SARS-CoV-2/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® SARS-CoV-2/Flu A/B/RSV Assay is a fully automated multiplexed real-time polymerase chain reaction (RT-PCR) *in vitro* diagnostic test intended for the qualitative detection and differentiation of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), influenza A virus (Flu A), influenza B virus (Flu B), and respiratory syncytial virus (RSV). Nucleic acids are isolated and purified from nasopharyngeal (NP) swab specimens and anterior nasal (AN) 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, Flu A, Flu B, and RSV infections in humans and is not intended to detect influenza C virus infections.

Nucleic acids from the viral organisms identified by this test are generally detectable in NP and AN swab specimens during the acute phase of infection. The detection and identification of specific viral nucleic acids from individuals exhibiting signs and symptoms of respiratory tract infection are indicative of the presence of the identified virus and aids in diagnosis if used in conjunction with other clinical and epidemiological information, and laboratory findings. The results of this test should not be used as the sole basis for diagnosis, treatment, or other patient management decisions.

Positive results do not rule out coinfection with other organisms. The organism(s) detected by the Panther Fusion SARS-CoV-2/Flu A/B/RSV Assay may not be the definite cause of disease. Negative results do not preclude SARS-CoV-2, influenza A virus, influenza B virus, or RSV infections. 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.[1,2,3]

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 flu),[4] decreasing the overall cost of care, reducing the potential for further development of antimicrobial resistance due to excessive and inappropriate use of antibiotics,[5] 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.[6]

Influenza is an acute respiratory illness caused by infection with the influenza virus, primarily types A and B.[7] Influenza A viruses are further categorized into subtypes based on the two major surface protein antigens: hemagglutinin (H) and neuraminidase (N).[8] Influenza B viruses are not categorized into subtypes.[8] 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, there are around a billion cases of seasonal influenza annually, including 3–5 million cases of severe illness, and about 290,000 to 650,000 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 to 80,000 hospitalizations and 2.1 million outpatient visits among children younger than 5 years, and 60,000 to 160,000 hospitalizations and 6,000 to 10,000 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 the outbreak in Wuhan, China, in December 2019.[10] 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.[11] On March 11, 2020, the COVID-19 outbreak was characterized as a pandemic by the World Health Organization (WHO).[12] Over 760 million cases and 6.9 million deaths have been recorded worldwide since December 2019, but the actual number is thought to be higher.[13]

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 where 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 medium (UTM) and viral transport medium (VTM) are transferred to a Specimen Lysis Tube containing specimen transport medium (STM). Alternatively, samples can be collected with the RespDirect® Collection Kit which contains enhanced specimen transport medium (eSTM). STM and eSTM lyse the cells, release target nucleic acid, and protect 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 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.

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

C. 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.

D. If infection with SARS-CoV-2 is suspected based on current clinical and epidemiological screening criteria recommended by public health authorities, specimens should be collected with appropriate infection control precautions. Refer to the CDC Interim Guidelines for Collecting and Handling of Clinical Specimens for COVID-19 Testing for more information. https://www.cdc.gov/covid/php/lab/. Viral culture should not be attempted in cases of positive results for SARS-CoV-2 and/or any similar microbial agents unless a facility with an appropriate level of laboratory biosafety (e.g., BSL 3 and BSL 3+, etc.) is available to receive and culture specimens.

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.

   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. Positive results for SARS-CoV-2 or suspected novel influenza should be reported to state, local, or federal health departments according to local reporting requirements.

G. Use only supplied or specified disposable laboratory ware.

H. 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.

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

J. 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.

K. 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.

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

M. Store assay components at the recommended storage condition. See *Reagent Storage and Handling Requirements* on *page 7*, and *Panther Fusion System Test Procedure* on *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 is compromised or if the assay cartridge foil is not intact. Contact Hologic Technical Support if either occurs.

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

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

T. 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.

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

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

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. For more information on the symbols, refer to the symbol legend on www.hologic.com/package-inserts.*

US Hazard Information

Panther Fusion Enhancer Reagent-S

Lithium Hydroxide, Monohydrate 5–10%

DANGER

H302 - Harmful if swallowed
H314 - Causes severe skin burns and eye damage
P264 - Wash face, hands and any exposed skin thoroughly after handling
P270 - Do not eat, drink or smoke when using this product
P330 - Rinse mouth
P501 - Dispose of contents/ container to an approved waste disposal plant
P260 - Do not breathe dusts or mists
P280 - Wear protective gloves/protective clothing/eye protection/face protection
P301 + P330 + P331 - IF SWALLOWED: rinse mouth. Do NOT induce vomiting
P303 + P361 + P353 - IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water/ shower
P304 + P340 - IF INHALED: Remove person to fresh air and keep 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
P321 - Specific treatment (see supplemental first aid instructions on the SDS)
P363 - Wash contaminated clothing before reuse
P405 - Store locked up
P301+P317 - IF SWALLOWED: Get medical help.
P316 - Get emergency medical help immediately.

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 (wFCR-S) and Panther Fusion Enhancer Reagent-S (FER-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 and AN swab specimens in VTM, UTM, or 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

Collect NP or AN 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. Specimens may also be collected with the RespDirect Collection Kit.

The following types of VTM/UTM were verified for use with the Panther Fusion SARS-CoV-2/Flu A/B/RSV Assay:

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

Specimen Processing

Specimen Processing with the Panther Fusion Specimen Lysis Tube

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)

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 maximum 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

Storing Specimens with the Panther Fusion Specimen Lysis Tube

1. After collection, NP and AN swab specimens in VTM/UTM can be stored at 2°C to 8°C up to 96 hours before transfer to the Panther Fusion Specimen Lysis Tube. Remaining specimen volumes can be stored at ≤–70°C. Freeze/thaw cycles should be minimized due to potential for sample degradation.
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. Freeze/thaw cycles should be minimized due to potential for sample degradation.
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 may 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.

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

1. NP and AN swab samples 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. Freeze/thaw cycles should be minimized due to potential for sample degradation.
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 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 Control tube, 5 per box Panther Fusion Negative Control tube, 5 per box	PRD-07401	2°C to 8°C
Panther Fusion Extraction Reagents-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 Module Upgrade	PRD-04173
Panther Fusion System	PRD-04172
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, conductive, liquid sensing, and disposable	901121 (10612513 Tecan)
	903031 (10612513 Tecan)
Not all products are available in all regions. Contact your representative for region-specific information	MME-04128
	MME-04134 (30180117 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 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 or nasal 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 the system 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 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*/*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: Results 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 detected.
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. The Panther Fusion SARS-CoV-2/Flu A/B/RSV Assay is a qualitative test that reports Ct values for individuals that test positive for SARS-CoV-2, Flu A, Flu B, and/or RSV. These Ct values should not be interpreted as a measure of viral levels.

