Hologic, Inc.
Vlada Rudenko
Regulatory Affairs Specialist
10210 Genetic Center Drive
San Diego, California 92121

Re: K222736
Trade/Device Name: Panther Fusion SARS-CoV-2/Flu A/B/RSV Assay
Regulation Number: 21 CFR 866.3981
Regulation Name: Device To Detect And Identify Nucleic Acid Targets In Respiratory Specimens From Microbial Agents That Cause The SARS-Cov-2 Respiratory Infection And Other Microbial Agents When In A Multi-Target Test
Regulatory Class: Class II
Product Code: QOF, OOI
Dated: September 8, 2022
Received: September 9, 2022

Dear Vlada Rudenko:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. Although this letter refers to your product as a device, please be aware that some cleared products may instead be combination products. The 510(k) Premarket Notification Database located at https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfpmn/pmn.cfm identifies combination product submissions. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you, however, that device labeling must be truthful and not misleading.

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

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal

statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Part 801 and Part 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR 803) for devices or postmarketing safety reporting (21 CFR 4, Subpart B) for combination products (see https://www.fda.gov/combination-products/guidance-regulatory-information/postmarketing-safety-reporting-combination-products); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820) for devices or current good manufacturing practices (21 CFR 4, Subpart A) for combination products; and, if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR 1000-1050.

Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to https://www.fda.gov/medical-devices/medical-device-safety/medical-device-reporting-mdr-how-report-medical-device-problems.

For comprehensive regulatory information about medical devices and radiation-emitting products, including information about labeling regulations, please see Device Advice (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance) and CDRH Learn (https://www.fda.gov/training-and-continuing-education/cdrh-learn). Additionally, you may contact the Division of Industry and Consumer Education (DICE) to ask a question about a specific regulatory topic. See the DICE website (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance/contact-us-division-industry-and-consumer-education-dice) for more information or contact DICE by email (DICE@fda.hhs.gov) or phone (1-800-638-2041 or 301-796-7100).

Sincerely,

Joseph Briggs -S

Joseph Briggs, Ph.D.
Deputy Branch Chief
Viral Respiratory and HPV Branch
Division of Microbiology Devices
OHT7: Office of In Vitro Diagnostics
Office of Product Evaluation and Quality
Center for Devices and Radiological Health

Enclosure

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: 06/30/2023
See PRA Statement below.

510(k) Number *(if known)*
K222736

Device Name
Panther Fusion SARS-CoV-2/Flu A/B/RSV Assay

Indications for Use *(Describe)*

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) 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 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.

The Hologic RespDirect Collection Kit can be used to collect NP specimens for testing with the Panther Fusion

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

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

CONTINUE ON A SEPARATE PAGE IF NEEDED.

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510(k) SUMMARY

K222736

Panther Fusion SARS-CoV-2/Flu A/B/RSV Assay

I. 510(k) Owner: Hologic, Inc.
10210 Genetic Center Drive
San Diego, CA 92121

Contact Person: Vlada Rudenko, MA
Regulatory Affairs Specialist
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Fax: N/A

Date Prepared: May 15, 2023

II. DEVICE

Proprietary Name:	Panther Fusion SARS-CoV-2/Flu A/B/RSV Assay
Classification Name:	Multi-Target Respiratory Specimen Nucleic Acid Test Including SARS-CoV-2 And Other Microbial Agents
Regulation Number:	21 CFR 866.3981
Regulatory Class:	Class II
Product Code:	QOF
Secondary Product Code:	OOI

III. PREDICATE DEVICE

The predicate device for the Panther Fusion SARS-CoV-2/Flu A/B/RSV assay is the BioFire Respiratory Panel 2.1 (BioFire RP2.1) Assay (DEN200031; granted March 17, 2021, BioFire Diagnostics, LLC).

IV. DEVICE DESCRIPTION

The Panther Fusion SARS-CoV-2/Flu A/B/RSV assay is a multiplex real-time reverse transcriptase PCR (RT-PCR) *in vitro* diagnostic test developed for use on the fully automated Panther Fusion system to detect and differentiate SARS-CoV-2, influenza A, influenza B, and respiratory syncytial virus (RSV) directly from nasopharyngeal (NP) swab specimens collected into UTM/VTM or with the Hologic RespDirect Collection Kit, from individuals exhibiting signs and symptoms of a respiratory tract infection.

