-	-
A/Indiana/08/2011	Flu A (H3N2)	1.1 TCID50/mL	-	+	-	-
A/Hong Kong/486/97	Flu A (H5N1)	0.01 ng/mL	-	+	-	-
B/Washington/02/2019*	Flu B (Victoria)	0.09 $TCID_{50}$/mL	-	-	+	-
B/Colorado/06/2017	Flu B (Victoria)	0.09 $TCID_{50}$/mL	-	-	+	-
B/Florida/78/2015	Flu B (Victoria)	0.30 $TCID_{50}$/mL	-	-	+	-
B/Alabama/2/17	Flu B (Victoria)	0.09 $TCID_{50}$/mL	-	-	+	-
B/Ohio/1/2005	Flu B (Victoria)	0.30 $TCID_{50}$/mL	-	-	+	-
B/Michigan/09/2011	Flu B (Victoria)	3^3 $TCID_{50}$/mL	-	-	+	-
B/Hawaii/01/2018 (NA D197N)	Flu B (Victoria)	0.90^1 $TCID_{50}$/mL	-	-	+	-
B/Brisbane/33/08	Flu B (Victoria)	0.09 $TCID_{50}$/mL	-	-	+	-
B/Phuket/3073/2013*	Flu B (Yamagata)	0.006 $TCID_{50}$/mL	-	-	+	-
B/Wisconsin/1/2010	Flu B (Yamagata)	2^1 $TCID_{50}$/mL	-	-	+	-
B/Utah/9/14	Flu B (Yamagata)	0.006 $TCID_{50}$/mL	-	-	+	-
B/St. Petersburg/04/06	Flu B (Yamagata)	0.06 $TCID_{50}$/mL	-	-	+	-

Table 3: Analytical Reactivity Summary for SARS-CoV-2, Flu A and Flu B and RSV Strains (Continued)

Description	Subtype	Concentration	SARS-CoV-2	Flu A	Flu B	RSV
B/Texas/81/2016	Flu B (Yamagata)	2 $TCID_{50}$/mL	-	-	+	-
B/Indiana/17/2017	Flu B (Yamagata)	0.60[1] $TCID_{50}$/mL	-	-	+	-
B/Oklahoma/10/2018	Flu B (Yamagata)	2[1] $TCID_{50}$/mL	-	-	+	-
B/Massachusetts/02/2012	Flu B (Yamagata)	0.2[2] $TCID_{50}$/mL	-	-	+	-
B/Lee/40	Flu B	0.09 $TCID_{50}$/mL	-	-	+	-
RSV-A/2006 Isolate*	RSVA	0.06 $TCID_{50}$/mL	-	-	-	+
RSV A/4/2015 isolate #1	RSVA	0.06 $TCID_{50}$/mL	-	-	-	+
RSV A/A2	RSVA	0.06 $TCID_{50}$/mL	-	-	-	+
RSV A/12/2014 isolate #2	RSVA	0.06 $TCID_{50}$/mL	-	-	-	+
RSV-B/CH93(18)-18*	RSVB	0.30 $TCID_{50}$/mL	-	-	-	+
RSV B/3/2015 isolate #1	RSVB	0.09 $TCID_{50}$/mL	-	-	-	+
RSV B/9320	RSVB	0.09 $TCID_{50}$/mL	-	-	-	+

*Strain used to establish LoD.

[1]In silico analysis showed 100% homology to amplification region. Virus stock degradation or error in $TCID_{50}$/mL quantification may have impacted the concentration at 100% detection.

[2] In silico analysis identified a single mismatch in the forward and reverse primers for A/Hong Kong/2671/2019 and a single mismatch in the reverse primer of B/Massachusetts/02/2012. Due to the location of the mismatches, amplification and detection are not expected to be impacted. Virus stock degradation or error in $TCID_{50}$/mL quantification may have impacted the concentration at 100% detection.

[3]Sequence of strain in targeted amplification regions are not available in NCBI or GISAID to further evaluate sensitivity.

Reactivity-In silico Analysis

The inclusivity of the Panther Fusion SARS-CoV-2/Flu A/B/RSV assay was evaluated using in silico analysis of the forward primers, reverse primers, and probes for the SARS-CoV-2, Flu A, Flu B and RSV target systems in relation to sequences available in the NCBI and GISAID gene databases. Any sequence with missing or ambiguous sequence information was removed from the analysis for that target region.