C. Results for this test must be correlated with the clinical history, epidemiological data, and other data available to the clinician evaluating the patient.

D. The Panther Fusion SARS-CoV-2/Flu A/B/RSV Assay has not been validated for the testing of pooled specimens or the screening of specimens from asymptomatic individuals that do not have signs and symptoms of respiratory infection.

E. The performance of the Panther Fusion SARS-CoV-2/Flu A/B/RSV Assay has not been specifically evaluated for NP and nasal swab specimens from immunocompromised individuals.

F. The performance of the Panther Fusion SARS-CoV-2/Flu A/B/RSV Assay has not been specifically evaluated in a population known to be vaccinated against illnesses caused by any of the Panther Fusion SARS-CoV-2/Flu A/B/RSV Assay analytes (e.g. SARS-CoV-2 (COVID-19) or, influenza, etc.).

G. The performance of the Panther Fusion SARS-CoV-2/Flu A/B/RSV Assay has not been established for monitoring treatment of infection with any of the panel organisms.

H. The performance of the Panther Fusion SARS-CoV-2/Flu A/B/RSV Assay has not been established for screening of blood or blood products.

I. The Panther Fusion SARS-CoV-2/Flu A/B/RSV Assay may not be able to distinguish between existing viral strains and new variants as they emerge.

J. Positive and negative predictive values are highly dependent on prevalence. The likelihood of a negative result being false is higher during peak activity when prevalence of disease is high. The likelihood of a positive result being false is higher during periods when prevalence is moderate to low.

K. Performance characteristics of the Panther Fusion SARS-CoV-2/Flu A/B/RSV Assay have only been determined with NP and AN swab specimens.

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

M. Accurate results are dependent on adequate specimen collection, transport, storage, and processing. Failure to observe proper procedures in any one of these steps can lead to incorrect results. There is a risk of false positive or false negative values resulting from improperly collected, transported, or handled specimens.

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

O. 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 diagnosis, treatment or other patient management decisions.

P. 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.

Q. A positive result indicates the detection of nucleic acid from the relevant virus. Nucleic acid may persist *in vivo* even after the virus is no longer viable. Detection of organism target(s) does not imply that the corresponding organisms are infectious or are the causative agents for clinical symptoms.

R. Interference was observed for SARS-CoV-2 when evaluated with high concentrations of RSV B, for Flu A when evaluated with high concentrations of SARS-CoV-2 or RSV B, and for Flu B when evaluated with high concentrations of RSV B. For more information, see Table 5 Competitive Interference in the Assay Performance section of this package insert.

S. Recent administration of nasal vaccines (e.g., FluMist®) prior to collection were not evaluated; Panther Fusion SARS-CoV-2/Flu A/B/RSV Assay may detect the agents in those vaccines but may not represent infection by those viruses.

T. Zinc was not evaluated in the Interfering Substance Study.

U. Performance characteristics for influenza A were established when influenza A/H1 and A/H3 were predominant. When other influenza A viruses are emerging, performance characteristics may differ.

V. Due to the small number or absence of positive results during the prospective clinical studies, performance characteristics for influenza B and RSV were established primarily with retrospective clinical specimens.

W. The clinical performance has not been established for all circulating variants of SARS-CoV-2 but is anticipated to be reflective of the prevalent variants in circulation at the time and location of the clinical evaluation. Performance at the time of testing may vary depending on the variants circulating, including newly emerging strains of SARS-CoV-2 and their prevalence, which change over time.

Analytical 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 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), Flu A (2 strains), Flu B (2 strains), RSV A (1 strain), and RSV B (1 strain). 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, 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 $TCID_{50}$/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	-	+	-	-
A/California/07/2009	Flu A (H1N1)	0.18 $TCID_{50}$/mL	-	+	-	-

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

Description	Subtype	Concentration	SARS-CoV-2	Flu A	Flu B	RSV
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 $TCID_{50}$/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	-	-	+	-
B/Texas/81/2016	Flu B (Yamagata)	2^{1} $TCID_{50}$/mL	-	-	+	-

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

Description	Subtype	Concentration	SARS-CoV-2	Flu A	Flu B	RSV
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 A/Peru/LIM-UPCH-4624/2024	RSVA	0.6[4] $TCID_{50}$/mL	-	-	-	+
RSV A/USA/LA-EVTL23380/2024	RSVA	0.6[5] $TCID_{50}$/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.

[4]In silico analysis identified a double mismatch in the forward primer. This sequence represents a minor variant of RSV. An in vitro transcribed RNA corresponding to this variant was tested.

[5]In silico analysis identified a triple mismatch in the reverse primer. This sequence represents a minor variant of RSV. An in vitro transcribed RNA corresponding to this variant was tested.

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 were 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 on the Panther Fusion SARS-CoV-2/Flu A/B/RSV Assay at the indicated 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[3]
HPIV-1	$1x10^5$ $TCID_{50}$/mL	*Mycoplasma pneumoniae*	$1x10^9$ rRNA copies/mL[3]
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.

[3]$1x10^9$ rRNA copies/mL is equivalent to ~$2x10^5$ CFU/mL.

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 virus was tested at 3X LoD, against a higher concentration of competing virus (up to 1.0E+4 $TCID_{50}$/mL). If less than 100% positivity was observed for the low concentration virus (Target 1), the high concentration virus (Target 2) was diluted until a concentration was reached where 100% positivity of Target 1 was achieved. The highest concentration of competing virus (Target 2) that maintained 100% positivity for the low concentration virus (Target 1) is shown in Table 5.