The Hologic RespDirect Collection Kit is intended for the collection of NP swab specimens. Each individual collection kit is comprised of a single flocked NP swab and an enhanced Direct Load Tube (eDLT) containing 2.9mL of enhanced Specimen Transport Media (eSTM) which are flow wrapped together for customer convenience.

The Panther Fusion SARS-CoV-2/Flu A/B/RSV assay involves the following steps:
a) Sample lysis; Prior to processing and testing on the Panther Fusion system, specimens are transferred to a tube containing specimen transport media (STM) that lyses the cells, releases target nucleic acid and protects them from degradation during storage. For specimens collected with the Hologic RespDirect Collection Kit, the swab is placed directly into the eDLT where the eSTM that lyses the cells, releases the nucleic acid and protects them from degradation during storage.
b) Nucleic acid capture and elution takes place in a single tube on the Panther Fusion system. The eluate is transferred to the Panther Fusion system reaction tube containing the assay reagents. The Internal Control-S (IC-S) is added to the Panther Fusion Capture Reagent-S (FCR-S) which gets added to each specimen. The IC-S in the reagent is used to monitor specimen processing, amplification, and detection. Magnetic particles with covalently bound oligonucleotides mediate the nucleic acid capture. Capture oligonucleotides hybridize to total nucleic acid in the test specimen. Hybridized nucleic acid is then separated from the lysed specimen in a magnetic field. Wash and aspiration steps remove extraneous components debris from the reaction tube. The elution step elutes purified nucleic acid.

c) Elution transfer and multiplex RT-PCR; Eluted nucleic acid is transferred to a Panther Fusion reaction tube already containing oil and reconstituted master mix. A reverse transcriptase generates a DNA copy of the target sequence. Target specific forward and reverse primers and probes then amplify targets while simultaneously detecting and discriminating multiple target types via multiplex RT-PCR. The Panther Fusion system compares the fluorescence signal to a predetermined cut-off to produce a qualitative result for the presence or absence of the analyte. The positive result for each analyte will be accompanied by the cycle threshold (Ct value). 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
SARS-CoV-2	ORF1ab	ROX
Influenza A Virus	Matrix	FAM
Respiratory Syncytial Virus A/B	Matrix	HEX
Influenza B Virus	Matrix	RED647
Internal Control	Not applicable	RED677

*Internal Control is a non-infectious synthetic nucleic acid sequence that is extracted and detected through targeted primers and probes.

Assay Components

The reagents required to perform the Panther Fusion SARS-CoV-2/Flu A/B/RSV assay are packaged and sold separately. There are 7 boxes containing 9 reagents which are required for sample processing. The Hologic RespDirect Collection Kit can be used to collect NP specimens for testing with the Panther Fusion SARS-CoV-2/Flu A/B/RSV Assay. Additionally, other NP swabs (not provided with the Hologic RespDirect Collection Kit) may be used to collect NP specimens in 3mL of VTM or UTM. A description of the components that are required to perform the Panther Fusion SARS-CoV-2/Flu A/B/RSV assay and the Hologic RespDirect Collection kit is detailed in **Table 1.** Boxes 3 - 7 were cleared by FDA as part of K171963.

Table 1: Reagents Required to Perform the Panther Fusion SARS-CoV-2/Flu A/B/RSV Assay

Box	Components Description	Part Number
1	**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	**Panther Fusion SARS-CoV-2/Flu A/B/RSV Controls** Positive Control tube, 5 per box Panther Fusion Negative Control tube, 5 per box	PRD-07401
3	**Panther Fusion Extraction Reagent-S - 960 Tests** Panther Fusion Capture Reagent-S bottle, 240 tests, 4 per box Panther Fusion Enhancer Reagent-S bottle, 240 tests, 4 per box	PRD-04331
4	**Panther Fusion Internal Control-S - 960 Tests** Panther Fusion Internal Control-S tube, 4 per box	PRD-04332
5	**Panther Fusion Reconstitution Buffer I - 1920 Tests** Panther Fusion Reconstitution Buffer I pack, 960 tests, 2 per box	PRD-04333
6	**Panther Fusion Elution Buffer - 2400 Tests** Panther Fusion Elution Buffer pack, 1200 tests, 2 per box	PRD-04334
7	**Panther Fusion Oil Reagent - 1920 Tests** Panther Fusion Oil Reagent pack, 960 tests, 2 per box	PRD-04335

Ancillary Kits:

1	**Panther Fusion Specimen Lysis Tubes** (100 tubes)	PRD-04339
2	**Hologic RespDirect Collection Kit** Collection kit composed of 1 flocked NP swab and 1 tube containing 2.9mL eSTM), 50 per box	PRD-07788

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

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

Instrumentation

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

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

The Panther Fusion SARS-CoV-2/Flu A/B/RSV assay has been designed for and validated on the Panther Fusion system. The Panther Fusion system fully automates all the steps necessary to perform the Panther Fusion SARS-CoV-2/Flu A/B/RSV assay.