Based on the in silico analysis of GISAID and NCBI sequences available up to June 25, 2022 for SARS-CoV-2 (10% random sampling of >9.3 million sequences), the Panther Fusion SARS-CoV-2/Flu A/B/RSV assay is predicted to detect all 934,493 SARS-CoV-2 sequences evaluated.

The sequences evaluated included lineages and variants of concern (VOC) or variants under investigation (VUI) that may have important epidemiological, immunological, or pathogenic properties from a public health perspective, such as Delta and Omicron variants. All lineages and variants of public health interest identified as of June 25, 2022 are predicted to be detected; new sequences and variants will continue to be monitored for impacts on detection by the Panther Fusion SARS-CoV-2/Flu A/B/RSV assay.

Based on in silico analysis of all sequences available from January 01, 2015 to February 15, 2022 in GISAID and NCBI databases, the Panther Fusion SARS-CoV-2/Flu A/B/RSV assay is

predicted to detect ≥99.998% of 88,128 Flu A, ≥99.94% of 31,801 Flu B, ≥98.12% of 1,599 RSV A, and ≥98.23% of 1,240 RSV B sequences evaluated.

Analytical Specificity and Microbial Interference

Analytical specificity (cross-reactivity) and microbial interference with the Panther Fusion SARS-CoV-2/Flu A/B/RSV assay was evaluated in the presence of closely related and non-targeted organisms. Panels consisting of 41 organisms (Table 4) were tested in processed negative clinical NP swab VTM/UTM matrix in the absence or presence of 3x LoD SARS-CoV-2, Flu A, Flu B and RSV. Bacteria were tested at 10^6 CFU/mL and viruses were tested at 10^5 $TCID_{50}$/mL, except where noted. No cross-reactivity or microbial interference was observed for any of the 41 organisms tested in the Panther Fusion SARS-CoV-2/Flu A/B/RSV assay at the following concentrations.

In silico cross-reactivity analysis of 143 respiratory organisms (545 GenBank accession numbers) predicted no cross reactivity or microbial interference with the exception of *S. marcescens* which had a possibility of low amplification without detection. Wet-testing in processed negative clinical NP swab VTM/UTM matrix of each target at 3X LoD in the presence of this organism at 10^6 CFU/mL demonstrated that no interference was observed.

Table 4: Cross Reactivity and Microbial Interference Microorganisms

Microorganism	Concentration[1]	Microorganism	Concentration[1]
Adenovirus 1	$1x10^5$ $TCID_{50}$/mL	*Bordetella pertussis*	$1x10^6$ CFU/mL
Adenovirus 7a	$1x10^5$ $TCID_{50}$/mL	*Candida albicans*	$1x10^6$ CFU/mL
CMV Strain AD 169	$1x10^4$ $TCID_{50}$/mL	*Chlamydophila pneumoniae*	$1x10^6$ IFU/mL
Human coronavirus 229E	$1x10^4$ $TCID_{50}$/mL	*Corynebacterium diphtheriae*	$1x10^6$ CFU/mL
Human coronavirus NL63	$1x10^4$ $TCID_{50}$/mL	*Escherichia coli*	$1x10^6$ CFU/mL
Human coronavirus OC43	$1x10^5$ $TCID_{50}$/mL	*Haemophilus influenzae*	$1x10^6$ CFU/mL
Epstein-Barr virus (EBV)	$1x10^6$ copies/mL	*Lactobacillus plantarum*	$1x10^6$ CFU/mL
Enterovirus (e.g. EV68)	$1x10^5$ $TCID_{50}$/mL	*Legionella pneumophila*	$1x10^6$ CFU/mL
Human coronavirus HKU1[2]	$1x10^6$ copies/mL	*Moraxella catarrhalis*	$1x10^5$ CFU/mL
Human Metapneumovirus (hMPV)	$1x10^5$ $TCID_{50}$/mL	*Mycobacterium tuberculosis*	$1x10^9$ rRNA copies/mL
HPIV-1	$1x10^5$ $TCID_{50}$/mL	*Mycoplasma pneumoniae*	$1x10^9$ rRNA copies/mL
HPIV-2	$1x10^5$ $TCID_{50}$/mL	*Neisseria spp*	$1x10^6$ CFU/mL
HPIV-3	$1x10^5$ $TCID_{50}$/mL	*Neisseria meningitides*	$1x10^6$ CFU/mL
HPIV-4	$1x10^4$ $TCID_{50}$/mL	*Neisseria mucosa*	$1x10^6$ CFU/mL
Measles	$1x10^4$ $TCID_{50}$/mL	*Pneumocystis jirovecii*	$1x10^6$ CFU/mL
MERS-Coronavirus	$5x10^4$ $TCID_{50}$/mL	*Pseudomonas aeruginosa*	$1x10^6$ CFU/mL
Mumps virus	$1x10^5$ $TCID_{50}$/mL	*Staphylococcus aureus*	$1x10^6$ CFU/mL
Rhinovirus 1A	$1x10^4$ $TCID_{50}$/mL	*Staphylococcus epidermidis*	$1x10^6$ CFU/mL
SARS coronavirus 1[2]	$1x10^6$ copies/mL	*Streptococcus pneumoniae*	$1x10^6$ CFU/mL
Varicella Zoster Virus	$1x10^3$ $TCID_{50}$/mL	*Streptococcus pyogenes*	$1x10^6$ CFU/mL
		Streptococcus salivarius	$1x10^6$ CFU/mL