Table 5: Competitive Interference

Target 1		Target 2		SARS-CoV-2 % detected	Flu A % detected	Flu B % detected	RSV % detected
Virus	**3X LoD ($TCID_{50}$/mL)**	**Virus**	**High Concentration ($TCID_{50}$/mL)**				
SARS-CoV-2	9.0E-2	Flu A	1.0E+4	**100%**	**100%**	0%	0%
		Flu B	1.0E+4	**100%**	0%	**100%**	0%
		RSV A	1.0E+4	**100%**	0%	0%	**100%**
		RSV B[1]	3.0E+1	**100%**	0%	0%	**100%**
Flu A	3.3E-1	SARS-CoV-2[2]	1.0E+2	**100%**	**100%**	0%	0%
		Flu B	1.0E+4	0%	**100%**	**100%**	0%
		RSV A	1.0E+4	0%	**100%**	0%	**100%**
		RSV B[1]	3.0E+1	0%	**100%**	0%	**100%**
Flu B	9.0E-2	SARS-CoV-2	1.0E+4	**100%**	0%	**100%**	0%
		Flu A	1.0E+4	0%	**100%**	**100%**	0%
		RSV A	1.0E+4	0%	0%	**100%**	**100%**
		RSV B[3]	1.0E+3	0%	0%	**100%**	**100%**
RSV A	6.0E-2	SARS-CoV-2	1.0E+4	**100%**	0%	0%	**100%**
		Flu A	1.0E+4	0%	**100%**	0%	**100%**
		Flu B	1.0E+4	0%	0%	**100%**	**100%**
RSV B	9.0E-2	SARS-CoV-2	1.0E+4	**100%**	0%	0%	**100%**
		Flu A	1.0E+4	0%	**100%**	0%	**100%**
		Flu B	1.0E+4	0%	0%	**100%**	**100%**

[1]Less than 100% positive results observed for Target 1 (3X LoD) with Target 2 at ≥1.0E+2 $TCID_{50}$/mL.

[2]Less than 100% positive results observed for Target 1 (3X LoD) with Target 2 at ≥1.0E+3 $TCID_{50}$/mL.

[3]Less than 100% positive results observed for Target 1 (3X LoD) with Target 2 at 1.0E+4 $TCID_{50}$/mL.

Interference

Interfering endogenous and exogenous substances (mucin, whole blood, 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 ng/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, days, 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.
**One SARS-CoV-2 false positive result was obtained for the negative panel member.
Low Pos = Both targets are at 2X LoD.
Mod Pos = Both targets are at 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 swab 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 swab 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 swab 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

Prospective Clinical Study — NP Swab Specimens in VTM/UTM

This study was performed to demonstrate clinical performance characteristics of the Panther Fusion SARS-CoV-2/Flu A/B/RSV Assay for NP swab specimens in VTM or UTM. A prospective multicenter study was conducted using remnant NP swab specimens from male and female individuals of all ages exhibiting signs and/or symptoms of respiratory infection consistent with COVID-19, influenza, or RSV. Five participating US pediatric/adolescent, private and/or university hospitals prospectively provided remnant NP swab specimens collected during portions of the 2020-2021 and 2021-2022 respiratory infection seasons. These specimens were tested at 3 US sites with the Panther Fusion SARS-CoV-2/Flu A/B/RSV Assay.

The Panther Fusion SARS-CoV-2/Flu A/B/RSV Assay was evaluated for SARS-CoV-2 performance by comparing the candidate device testing results to a composite comparator algorithm (CCA) consisting of two highly sensitive US FDA EUA SARS-CoV-2 molecular tests and a validated PCR followed by bi-directional sequencing (PCR/BDS) assay. A final CCA result was assigned when two of the three composite comparator assays were in concordance. The comparator method utilized to establish performance for the Flu A, Flu B, and RSV targets was a US FDA-cleared molecular Flu A/B/RSV Assay.

Of the 1949 remnant specimens enrolled during the study, 1056 were collected between January 2022 and March 2022, while the remaining 893 were collected between November 2020 and March 2021. Forty-five (45) of these specimens were withdrawn; mishandling during transport was the most common reason for withdrawal. A total of 1905 NP swab specimens were tested in valid Panther Fusion SARS-CoV-2/Flu A/B/RSV Assay runs, including 12 (0.6%) with initial invalid results. Upon retest, 8 of the 12 specimens yielded valid results and 4 yielded invalid results, for a total of 1901 (99.8%) specimens with final valid results. One specimen with valid Panther Fusion assay results was withdrawn following testing with the Panther Fusion assay upon determination that the sample had not been stored according to the Panther Fusion assay package insert instructions. The final data set consisted of 1900 evaluable NP swab specimens; not all were evaluable for all analytes. For the SARS-CoV-2 target, 13 of these 1900 NP swab specimens were excluded from analysis due to unknown infection status obtained from the CCA tests. A total of 1887 prospective samples were evaluated for SARS-CoV-2, including 790 (41.9%) tested fresh and 1097 (58.1%) tested after freezing. For the Flu A, Flu B, and RSV targets, 63 specimens were excluded from analysis due to obtaining an invalid result from the comparator test. A total of 1837 valid prospective specimens were evaluated for Flu A, Flu B, and RSV, including 798 (43.4%) tested fresh and 1039 (56.6%) tested after freezing. Demographic information for the 1900 evaluable prospective specimens is provided in Table 9.

Table 9: Summary of Subject Demographics for Evaluable Prospectively Collected NP Swab Specimens

		N (%)
Total		1900 (100)
Sex	Female	1049 (55.2)
	Male	850 (44.7)
	Unknown	1 (0.1)
Age Group	< 5 years	388 (20.4)
	5 to 21 years	435 (22.9)
	22 to 40 years	372 (19.6)
	41 to 60 years	326 (17.2)
	> 60 years	379 (19.9)

The performance of the Panther Fusion SARS-CoV-2/Flu A/B/RSV Assay with prospective NP swab specimens is summarized in Table 10. Positive Percent Agreement (PPA) was calculated as 100% × (TP / (TP + FN)). True positive (TP) indicates that both the Panther Fusion SARS-CoV-2/Flu A/B/RSV Assay and the comparator method had a positive result for the specific analyte, and false negative (FN) indicates that the Panther Fusion SARS-CoV-2/Flu A/B/RSV was negative while the comparator method result was positive. Negative Percent Agreement (NPA) was calculated as 100% × (TN / (TN + FP)). True negative (TN) indicates that both the Panther Fusion SARS-CoV-2/Flu A/B/RSV Assay and the comparator method had negative results, and false positive (FP) indicates that the Panther Fusion SARS-CoV-2/Flu A/B/RSV Assay was positive while the comparator method result was negative. Specimens that obtained discordant results underwent additional testing with a US FDA EUA SARS-CoV-2/Flu A/B/RSV molecular test, volume permitting.