V. INDICATIONS FOR USE

Panther Fusion SARS-CoV-2/Flu A/B/RSV Assay

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) 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 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.

The Hologic® RespDirect™ Collection Kit can be used to collect NP specimens for testing with the Panther Fusion SARS-CoV-2/Flu A/B/RSV Assay. Additionally, other NP swabs (not provided with the Hologic RespDirect Collection Kit) may be used to collect NP specimens in 3mL of VTM or UTM.

Ancillary Collection Kit:

Hologic RespDirect Collection Kit

The Hologic® RespDirect™ Collection Kit is intended to be used for the collection of nasopharyngeal (NP) swab specimens (collected by a healthcare provider) for testing with the Panther Fusion SARS-CoV-2/Flu A/B/RSV Assay.

VI. COMPARISON OF TECHNOLOGICAL CHARACTERISTICS WITH THE PREDICATE DEVICE

A comparison of the Panther Fusion SARS-CoV-2/Flu A/B/RSV assay to the predicate BioFire Respiratory Panel 2.1 (DEN200031) is summarized in **Table 2**.

Table 2: Comparison of Similarities and Differences Between the Subject Device (Panther Fusion SARS-CoV-2/Flu A/B/RSV Assay) and the Predicate Device (BioFire Respiratory Panel 2.1 (RP2.1))

Item	Panther Fusion SARS-CoV-2/Flu A/B/RSV Assay (Subject Device)	BioFire Respiratory Panel 2.1 (RP2.1) (Predicate Device) DEN200031
Prescription Use Only	Yes	Same
Specimen Types	Nasopharyngeal (NP) swab specimens	Same
Intended User	Professional use	Same
Technology Principle of Operation	Reverse transcriptase multiplexed polymerase chain reaction test	Same
Organisms Detected	4 targets including: **SARS-CoV-2** **Flu A** **Flu B** **RSV (A and B)**	Same, however the BioFire RP 2.1 includes additional targets (listed below) Adenovirus Coronavirus 229E Coronavirus HKU1 Coronavirus NL63 Coronavirus OC43 **Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2)** Human Metapneumovirus Human Rhinovirus/Enterovirus

		Influenza A, including subtypes H1, H1-2009, and H3 **Influenza B** Parainfluenza Virus 1 Parainfluenza Virus 2 Parainfluenza Virus 3 Parainfluenza Virus 4 **Respiratory Syncytial Virus** *Bordetella parapertussis* (IS1001) *Bordetella pertussis* (ptxP) *Chlamydia pneumoniae* *Mycoplasma pneumoniae*
Assay Controls	Internal and external controls	Same
Platform	Automated nucleic acid amplification platform. Uses Panther Fusion system for all steps including nucleic acid extraction, amplification, detection and result processing.	Automated nucleic acid amplification platform. Uses BioFire FilmArray 2.0 or BioFire FilmArray Torch systems including integrated sample preparation, amplification, detection, and analysis.
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) 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.	The BioFire Respiratory Panel 2.1 (RP2.1) is a PCR-based multiplexed nucleic acid test intended for use with the BioFire FilmArray 2.0 or BioFire FilmArray Torch systems for the simultaneous qualitative detection and identification of multiple respiratory viral and bacterial nucleic acids in nasopharyngeal swabs (NPS) obtained from individuals suspected of respiratory tract infections, including COVID-19. The following organism types and subtypes are identified using the BioFire RP2.1: • Adenovirus, • Coronavirus 229E, • Coronavirus HKU1, • Coronavirus NL63, • Coronavirus OC43, • Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV-2),