[1]CFU = Colony Forming Units; IFU = Inclusion Forming Units; $TCID_{50}$ = Median Tissue Culture Infectious Dose

[2]Cultured virus and whole genome purified nucleic acid for Human HKU1 and SARS-coronavirus are not readily available. HKU1 and SARS-coronavirus *in vitro* transcript (IVT) corresponding to the ORF1a gene regions targeted by the assay were used to evaluate cross-reactivity and microbial interference.

Competitive Interference

Competitive interference in the Panther Fusion SARS-CoV-2/Flu A/B/RSV assay was evaluated in triplicate using pairs of targeted viruses at low/high concentrations in processed negative clinical NP swab VTM/UTM matrix. The low concentration was tested at 3x LoD, while the high concentration virus was tested at 1000x LoD. Results of the study are shown in Table 5. The presence of two viruses at varying concentrations had no effect on the analytical sensitivity of one target in the presence of high concentrations of the other target.

Table 5: Competitive Interference

Low Target		High Target		SARS-CoV-2 (detected)	Flu A (detected)	Flu B (detected)	RSV (detected)
Virus	3x LoD ($TCID_{50}$/mL)	Virus	1000x LoD ($TCID_{50}$/mL)				
SARS-CoV-2	0.09	Flu A	110	+	+	-	-
SARS-CoV-2	0.09	Flu B	30	+	-	+	-
SARS-CoV-2	0.09	RSV	30	+	-	-	+
Flu A	0.33	SARS-CoV-2	30	+	+	-	-
Flu A	0.33	Flu B	30	-	+	+	-
Flu A	0.33	RSV	30	-	+	-	+
Flu B	0.09	SARS-CoV-2	30	+	-	+	-
Flu B	0.09	Flu A	110	-	+	+	-
Flu B	0.09	RSV	30	-	-	+	+
RSV	0.09	SARS-CoV-2	30	+	-	-	+
RSV	0.09	Flu A	110	-	+	-	+
RSV	0.09	Flu B	30	-	-	+	+

Interference

Interfering endogenous and exogenous substances (mucin, whole blood, other potential medications and over-the-counter products) that may be present in a specimen were evaluated in the Panther Fusion SARS-CoV-2/Flu A/B/RSV assay. Clinically relevant concentrations of potentially interfering substances were added to processed clinical negative NP swab VTM/UTM matrix and tested in the absence and presence of SARS-CoV-2, Flu A, Flu B and RSV cultured virus at their respective 3X LoD concentrations. Tests were performed in triplicate. The substances and concentrations are shown in Table 6.

No impact on the performance of the Panther Fusion SARS-CoV-2/Flu A/B/RSV assay was seen for any of the substances at the concentrations tested.