Table 10: *Panther Fusion SARS-CoV-2/Flu A/B/RSV Assay Performance with Prospectively Collected NP Swab Specimens*

Analyte		Positive Percent Agreement			Negative Percent Agreement		
		TP/ (TP+FN)	%	95% CI	TN/ (TN+FP)	%	95% CI
SARS-CoV-2	Fresh	60/64	93.8	85.0–97.5	716/726	98.6	97.5–99.3
	Frozen	318/326	97.5	95.2–98.8	758/771	98.3	97.1–99.0
	Overall	**378/390[1]**	**96.9**	**94.7–98.2**	**1474/1497[2]**	**98.5**	**97.7–99.0**
Flu A	Fresh	98/100	98.0	93.0–99.5	696/698	99.7	99.0–99.9
	Frozen	23/23	100	85.7–100	1013/1016	99.7	99.1–99.9
	Overall	**121/123[3]**	**98.4**	**94.3–99.6**	**1709/1714[4]**	**99.7**	**99.3–99.9**
Flu B	Fresh	0/0	NC	NC	796/798	99.7	99.1–99.9
	Frozen	0/0	NC	NC	1037/1039	99.8	99.3–99.9
	Overall	**0/0**	**NC**	**NC**	**1833/1837[5]**	**99.8**	**99.4–99.9**
RSV	Fresh	11/13	84.6	57.8–95.7	785/785	100	99.5–100
	Frozen	0/0	NC	NC	1039/1039	100	99.6–100
	Overall	**11/13[6]**	**84.6**	**57.8–95.7**	**1824/1824**	**100**	**99.8–100**

CI = Score confidence interval; FN = false negative; FP = false positive; NC = Not calculable; TN = true negative; TP = true positive

[1]Five (5) specimens with false negative SARS-CoV-2 results had sufficient volume remaining for discordant testing. All five specimens were positive for SARS-CoV-2 by a US FDA EUA SARS-CoV-2/Flu A/B/RSV molecular test.

[2]Eleven (11) specimens with false positive SARS-CoV-2 results had sufficient volume remaining for discordant testing. Seven of the specimens were negative for SARS-CoV-2 by a US. FDA EUA SARS-CoV-2/Flu A/B/RSV molecular test.

[3]No specimens with false negative Flu A results had sufficient volume remaining for discordant testing.

[4]Two (2) specimens with false positive Flu A results had sufficient volume remaining for discordant testing. Both specimens were negative for Flu A by a US FDA EUA SARS-CoV-2/Flu A/B/RSV molecular test.

[5]One (1) specimen with false positive Flu B result had sufficient volume remaining for discordant testing. This specimen was negative for Flu B by a US FDA EUA SARS-CoV-2/Flu A/B/RSV molecular test.

[6]No specimens with false negative RSV results had sufficient volume remaining for discordant testing. The specimens had Ct values of 41.3 and 43.5 with a comparator molecular assay.

Five co-infections were detected by the Panther Fusion SARS-CoV-2/Flu A/B/RSV Assay: 4 SARS-CoV-2 positive/Flu A positive and 1 SARS-CoV-2 positive/Flu B positive; 3 of the 4 SARS-CoV-2 / Flu A coinfections were also detected by comparator testing.

Retrospective Clinical Study — NP Swab Specimens in VTM/UTM

Flu B and RSV were of low prevalence during the prospective clinical study and were therefore not encountered in large enough numbers to adequately demonstrate assay performance for NP swab specimens. To supplement the results of the prospective specimen population,

retrospective specimen testing was performed. This study included 95 preselected archived retrospective NP swab specimens in VTM or UTM that were collected between December 2019 and March 2020. Specimens were selected for enrollment in the study based solely on the historic qualitative positive result. In addition to evaluating Flu B and RSV positive specimens, Flu A positive specimens were included in the study. All known positive specimens underwent confirmatory testing using a US FDA-cleared molecular Flu A/B/RSV Assay. The 95 specimens were distributed uniformly across all three clinical testing sites. Demographic information for the 95 evaluable retrospective specimens is provided in Table 11.

Table 11: Summary of Subject Demographics for Evaluable Retrospective for NP Swab Specimens

		N (%)
Total		95 (100)
Sex	Female	51 (53.7)
	Male	44 (46.3)
Age Group	< 5 years	16 (16.8)
	5 to 21 years	12 (12.6)
	22 to 40 years	15 (15.8)
	41 to 60 years	16 (16.8)
	> 60 years	36 (37.9)

The PPA and NPA of the Panther Fusion SARS-CoV-2/Flu A/B/RSV Assay with retrospective NP swab specimens were calculated against the results from confirmatory testing. Specimens that obtained discordant results underwent additional testing with a US FDA EUA SARS-CoV-2/Flu A/B/RSV molecular test, volume permitting. Table 12 shows the PPA for specimens that were confirmed positive for at least one target analyte.

Table 12: Panther Fusion SARS-CoV-2/ Flu A/B/RSV Assay Clinical Performance with Confirmed Positive Retrospective NP Swab Specimens

Analyte	Positive Percent Agreement		
	TP/ (TP+FN)	%	95% CI
Flu A	27/29[1]	93.1	78.0–98.1
Flu B	21/22[2]	95.5	78.2–99.2
RSV	47/47	100	92.4–100

CI = Score confidence interval; FN = false negative; TP = true positive

[1]One (1) specimen with a false negative Flu A result tested positive for Flu A with a US FDA EUA SARS-CoV-2/ Flu A/B/RSV molecular test, while one (1) specimen with a false negative Flu A result tested negative for Flu A with a US FDA EUA SARS-CoV-2/Flu A/B/RSV molecular test.

[2]One (1) specimen with a false negative Flu B result tested positive for Flu B with a US FDA EUA SARS-CoV-2/ Flu A/B/RSV molecular test.

Table 13 shows the NPA for specimens that had a negative result on the comparator assay although they were confirmed positive for one of the other target analytes.

Table 13: Panther Fusion SARS-CoV-2/ Flu A/B/RSV Assay Clinical Performance with Confirmed Negative Retrospective NP Swab Specimens

Analyte	Negative Percent Agreement		
	TN/ (TN+FP)	%	95% CI
Flu A	66/66	100	94.5–100
Flu B	73/73	100	95.0–100
RSV	48/48	100	92.6–100

CI = Score confidence interval; FP = false positive; TN = true negative

Two co-infections were detected by the Panther Fusion SARS-CoV-2/Flu A/B/RSV Assay were also detected by comparator testing: 1 Flu A positive/ Flu B positive and 1 Flu A positive/RSV positive.