	Nucleic acids from the viral organisms identified by this test are generally detectable in NP 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. The Hologic RespDirect Collection Kit can be used to collect NP specimens for testing with the Panther Fusion SARS-CoV-2/Flu A/B/RSV Assay. Additionally, other NP swabs (not provided with the Hologic RespDirect Collection Kit) may be used to collect NP specimens in 3mL of VTM or UTM. **Ancillary Collection Kit:** <u>Hologic RespDirect Collection Kit</u> The Hologic RespDirect Collection Kit is intended to be used for the collection of nasopharyngeal (NP) swab specimens (collected by a healthcare provider) for	• Human Metapneumovirus, • Human Rhinovirus/Enterovirus, • Influenza A, including subtypes H1, H1-2009, and H3, • Influenza B, • Parainfluenza Virus 1, • Parainfluenza Virus 2, • Parainfluenza Virus 3, • Parainfluenza Virus 4, • Respiratory Syncytial Virus, • *Bordetella parapertussis* (IS1001), • *Bordetella pertussis* (ptxP), • *Chlamydia pneumoniae*, and • *Mycoplasma pneumoniae* Nucleic acids from the respiratory viral and bacterial organisms identified by this test are generally detectable in NPS specimens during the acute phase of infection. The detection and identification of specific viral and bacterial nucleic acids from individuals exhibiting signs and/or symptoms of respiratory infection is indicative of the presence of the identified microorganism and aids in the diagnosis of respiratory infection if used in conjunction with other clinical and epidemiological information. The results of this test should not be used as the sole basis for diagnosis, treatment, or other patient management decisions. Negative results in the setting of a respiratory illness may be due to infection with pathogens that are not detected by this test, or lower respiratory tract infection that may not be detected by an NPS specimen. Positive results do not rule out coinfection with other organisms. The agent(s) detected by the BioFire RP2.1 may not be the definite cause of disease. Additional laboratory testing (e.g., bacterial

	testing with the Panther Fusion SARS-CoV-2/Flu A/B/RSV Assay.	and viral culture, immunofluorescence, and radiography) may be necessary when evaluating a patient with possible respiratory tract infection.
Time to Obtain Test Results	Approximately 2.5 hours	Approximately 45 minutes
Flu A Subtyping	No	Yes

VII. PERFORMANCE DATA

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

Brief Description of Analytical (Non-Clinical) Studies

The following analytical studies (non-clinical) were conducted to support the clearance of the Panther Fusion SARS-CoV-2/Flu A/B/RSV Assay on the Panther Fusion system.

Specimen Stability (VTM/UTM)

The purpose of this study was to demonstrate the stability of NP specimens collected in Viral Transport Media/Universal Transport Media (VTM/UTM) when stored under recommended temperature conditions and durations for the Panther Fusion SARS-CoV-2/Flu A/B/RSV Assay on the Panther Fusion system. Additionally, freeze/thaw stability was demonstrated. Specimen positive panels were prepared by spiking virus strains of each assay target at 3xLoD or 10xLoD into 20 negative unique clinical matrix pools and tested at 20 replicates per storage condition. This study demonstrates stability for SARS-CoV-2, Flu A, Flu B, and RSV for the following:

<u>Primary Specimens (NP swab stored in VTM/UTM)</u>

a) Stored refrigerated (2-8ºC) for up to 96 hours prior to transfer into a specimen lysis tube.

b) Frozen at -70ºC. Freeze/thaw cycles should be minimized due to potential for sample degradation.

<u>Processed Specimens (NP swab in VTM/UTM transferred to a specimen lysis tube)</u>

c) Stored at room temperature (15-30ºC) for up to 6 days.

d) Refrigerated (2-8ºC) for up to 3 months.

e) Frozen at-20ºC for up to 3 months. Freeze/thaw cycles should be minimized due to potential for sample degradation.

f) Frozen at -70ºC for up to 3 months. Freeze/thaw cycles should be minimized due to potential for sample degradation.

Hologic RespDirect Specimen Stability at Room Temperature

The purpose of this study was to demonstrate the stability of specimens collected into eSTM using the RespDirect Collection Kit when stored at room temperature (15°C to 30°C) up to 7 days and tested in the Panther Fusion SARS-CoV-2/ Flu A/B/RSV assay. The Hologic RespDirect specimen storage condition at 30°C for 7 days was tested to support 15°C to 30°C specimen stability. An unspiked negative panel was prepared from pooled negative clinical eSTM matrix. Positive panels were prepared by spiking one virus strain of each assay target at 3xLoD or 10xLoD. Testing included a minimum of 20 replicates per target concentration per panel on day 0 (baseline) and day 7. A day 0 baseline testing was set. All 20 replicates of positive panels (3x and 10xLoD) showed 100% positivity for all targets on day 0 and after storage at 30 °C for 7 days. All 20 replicates of negative panels tested on both Day 0 and Day 7 were negative.