Table 6: Potentially Interfering Substances

Substance Type	Substance Name	Active Ingredient(s)	Concentration[1]
Endogenous	Mucin	Purified mucin protein	60 µg/mL
	Blood (human)	N/A	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[2]	Albuterol	45 ng/mL
Nasal corticosteroids	QVAR® Beconase AQ[2]	Beclomethasone	15 ng/mL
	Dexacort[2]	Dexamethasone	12 µg/mL
	Nasacort	Triamcinolone	5% v/v
	Flonase	Fluticasone	5% v/v
	Rhinocort	Budesonide	5% v/v
	Nasonex[2]	Mometasone	0.5 ng/mL
	AEROSPAN®[2]	Flunisolide	10 µg/mL
Nasal gel	Zicam® (Allergy Relief)	Luffa opperculata, Galphimia, Glauca, Histaminum hydrochloricum, Sulfur	5% v/v
Throat lozenge	Cepacol Extra Strength	Benzocaine, Menthol	0.7 mg/mL
Anti-viral drug	Relenza®[2]	Zanamivir	3.3 mg/mL
	TamiFlu[2]	Oseltamivir	400 µg/mL
	Virazole[2]	Ribavirin	10.5 µg/mL
Antibiotic, nasal ointment	Bactroban cream[2]	Mupirocin	1.6 µg/mL
Antibiotic, systemic	Tobramycin	Tobramycin	33.1 µg/mL

[1] v/v: volume by volume

[2] Active ingredients tested

Assay Precision

Panther Fusion SARS-CoV-2/Flu A/B/RSV assay within-lab precision was evaluated with a 5-member panel consisting of virus in negative clinical NP swab VTM/UTM matrix. The 5-member panel included one negative and four dual positive panel members. The panels were tested by two operators on two runs per day, using three reagent lots on three Panther Fusion systems over twelve days.

The panel members are described in Table 7, along with a summary of the agreement with the expected results and the Ct mean and variability analysis between reagent lots, operators, instruments, between and within runs, and overall (total).

Table 7: Signal Variability of the Panther Fusion SARS-CoV-2/Flu A/B/RSV Assay by Panel Member

Panel	Description	Analyte	Agreed/N*	Agreement (%)	Mean Ct	Between Lots		Between Instrument		Between Operators		Between Days		Between Runs		Within Run		Total	
						SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
1	Neg	Internal Control	95/96	99	33.7	0.19	0.57	0.08	0.23	0.00	0.00	0.00	0.00	0.21	0.62	0.29	0.86	0.42	1.23
2	SARS-CoV-2/ Flu A Low Pos	Flu A	96/96	100	35.1	0.33	0.93	0.06	0.17	0.00	0.00	0.00	0.00	0.30	0.85	0.56	1.59	0.72	2.04
		SARS-CoV-2	96/96	100	35.9	0.00	0.00	0.13	0.36	0.00	0.00	0.00	0.00	0.00	0.00	0.60	1.67	0.61	1.71
3	Flu B/ RSV Low Pos	Flu B	96/96	100	36.0	0.14	0.40	0.09	0.25	0.00	0.00	0.00	0.00	0.00	0.00	0.36	0.99	0.39	1.09
		RSV	96/96	100	36.1	0.12	0.33	0.28	0.77	0.00	0.00	0.00	0.00	0.37	1.04	0.53	1.46	0.71	1.97
4	SARS-CoV-2/ Flu A Mod Pos	Flu A	96/96	100	33.9	0.23	0.66	0.00	0.00	0.00	0.00	0.19	0.56	0.00	0.00	0.47	1.37	0.55	1.63
		SARS-CoV-2	96/96	100	34.7	0.21	0.62	0.16	0.45	0.06	0.17	0.00	0.00	0.00	0.00	0.45	1.30	0.52	1.51
5	Flu B/ RSV Mod Pos	Flu B	96/96	100	34.7	0.15	0.44	0.00	0.00	0.00	0.00	0.00	0.00	0.06	0.18	0.28	0.80	0.32	0.93
		RSV	96/96	100	34.5	0.10	0.30	0.18	0.51	0.00	0.00	0.00	0.00	0.00	0.00	0.40	1.15	0.44	1.29

*Agreement to expected panel positivity result.
Low Pos = Low positive 2X LoD.
Mod Pos = Moderate positive 5X LoD.
Note: Variability from some factors may be numerically negative, which can occur if the variability due to those factors is very small. When this occurs, SD=0 and CV=0%.

Carryover Contamination

The carryover contamination rate of the assay was demonstrated using the Enhanced Direct Load Tube (RespDirect Collection Kit) using a checkerboard design, with panels made of pooled clinical matrix. A total of 300 negatives interspersed with 301 positive samples (spiked with Flu A to 1 x 10^4 $TCID_{50}$/mL or 90,909X LoD) were tested across 5 runs on two Panther Fusion instruments. The Panther Fusion SARS-CoV-2/Flu A/B/RSV assay had a 0% carryover rate.