Prospective Clinical Study — Anterior Nasal Swab Specimens in VTM/UTM

A multicenter study was conducted using AN swab specimens prospectively collected from male and female individuals of all ages exhibiting signs and/or symptoms of respiratory infection consistent with COVID-19, influenza, or RSV. Study participants were enrolled at nine participating US pediatric/adolescent, private and/or university hospitals during the 2022–2023 respiratory infection season. Two AN specimens were collected from each enrolled individual and stored in VTM/UTM, one specimen alternated between collection by the HCP or by the individual (under HCP supervision). These specimens were tested at 3 US sites with the Panther Fusion SARS-CoV-2/Flu A/B/RSV Assay.

The Panther Fusion SARS-CoV-2/Flu A/B/RSV Assay was evaluated for SARS-CoV-2 performance by comparing the candidate device testing results to a composite comparator algorithm (CCA) consisting of up to three highly sensitive US FDA EUA SARS-CoV-2 molecular tests. A final CCA result was assigned when two of the three composite comparator assays were

in concordance. The comparator method utilized to establish performance for the Flu A, Flu B, and RSV targets was a US FDA-cleared molecular Flu A/B/RSV Assay. All specimens that obtained discordant results underwent additional testing with an alternate molecular test.

A total of 1268 individuals were enrolled in the study between October 2022 and May 2023. Two (2) subjects were withdrawn. A total of 1230 nasal swab specimens in VTM/UTM were tested in valid Panther Fusion SARS-CoV-2/Flu A/B/RSV Assay runs, including 10 (0.8%) with initial invalid results. Upon retest, 6 of the 10 specimens yielded valid results and 4 yielded invalid results, for a total of 1226 (99.7%) specimens with final valid results.

The final data set consisted of 1189 evaluable nasal swab specimens in VTM/UTM; not all were evaluable for all analytes. Non-evaluable specimens were excluded from the performance analyses for the following reasons: withdrawn Panther Fusion SARS-CoV-2/Flu A/B/RSV Assay specimen (40 VTM/UTM), invalid or missing Panther Fusion SARS-CoV-2/Flu A/B/RSV Assay result (4 VTM/UTM), unknown comparator method result (33 VTM/UTM each for SARS-CoV-2, Flu A, Flu B, and RSV). All 1189 prospective AN swab specimens were tested fresh with the Panther Fusion SARS-CoV-2/Flu A/B/RSV Assay. Demographic information for the evaluable prospective nasal swab specimens is provided in Table 14.

Table 14: Summary of Subject Demographics for Evaluable Prospectively Collected AN Swab Specimens in VTM/UTM

		N (%)
Total		1189 (100)
Sex	Female	715 (60.1)
	Male	474 (39.9)
Age (years)	< 5 years	49 (4.1)
	5 to 21 years	162 (13.6)
	22 to 40 years	419 (35.2)
	41 to 60 years	362 (30.4)
	> 60 years	197 (16.6)

The performance of the Panther Fusion SARS-CoV-2/Flu A/B/RSV Assay with prospective AN swab specimens is summarized in Table 15. Both PPA and NPA were calculated against the target-specific comparator method using the same calculation method described above for prospective NP swab specimens.

Table 15: Panther Fusion SARS-CoV-2/Flu A/B/RSV Assay Performance with Prospectively Collected AN Swab Specimens in VTM/UTM

Analyte	Positive Percent Agreement			Negative Percent Agreement		
	TP/(TP+FN)	%	95% CI	TN/(TN+FP)	%	95% CI
SARS-CoV-2	146/154[1]	94.8	90.1–97.3	1023/1035[2]	98.8	98.0–99.3
Flu A	45/49[3]	91.8	80.8–96.8	1135/1140[4]	99.6	99.0–99.8
Flu B	2/3[5]	66.7	20.8–93.9	1183/1186[6]	99.7	99.3–99.9
RSV	17/18[7]	94.4	74.2–99.0	1169/1171[8]	99.8	99.4–100

CI = Score confidence interval; FN = false negative; FP = false positive; TP = true positive; TN = true negative

[1]Three (3) of the 8 specimens with a false negative SARS-CoV-2 results were negative for SARS-CoV-2 by a US FDA EUA SARS-CoV-2/Flu A/B/RSV molecular test and 4 of the 8 specimens were positive for SARS-CoV-2; 1 specimen was not retested due to insufficient volume. When retested by a second US FDA EUA SARS-CoV-2/Flu A/B/RSV molecular test, 5 of the 8 specimens were negative and 3 were positive for SARS-CoV-2.

[2]One (1) of the 12 specimens with a false positive SARS-CoV-2 result was positive for SARS-CoV-2 by a US FDA EUA SARS-CoV-2/Flu A/B/RSV molecular test and 11 of the 12 specimens were negative for SARS-CoV-2. When retested by a second US FDA EUA SARS-CoV-2/Flu A/B/RSV molecular test, 1 of the 12 specimens was positive and 10 were negative for SARS-CoV-2; 1 specimen was not retested due to insufficient volume.

[3]One (1) specimen with a false negative Flu A result tested negative for Flu A by a US FDA EUA SARS-CoV-2/Flu A/B/RSV molecular test; the other 3 specimens were positive for Flu A.

[4]Three (3) specimens with a false positive Flu A result tested positive for Flu A by a US FDA EUA SARS-CoV-2/Flu A/B/RSV molecular test; the other 2 specimens were negative for Flu A.

[5]The specimen with a false negative Flu B result tested negative for Flu B by a US FDA EUA SARS-CoV-2/Flu A/B/RSV molecular test.

[6]All 3 specimens with a false positive Flu B result tested negative for Flu B by a US FDA EUA SARS-CoV-2/Flu A/B/RSV molecular test.

[7]The specimen with a false negative RSV result tested positive for RSV by a US FDA EUA SARS-CoV-2/Flu A/B/RSV molecular test.

[8]Both specimens with a false positive RSV result tested positive for RSV by a US FDA EUA SARS-CoV-2/Flu A/B/RSV molecular test.