This study demonstrates stability for SARS-CoV-2, Flu A, Flu B and RSV for specimens collected in the Hologic RespDirect Collection Kit stored at 15°C-30°C for up to 6 days.

Hologic RespDirect Specimen Stability at 2-8ºC, -20 ºC, and -70 ºC

The purpose of this study was to demonstrate the stability of specimens collected in eSTM using the Hologic RespDirect Collection Kit when stored refrigerated (2-8ºC) or frozen at -20°C or-70°C for 3 months and tested in the Panther Fusion SARS-CoV-2/Flu A/B/RSV assay. An unspiked negative panel was prepared from pooled negative clinical eSTM matrix. Positive panels were prepared by spiking one virus strain of each assay target at 3xLoD or 10xLoD in each negative clinical eSTM matrix pool. Baseline testing was performed on day 0 with 20 replicates per panel member. At each time point (33 days, 68 days and 99 days), 20 panel aliquots per temperature condition and target concentration were removed from the storage condition (2-8ºC, -20 ºC, and -70 ºC) and tested. Stability results showed 100% positivity for all assay targets (SARS-CoV-2, Flu A, Flu B, and RSV) at 3xLoD and 10xLoD for all timepoints and storage conditions tested, with the exception of SARS-CoV-2 at 3xLoD in the baseline of 2-8°C condition which was 95% positive. All 20 replicates of negative panels tested on day 0 and the various timepoints were negative.

This study demonstrates the following stability for SARS-CoV-2, Flu A, Flu B and RSV for specimens collected in the Hologic RespDirect Collection Kit and tested with the Panther Fusion SARS-CoV-2/Flu A/B/RSV Assay:

- Refrigerated (2-8˚C) for up to 3 months or,
- Frozen at -20˚C for up to 3 months or,
- Frozen at -70˚C for up to 3 months.

Hologic RespDirect Specimen Freeze Thaw Stability

The purpose of this study was to demonstrate that specimens collected with the RespDirect Collection Kit are stable when stored frozen at -20˚C and -70˚C and subjected to 4 freeze-thaw cycles. A negative panel was prepared from the pooled negative clinical eSTM matrix. Positive panels were prepared by spiking one virus strain of each assay target at 3xLoD or 10xLoD. Multiple panels were prepared and placed on stability in the appropriate test conditions (storage cycled between -20°C and 30°C for 2 and 4 freeze/thaw (F/T) cycles or cycled between -70°C and 30°C for 2 and 4 freeze/thaw (F/T) cycles). At each timepoint, samples were removed from their specified storage temperature and tested using the Panther Fusion SARS-CoV-2/Flu A/B/RSV assay. Testing included a minimum of 20 replicates per target concentration per condition at each time point. Results showed 100.0% positivity for all assay targets (SARS-CoV-2, Flu A, Flu B, and RSV) at 3xLoD and 10xLoD in both storage conditions (-20°C and -70°C), with the exception of SARS-CoV-2 at 3xLoD in the 4 F/T condition which was 95.0% positive.

This study demonstrates that NP swab specimens in the Hologic RespDirect Collection Kit may undergo up to 3 freeze-thaw cycles when stored at either -20°C or -70°C.

Transport Media Equivalency

The purpose of this study was to demonstrate equivalency between viral transport media (VTM) and universal transport media (UTM) vendor types when used with the Panther Fusion SARS-CoV-2/Flu A/B/RSV assay. Multiple viral transport media (VTM) and universal transport media (UTM) types were tested and included Remel Micro Test M4RT, Remel Micro Test M5, Remel Micro Test M6, BD Universal Viral Transport Media, Copan Universal Transport Medium, and Hardy Diagnostics Viral Transport Media. To demonstrate equivalency between the viral transport medias, contrived positive samples were created by spiking one strain of SARS-CoV-2, Flu A H3N2, Flu B Victoria, and RSV B into either true negative clinical NP swab matrix or simulated NP swab matrix (i.e., transport media spiked with HeLa cells at a concentration of $2x10^4$ cells/mL) at 3 concentrations (0.5x, 1x, and 5x LoD). Flu A, Flu B, and RSV B were co-spiked while SARS-CoV-2 was evaluated independently. Contrived positives generated with true clinical NP matrix were included to demonstrate equivalency between clinical NP matrix and simulated NP

matrix, supporting the use of simulated viral transport media in the study. The Panther Fusion SARS-CoV-2/Flu A/B/RSV assay demonstrated equivalency between VTM/UTM vendor medias and NP clinical VTM/UTM matrix based on comparable positivity observed at the 1x LoD and 5x LoD concentrations for SARS-CoV-2, Flu A (H3N2), Flu B (Victoria lineage), and RSV B.