Collection Device Equivalency

Equivalence between NP specimens collected into VTM/UTM and eSTM was evaluated by testing individual negative specimens and contrived positive panels prepared from paired negative clinical NP swab specimens collected from patients with symptoms of respiratory infection. Contrived panels were prepared by spiking individual donor paired NP specimens with SARS-CoV-2, Flu A, Flu B, and RSV to 2X and 5X LoD.

The results of the negative and contrived panels demonstrated similar agreement between the two collection devices (Table 8).

Table 8: Results of negative and contrived panels composed of paired individual donor NP clinical specimens, collected with each collection device spiked with SARS-CoV-2, Flu A, Flu B, and RSV

Analyte	Sample Concentration	N per Collection Device	VTM/UTM % Positive	RespDirect % Positive
None (negative sample)	0	181	0	0
SARS-CoV-2	2X LoD	50	100	98
	5X LoD	50	100	100
Flu A	2X LoD	25	100	100
	5X LoD	25	100	100
Flu B	2X LoD	25	100	100
	5X LoD	25	100	100
RSV	2X LoD	25	100	100
	5X LoD	25	100	100

Clinical Performance

The clinical performance of the Panther Fusion SARS-CoV-2/Flu A/B/RSV assay was evaluated in comparison to an FDA Emergency Use Authorization (EUA) nucleic acid amplification test (NAAT) assay and an FDA-cleared Flu/RSV NAAT assay using individual remnant clinical NP specimens in VTM/UTM collected from patients with signs and symptoms of respiratory infection. For the evaluation, a combination of negative, SARS-CoV-2 positive, Flu A positive, Flu B positive, and RSV positive specimens were tested with each assay.

The Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA) for SARS-CoV-2 was calculated in relation to the FDA EUA authorized NAAT assay as the reference result, as shown in Table 9. The assay showed positive and negative percent agreements of 98.1% and 98.5% respectively for SARS-CoV-2.

For Flu A, Flu B and RSV, the PPA and NPA were calculated in relation to the FDA-cleared Flu/RSV NAAT assay as the reference result, as shown in Table 10 for Flu A, Table 11 for Flu B and Table 12 for RSV. The assay showed positive and negative percent agreements of 100.0% and 99.6% respectively, for Flu A, 98.1% and 99.6% for Flu B and 98.1% and 100.0% for RSV.

Table 9: Clinical Performance for SARS-CoV-2

SARS-CoV-2		FDA EUA Authorized NAAT Assay		
		Positive	**Negative**	**Total**
Panther Fusion SARS/Flu A/B/RSV Assay	**Positive**	52	4	56
	Negative	1	256	257
	Total	53	260	313
Positive Agreement (95% CI)		98.1%	(90.1% - 99.7%)	
Negative Agreement (95% CI)		98.5%	(96.1% - 99.4%)	

Table 10: Clinical Performance for Flu A

Flu A		FDA-Cleared Assay		
		Positive	**Negative**	**Total**
Panther Fusion SARS/Flu A/B/RSV Assay	**Positive**	52	1	53
	Negative	0	260	260
	Total	52	261	313
Positive Agreement (95% CI)		100.0%	(93.1% - 100.0%)	
Negative Agreement (95% CI)		99.6%	(97.9% - 99.9%)	

Table 11: Clinical Performance for Flu B

Flu B		FDA-Cleared Assay		
		Positive	**Negative**	**Total**
Panther Fusion SARS/Flu A/B/RSV Assay	**Positive**	52	1	53
	Negative	1	259	260
	Total	53	260	313
Positive Agreement (95% CI)		98.1%	(90.1% - 99.7%)	
Negative Agreement (95% CI)		99.6%	(97.9% - 99.9%)	

Table 12: Clinical Performance for RSV

RSV		FDA-Cleared Assay		
		Positive	**Negative**	**Total**
Panther Fusion SARS/Flu A/B/RSV Assay	**Positive**	52	0	52
	Negative	1	260	261
	Total	53	260	313
Positive Agreement (95% CI)		98.1%	(90.1% - 99.7%)	
Negative Agreement (95% CI)		100.0%	(98.5% - 100.0%)	

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