Three co-infections were detected by the Panther Fusion SARS-CoV-2/Flu A/B/RSV Assay in AN swab specimens in VTM/UTM: 1 SARS-CoV-2 positive/Flu A positive, 1 SARS-CoV-2 positive/RSV positive, and 1 Flu A positive/RSV positive; only the SARS-CoV-2 positive/RSV positive coinfection was detected by comparator testing.

Retrospective Clinical Study — Anterior Nasal Swab Specimens in VTM/UTM

Flu B and RSV were of lower prevalence during the prospective clinical study and were therefore not encountered in large enough numbers to adequately demonstrate assay performance in AN swab specimens in VTM/UTM. Testing of 175 preselected retrospective specimens was performed to supplement the results of the prospective specimen population. In addition to evaluating Flu B and RSV positive specimens, Flu A positive specimens were included in the study. All known positive specimens underwent confirmatory testing using a US FDA-cleared molecular Flu A/B/RSV Assay. Two (2) specimens were not included in the performance analyses because of a missing comparator results. Demographic information for the 173 evaluable retrospective specimens is provided in Table 16.

Table 16: Summary of Subject Demographics for Evaluable Retrospective AN Swab Specimens in VTM/UTM

		N (%)
Total		173 (100)
Sex	Female	94 (54.3)
	Male	79 (45.7)
Age (years)	< 5 years	63 (36.4)
	5 to 21 years	60 (34.7)
	22 to 40 years	26 (15.0)
	41 to 60 years	15 (8.7)
	> 60 years	9 (5.2)

The PPA and NPA of the Panther Fusion SARS-CoV-2/Flu A/B/RSV Assay with retrospective specimens were calculated against the results from confirmatory testing. All specimens that obtained discordant results underwent additional testing with an alternate molecular test. Table 17 shows the PPA for specimens that were confirmed positive for at least one target analyte.

Table 17: Panther Fusion SARS-CoV-2/Flu A/B/RSV Assay Clinical Performance with Confirmed Positive Retrospective AN Swab Specimens in VTM/UTM

Analyte	Positive Percent Agreement		
	TP/(TP+FN)	%	95% CI
Flu A	47/48[1]	97.9	89.1–99.6
Flu B	69/71[2]	97.2	90.3–99.2
RSV	49/50[3]	98.0	89.5–99.6

CI = Score confidence interval; FN = false negative; TP = true positive

[1]The specimen with a false negative Flu A result tested positive for Flu A by a US FDA EUA SARS-CoV-2/Flu A/B/RSV molecular test.

[2]Both specimens with a false negative Flu B result tested positive for Flu B by a US FDA EUA SARS-CoV-2/Flu A/B/RSV molecular test.

[3]The specimen with a false negative RSV result tested negative for RSV by a US FDA EUA SARS-CoV-2/Flu A/B/RSV molecular test.

Table 18 shows the NPA for specimens that had a negative result on the comparator assay although they were confirmed positive for one of the other target analytes.

Table 18: Panther Fusion SARS-CoV-2/Flu A/B/RSV Assay Clinical Performance with Confirmed Negative Retrospective AN Swab Specimens in VTM/UTM

Analyte	Negative Percent Agreement		
	TN/(TN+FP)	%	95% CI
Flu A	123/125[1]	98.4	94.4–99.6
Flu B	102/102	100	96.4–100
RSV	122/123[2]	99.2	95.5–99.9

CI = Score confidence interval; FP = false positive; TN = true negative

[1]One (1) specimen with false positive Flu A results tested positive for Flu A by a US FDA EUA SARS-CoV-2/Flu A/B/RSV molecular test; the other specimen was negative for Flu A.

[2]The specimen with a false positive RSV result tested negative for RSV by a US FDA EUA SARS-CoV-2/Flu A/B/RSV molecular test.

One SARS-CoV-2 positive/RSV positive co-infection and three Flu A positive/RSV positive co-infections were detected by the Panther Fusion SARS-CoV-2/Flu A/B/RSV Assay. One co-infection (Flu A positive/RSV positive) was also detected by comparator testing.

Prospective Clinical Studies – Anterior Nasal Swab Specimens in RespDirect eSTM

A multicenter study was conducted using AN swab specimens prospectively collected from male and female individuals of all ages exhibiting signs and/or symptoms of respiratory infection consistent with COVID-19, influenza, or RSV.

Individuals with unknown standard of care molecular test results for SARS-CoV-2, Flu A, Flu B, and/or RSV were prospectively enrolled at nine participating US clinical facilities during the 2022-2023 respiratory infection season. Two AN swab specimens were collected from each enrolled individual: one specimen always collected by a healthcare professional (HCP) using a synthetic flocked swab and stored in VTM/UTM, and one specimen alternated between collection by the HCP or by the individual (under HCP supervision) using the RespDirect flocked swab and stored in RespDirect eSTM. All Panther Fusion SARS-CoV-2/Flu A/B/RSV Assay testing was performed at three US sites. Specimens from this study were included in assay performance assessments for SARS-CoV-2, Flu A, Flu B, and RSV.

The Panther Fusion SARS-CoV-2/Flu A/B/RSV Assay was evaluated for SARS-CoV-2 performance in the prospective study by comparing the candidate device testing results to a composite comparator algorithm (CCA) consisting of up to three highly sensitive US FDA EUA SARS-CoV-2 molecular test results from AN swab specimens stored in VTM/UTM. A final CCA result was assigned when two of the three composite comparator assays were in concordance. The comparator method utilized to establish performance for the Flu A, Flu B, and RSV targets for the prospective study was a US FDA-cleared molecular Flu A/B/RSV Assay. PPA and NPA for the prospective study were calculated against the target-specific comparator result using the same calculation described above for prospective NP swab specimens.

There were 1033 individuals enrolled in the prospective study. Four individuals were withdrawn, and 10 individuals were not evaluable for at least 1 target: one individual had their Panther Fusion SARS-CoV-2/Flu A/B/RSV specimen withdrawn, seven did not have a valid final Panther Fusion SARS-CoV-2/Flu A/B/RSV Assay result, and two had unknown CCA results.

A total of 1032 nasal swab specimens from the prospective study were tested in valid Panther Fusion SARS-CoV-2/Flu A/B/RSV Assay runs, including 11 (1.1%) with initial invalid results. Upon retest, four specimens yielded valid results and seven yielded invalid results, for a total of 1025 (99.3%) specimens with final valid results.