Analytical Sensitivity - Limit of Detection (LoD)

The analytical sensitivity (limit of detection) 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), Influenza A (one H1N1 strain and one H3N2 strain), Influenza B (one Victoria lineage strain and one Yamagata lineage strain) , RSV (one RSV-A strain and one RSV-B strain). Dilutions of cultured virus in $TCID_{50}$/mL and SARS-CoV-2 WHO standard in IU/mL were prepared in pooled negative clinical nasopharyngeal (NP) swab VTM/UTM matrix processed into STM (1:1.56 ratio). The dilutions were tested at a minimum of 24 replicates per concentration, per reagent lot, using 3 reagent lots for a total of at least 72 replicates per strain. 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.

The results from this study demonstrated that the Panther Fusion SARS-CoV-2/Flu A/B/RSV assay has the following LoD for processed NP specimens:

Table 3: Analytical Sensitivity

Viral Strain	LoD Concentration
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
SARS-CoV-2 WHO International Standard, NIBSC (20/146)	47.20 IU/mL

LoD testing was also performed with the Hologic 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 Hologic RespDirect Collection kit, this is the concentration in the Enhanced Direct Load tube.*

Interfering Substances

This study evaluated the performance of the Panther Fusion SARS-CoV-2/Flu A/B/RSV Assay in the presence of potentially interfering endogenous and exogenous substances. Potentially interfering substances were prepared in processed negative clinical NP swab VTM/UTM matrix in STM with up to three substances in each pool. Pools were tested with and without the presence of assay analytes. The analyte negative pools contained the negative clinical matrix and the potentially interfering substances only. The analyte positive pools contained the negative clinical matrix, the potentially interfering substances and one representative strain of each targeted analyte spiked to a final testing concentration of 3xLoD for each analyte. No cross-reactivity was observed in analyte negative pools in the presence of interfering substances which produced 0% positive results. No interference to analyte positive pools at 3xLoD was observed in the presence of interfering substances which produced 100% positive results. All pools met the acceptance criteria for each condition tested. The results from this study demonstrate that the evaluated substances do not interfere with the Panther Fusion SARS-CoV-2/Flu A/B/RSV assay.

Table 4: 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
	Neo-Synephrine®	Phenylephrine	15% v/v

Nasal sprays or drops	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.

Cross-Reactivity and Microbial Interference Wet Testing

The purpose of this study was to demonstrate that the Panther Fusion SARS-CoV-2/Flu A/B/RSV assay does not cross-react with genetically closely related microorganisms or microorganisms commonly encountered in a respiratory specimen. Microorganism wet testing consisted of preparing live or inactivated organisms in processed negative clinical NP swabs VTM/UTM matrix. In total, 41 microorganisms, including bacteria, fungi, and viruses were evaluated. Bacteria/fungi were tested at 10^6 CFU/mL and viruses were tested at 10^5 $TCID_{50}$/mL, except where noted. *In vitro* transcripts (IVTs) corresponding to the ORF1ab regions targeted by the assay or SARS coronavirus and coronavirus HKU1 were used to evaluate cross-reactivity. Pools were tested with and without the presence of assay analytes. The analyte negative

pools contained the negative clinical matrix and the microorganisms. The analyte positive pools contained the negative clinical matrix, microorganisms and one representative strain of each targeted analyte (SARS-CoV-2, Flu A H3N2, Flu B Victoria lineage, RSV B) spiked to a final testing concentration of 3xLoD for each analyte. A minimum of three replicates of each microorganism panel were evaluated using one reagent lot and one Panther Fusion system. All pools had the expected results of no interference or cross reactivity and observed the expected SARS-CoV-2/Flu A/B/RSV results.

Table 5: 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*	1x106 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.