The final data set consisted of 1021 evaluable AN swab specimens in the prospective study, of which 1011 were tested fresh (Category I specimens) with the Panther Fusion SARS-CoV-2/Flu A/B/RSV Assay and 10 were tested after freezing (Category II specimens); not all were evaluable for all analytes. Demographic information for the evaluable nasal swab specimens for the prospective study is provided in Table 19.

Table 19: Summary of Subject Demographics for Evaluable Prospectively Collected AN Swab Specimens in RespDirect eSTM

		Overall N (%)
Total		1021 (100)
Sex	Male	427 (41.8)
	Female	594 (58.2)
Age (years)	<5 years	18 (1.8)
	5–21 years	120 (11.8)
	22–40 years	386 (37.8)
	41–60 years	319 (31.2)
	>60 years	178 (17.4)

The performance of the Panther Fusion SARS-CoV-2/Flu A/B/RSV Assay with AN swab specimens in RespDirect eSTM for the prospective study is summarized in Table 20.

Table 20: Panther Fusion SARS-CoV-2/Flu A/B/RSV Assay Performance with Prospectively Collected AN Swab Specimens in RespDirect eSTM

Analyte	Specimen Type	Positive Percent Agreement			Negative Percent Agreement		
		TP/(TP+FN)	%	95% CI	TN/(TN+FP)	%	95% CI
SARS-CoV-2	Fresh	107/108	99.1	94.9-99.8	892/901	99.0	98.1-99.5
	Frozen	2/2	100	34.2-100	8/8	100	67.6-100
	Overall	**109/110**	**99.1**	**95.0-99.8**	**900/909**	**99.0**	**98.1-99.5**
Flu A	Fresh	11/11	100	74.1-100	999/1000	99.9	99.4-100
	Frozen	0/0	NC	NC	10/10	100	72.2-100
	Overall	**11/11**	**100**	**74.1-100**	**1009/1010**	**99.9**	**99.4-100**
Flu B	Fresh	5/6	83.3	43.6-97.0	1003/1005	99.8	99.3-99.9
	Frozen	0/0	NC	NC	10/10	100	72.2-100
	Overall	**5/6**	**83.3**	**43.6-97.0**	**1013/1015**	**99.8**	**99.3-99.9**
RSV	Fresh	1/1	100	20.7-100	1009/1010	99.9	99.4-100
	Frozen	0/0	NC	NC	10/10	100	72.2-100
	Overall	**1/1**	**100**	**20.7-100**	**1019/1020**	**99.9**	**99.4-100**

FN = false negative; FP = false positive; NC = not calculable; TP = true positive; TN = true negative

One co-infection was detected by the Panther Fusion SARS-CoV-2/Flu A/B/RSV Assay in AN swab specimens in RespDirect eSTM in the prospective study: one SARS-CoV-2 positive/Flu B positive. The sample was negative for all analytes by the comparator methods.

Supplemental Clinical Data for Low Prevalence Analytes (Category III Specimens) - Anterior Nasal Swab Specimens in RespDirect eSTM

Flu A, Flu B and RSV were of lower prevalence and were not encountered in sufficiently large numbers during the prospective clinical study to adequately demonstrate assay performance with AN swab specimens collected with the RespDirect Collection Kit in eSTM. To supplement the results of the prospective clinical study, an enrichment phase of the study was initiated which enrolled only symptomatic individuals with recent positive standard of care PCR-based results for Flu A, Flu B, and/or RSV. Subjects were enrolled at six participating US clinical facilities during the 2023-2024 respiratory infection season. Two AN swab specimens were collected from each enrolled individual: one specimen always collected by a healthcare professional (HCP) using a synthetic flocked swab and stored in VTM/UTM and one specimen alternated between collection by the HCP or by the individual (under HCP supervision) using the RespDirect flocked swab and stored in RespDirect eSTM. All enrichment study specimens were frozen prior to testing with the Panther Fusion SARS-CoV-2/Flu A/B/RSV Assay at one US site. Specimens from this study were included in assay performance assessments for Flu A, Flu B, and RSV only.

The comparator method utilized to establish performance for the Flu A, Flu B, and RSV targets for the enrichment study was a US FDA-cleared molecular Flu A/B/RSV Assay. PPA and NPA for the enrichment study were calculated against the target-specific comparator result using the same calculation described above for prospective NP swab specimens.There were 210 individuals enrolled in the enrichment study. Six individuals were withdrawn.

A total of 205 nasal swab specimens from the enrichment study were tested in valid Panther Fusion SARS-CoV-2/Flu A/B/RSV Assay runs. Two (2) of these specimens were invalid by the

candidate device during testing, for an initial invalid rate of 1.0% (2/205). Upon retesting, both specimens obtained valid results for a total of 205/205 (100%) specimens with final valid results.

Demographic information for the 204 evaluable nasal swab specimens for the enrichment study is provided in Table 21.

Table 21: Summary of Subject Demographics for Evaluable AN Nasal Swab Specimens in RespDirect eSTM in the Enrichment Study

		Overall N (%)
Total		204 (100)
Sex	Male	85 (41.7)
	Female	119 (58.3)
Age (years)	<5 years	43 (21.2)
	5–21 years	54 (26.5)
	22–40 years	34 (16.7)
	41–60 years	37 (18.1)
	>60 years	36 (17.6)

Table 22 shows the PPA for enrichment study specimens that were confirmed positive for at least one target analyte.

Table 22: Panther Fusion SARS-CoV-2/Flu A/B/RSV Assay Performance with Confirmed Positive AN Swab Specimens in RespDirect eSTM

Analyte	**Positive Percent Agreement**		
	TP/ (TP+FN)	**%**	**95% CI**
Flu A	69/71	97.2	90.3–99.2
Flu B	44/45	97.8	88.4–99.6
RSV	60/61	98.4	91.3–99.7

FN = false negative; TP = true positive;

Table 23 shows the NPA for enrichment study specimens that had a negative result on the comparator assay although they were confirmed positive for one of the other target analytes.

Table 23: Panther Fusion SARS-CoV-2/Flu A/B/RSV Assay Performance with Confirmed Negative AN Swab Specimens in RespDirect eSTM

Analyte	Negative Percent Agreement[1]		
	TN/ (TN+FP)	%	95% CI
Flu A	124/133	93.2	87.6–96.4
Flu B	158/159	99.4	96.5–99.9
RSV	137/143	95.8	91.1–98.1

FN = false negative; TP = true positive

[1]All samples enrolled in the enrichment study were SOC positive for Flu A, Flu B, and/or RSV. NPA for the enrichment study was calculated using results from all evaluable samples with a negative comparator result for the analyte of interest.