Analytical Reactivity – Strain Wet Testing

The purpose of this study was to verify that the Panther Fusion SARS-CoV-2/Flu A/B/RSV Assay on the Panther Fusion system can detect multiple strains of SARS-CoV-2, Flu A, Flu B, RSV A, and RSV B. Multiple strains of each assay target were tested at ~3x the assay Limit of Detection (LoD). For strains not detected at 3x LoD, additional testing at higher concentrations was performed until 100% positivity was observed. Samples consisted of 15 strains of SARS-CoV-2, 28 strains of Flu A (13 H1N1, 14 H3N2 and 1 H5N1), 17 strains of Flu B (8 Victoria, 8 Yamagata and 1 unknown), 4 strains of RSV A, and 3 strains of RSV B. The Panther Fusion SARS-CoV-2/Flu A/B/RSV Assay demonstrates SARS-CoV-2, Flu A, Flu B and RSV strain wet testing inclusivity for the strains tested at the concentrations illustrated in Table 6.

Table 6: Analytical Reactivity Summary for SARS-CoV-2, Flu A and Flu B and RSV Strains

Description	Subtype	Concentration	SARS-CoV-2	Flu A	Flu B	RSV
USA-WA1/2020*	SARS-CoV-2	0.09 $TCID_{50}$/mL	+	-	-	-
USA-CA1/2020	SARS-CoV-2	0.09 $TCID_{50}$/mL	+	-	-	-
USA-AZ1/2020	SARS-CoV-2	0.09 $TCID_{50}$/mL	+	-	-	-
USA-WI1/2020	SARS-CoV-2	0.09 $TCID_{50}$/mL	+	-	-	-
USA/OR-OHSU-PHL00037/ 2021|B.1.1.7	SARS-CoV-2	0.09 $TCID_{50}$/mL	+	-	-	-
Uganda/MUWRP-20200195568/ 2020|A.23.1	SARS-CoV-2	0.09 $TCID_{50}$/mL	+	-	-	-
USA/PHC658/2021|B.1.617.2	SARS-CoV-2	0.09 $TCID_{50}$/mL	+	-	-	-
USA/MD-HP05285/2021|B.1.617.2	SARS-CoV-2	0.09 $TCID_{50}$/mL	+	-	-	-
USA/CA/VRLC009/2021|B.1.427	SARS-CoV-2	0.09 $TCID_{50}$/mL	+	-	-	-
USA/CA/VRLC012/2021|P.2	SARS-CoV-2	0.30 $TCID_{50}$/mL	+	-	-	-
USA/MD-HP03056/2021|B.1.525	SARS-CoV-2	0.30 $TCID_{50}$/mL	+	-	-	-
USA/CA-Stanford-16_S02/ 2021|B.1.617.1	SARS-CoV-2	0.09 $TCID_{50}$/mL	+	-	-	-

SARS-CoV-2/Flu A/B/RSV Assay Performance Panther Fusion®

Description	Subtype	Concentration	SARS-CoV-2	Flu A	Flu B	RSV
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 TCID50/mL	-	+	-	-
A/Michigan/45/2015	Flu A (H1N1)	0.18 TCID50/mL	-	+	-	-
A/Christ Church/16/2010	Flu A (H1N1)	180[1] TCID50/mL	-	+	-	-
A/Kentucky/2/06	Flu A (H1N1)	0.60 TCID50/mL	-	+	-	-
A/Solomon Islands/03/06	Flu A (H1N1)	0.60 TCID50/mL	-	+	-	-
A/Guangdong-maonan/ 1536/ 2019	Flu A (H1N1)	180[1] TCID50/mL	-	+	-	-
A/Taiwan/42/2006	Flu A (H1N1)	0.60 TCID50/mL	-	+	-	-
A/Henan/8/05	Flu A (H1N1)	0.60 TCID50/mL	-	+	-	-
A/Hawaii/15/01	Flu A (H1N1)	18[3] TCID50/mL	-	+	-	-
A/California/07/2009	Flu A (H1N1)	0.18 TCID50/mL	-	+	-	-
A/Hawaii/66/2019	Flu A (H1N1)	180 CEID50/mL	-	+	-	-
A/Indiana/02/2020	Flu A (H1N1)	60 CEID50/mL	-	+	-	-
A/Michigan/45/2015 pdm09-like virus	Flu A (H1N1)	0.60 TCID50/mL	-	+	-	-
A/Kansas/14/17*	Flu A (H3N2)	0.33 TCID50/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] TCID50/mL	-	+	-	-
A/Hiroshima/52/05	Flu A (H3N2)	1.1 TCID50/mL	-	+	-	-