Seven co-infections were detected by the Panther Fusion SARS-CoV-2/Flu A/B/RSV Assay in AN swab specimens in RespDirect eSTM in the enrichment study: one SARS-CoV-2 positive/ RSV positive, and four Flu A positive/RSV positive, and one Flu B positive/RSV positive. Two Flu A positive/RSV positive and one Flu B positive/RSV positive co-infections were also detected by comparator testing. The U.S. FDA cleared molecular assay used for the comparator for Flu A, Flu B and RSV does not detect SARS-CoV-2; therefore, co-infections detected by the candidate device that contained SARS-CoV-2 could not be confirmed.

Reproducibility

Panther Fusion SARS-CoV-2/Flu A/B/RSV Assay reproducibility was evaluated at three US sites using one negative and four dual positive 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 pooled negative clinical NP swab specimens in VTM/UTM processed into STM (i.e., negative matrix). Positive panel members were created by spiking 1–2X LoD (low–positive) or 3–5X LoD (moderate–positive) concentrations of the target analyte into the negative clinical matrix.

The agreement with expected results was 100% for all panel member components for SARS-CoV-2, Flu A, Flu B, and RSV (Table 24) except the following: 98.9% in both the negative panel member and in the low positive Flu A panel member component.

Table 24: Agreement of Panther Fusion SARS-CoV-2/Flu A/B/RSV Assay Results with Expected Results

Panel	Description	Analyte	Agreement with Expected Results	
			N	(%) 95% CI
1	Neg	Internal Control	89/90[1]	98.9 (94.0–99.8)
2	SARS-CoV-2/Flu A Low Pos	Flu A	89/90	98.9 (94.0–99.8)
		SARS-CoV-2	90/90	100 (95.9–100)
3	Flu B/RSV Low Pos	Flu B	90/90	100 (95.9–100)
		RSV	90/90	100 (95.9–100)
4	SARS-CoV-2/Flu A Mod Pos	Flu A	90/90	100 (95.9–100)
		SARS-CoV-2	90/90	100 (95.9–100)
5	Flu B/RSV Mod Pos	Flu B	90/90	100 (95.9–100)
		RSV Pos	90/90	100 (95.9–100)

[1]One false positive Flu B result was obtained for a negative panel member.
CI = Score confidence interval; Mod = moderate; Neg = negative; Pos = positive.
Low Pos = Both targets are at 1–2X LoD.
Mod Pos = Both targets are at 3–5X LoD.

The total SARS-CoV-2, Flu A, Flu B, and RSV signal variability measured as %CV was ≤1.82% (SD 0.30 to 0.65) for all moderate positive panel components and for low positive panel components for SARS-CoV-2, Flu B, and RSV (Table 25). The %CV and SD for the Flu A low positive panel component were 10.92% and 3.77, respectively, due to the false negative result for one replicate. For the sources of variation except the 'within run' factor, %CV values were ≤0.62% for all panel member components.

Table 25: Signal Variability of the Panther Fusion SARS-CoV-2/Flu A/B/RSV Assay by Panel Member

					Between Sites		Between Operators/ Runs[1]		Between Days		Within Runs		Total	
Panel	**Description**	**Analyte**	**N**	**Mean Ct**	**SD**	**CV (%)**	**SD**	**CV (%)**	**SD**	**CV (%)**	**SD**	**CV (%)**	**SD**	**CV (%)**
2	SARS-CoV-2/Flu A Low Pos	Flu A	90	34.55	0.57	1.66	0.62	1.81	0.00	0.00	3.68	10.64	3.77	10.92
		SARS-CoV-2	90	35.53	0.24	0.68	0.18	0.50	0.19	0.52	0.49	1.38	0.60	1.70
3	Flu B/RSV Low Pos	Flu B	90	35.80	0.12	0.35	0.00	0.00	0.22	0.60	0.39	1.10	0.47	1.30
		RSV	90	35.78	0.07	0.20	0.23	0.65	0.14	0.39	0.59	1.64	0.65	1.82
4	SARS-CoV-2/Flu A Mod Pos	Flu A	90	33.55	0.09	0.27	0.03	0.10	0.17	0.49	0.48	1.42	0.51	1.53
		SARS-CoV-2	90	34.15	0.11	0.32	0.00	0.00	0.00	0.00	0.40	1.16	0.41	1.20
5	Flu B/RSV Mod Pos	Flu B	90	34.56	0.00	0.00	0.10	0.29	0.00	0.00	0.29	0.83	0.30	0.88
		RSV	90	34.41	0.05	0.14	0.00	0.00	0.00	0.00	0.43	1.25	0.43	1.26

Ct = threshold cycle, CV = coefficient of variation, Mod = moderate, Pos = positive, SD = standard deviation.
Note: Variability from some factors may be numerically negative; in these cases, SD and %CV are displayed as 0.
Low Pos = Both targets are at 1–2X LoD.
Mod Pos = Both targets are at 3–5X LoD.

[1]Between Operator may be confounded with Between Run; therefore, Between Operator and Between Run estimates are combined in Between Operator/Run.

The signal variability measured as %CV was ≤1.50% (SD ≤0.48) between sites, between operators, between days, or overall for the Panther Fusion SARS-CoV-2/Flu A/B/RSV Assay positive controls (Table 26).

Table 26: Signal Variability of the Panther Fusion SARS-CoV-2/Flu A/B/RSV Assay Controls

Control	Analyte	N	Mean Ct	Between Sites		Between Operators		Between Days		Within Days		Total	
				SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
Pos	SARS-CoV-2	30	33.44	0.06	0.18	0.14	0.41	0.19	0.57	0.29	0.87	0.38	1.13
	Flu A	30	31.75	0.27	0.86	0.00	0.00	0.00	0.00	0.39	1.22	0.48	1.50
	Flu B	30	31.28	0.14	0.43	.005	0.15	0.02	0.07	0.24	0.76	0.28	0.89
	RSV	30	32.55	0.06	0.20	0.00	0.00	0.00	0.00	0.28	0.87	0.29	0.89

Ct = threshold cycle; CV = coefficient of variation; Pos = positive; SD = standard deviation.
Note: Variability from some factors may be numerically negative; in these cases, SD and %CV are displayed as 0.

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