SARS-CoV-2/Flu A/B/RSV Assay Performance Panther Fusion®

Description	Subtype	Concentration	SARS-CoV-2	Flu A	Flu B	RSV
A/Costa Rica/07/99	Flu A (H3N2)	11[3] TCID50/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 TCID50/mL	-	-	+	-
B/Texas/81/2016	Flu B (Yamagata)	2[1] TCID50/mL	-	-	+	-
B/Indiana/17/2017	Flu B (Yamagata)	0.60[1]TCID50/mL	-	-	+	-
B/Oklahoma/10/2018	Flu B (Yamagata)	2[1] TCID50/mL	-	-	+	-
B/Massachusetts/02/2012	Flu B (Yamagata)	0.2[2] TCID50/mL	-	-	+	-

Description	Subtype	Concentration	SARS-CoV-2	Flu A	Flu B	RSV
B/Lee/40	Flu B	0.09 $TCID_{50}$/mL	-	-	+	-
RSV-A/2006 Isolate*	RSVA	0.06 $TCID_{50}$/mL	-	-	-	+
RSV A/4/2015 isolate #1	RSVA	0.06 $TCID_{50}$/mL	-	-	-	+
RSV A/A2	RSVA	0.06 $TCID_{50}$/mL	-	-	-	+
RSV A/12/2014 isolate #2	RSVA	0.06 $TCID_{50}$/mL	-	-	-	+
RSV-B/CH93(18)-18*	RSVB	0.30 TCID50/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 TCID50/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 TCID50/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.

Analytical Reactivity – *In Silico* Inclusivity

The purpose of this analysis is to document SARS-CoV-2, Flu A, Flu B, RSV A, and RSV B strain and isolate inclusivity of the Panther Fusion SARS-CoV-2/Flu A/B/RSV assay. Inclusivity was assessed based on *in silico* analysis of the assay forward primers, reverse primers, and probe oligos compared to available sequences in the GISAID and NCBI gene databases. For SARS-CoV-2, sequences on or before 6/25/2022 were evaluated (10% of the database was randomly sampled for analysis); for Flu A, Flu B, RSV A and RSV B, sequences from between January 01, 2015 and February 15, 2022 were evaluated. Exclusion criteria removed all non-human isolates and partial sequences that do not span the amplicon regions of the assay. Sequence alignment for the analysis was performed using MAFFT (multiple alignment using fast Fourier transform) and MSAE (Hologic Multiple Sequence Alignment Editor) alignment programs. Based on *in silico* analysis of GISAID and NCBI sequences available up to June 25, 2022 for SARS-CoV-2 (10% random sampling), 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.

Cross-Reactivity in the Presence of Microorganisms – *In Silico*

The purpose of this study was to demonstrate that the Panther Fusion SARS-CoV-2/Flu A/B/RSV assay does not cross-react with genetically closely related or commonly encountered microorganisms through in silico BLAST analysis. The 143 microorganisms were evaluated with *in silico* BLAST analysis using multiple representative strains from GenBank. BLAST results were analyzed for homology to the amplification primers (forward and reverse primer) and probes for the SARS-CoV-2 (Region 1 and Region 2), Flu A (Region A1 and Region A2), Flu B, RSV A, and RSV B targets included in the assay. Any microorganism sequence showing ≥80% homology to a single primer or probe was further evaluated for homology of ≥50% to the other target region primers and probes. If homology of a primer pair was detected, additional analysis included determining the proximity of the pair and evaluation the probability of generating and/or detecting amplicon from the microorganism sequences.

A total of 545 GenBank sequences for the 143 non-target microorganisms were analyzed. Homology to each of the sequences was analyzed per primer or probe oligo for the SARS-CoV-2, Flu A, Flu B, and RSV assay target regions. The *in-silico* BLAST analysis of the primers and probes of the Panther Fusion SARS-CoV-2/Flu A/B/RSV assay demonstrates that there is no predicted cross reactivity or interference to genetically closely related or commonly encountered respiratory microorganisms, 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.

Analytical Exclusivity

The purpose of this study was to demonstrate the influenza analytical specificity in relation to influenza types and subtypes not intended to be detected with the Panther Fusion SARS CoV-2/Flu A/B/RSV Assay on the Panther Fusion System. Two non-indicated influenza types, influenza type C and influenza type D, were tested. The Panther Fusion SARS-CoV-2/Flu A/B/RSV Assay did not detect either.

Within Laboratory Precision and Repeatability

This study demonstrated the within laboratory precision and repeatability of results generated by the Panther Fusion SARS-CoV-2/Flu A/B/RSV Assay on the Panther Fusion system when evaluated under different conditions of variability (i.e., operators, days, reagent lots, instruments). The panels tested consisted of a negative panel, a low level (2xLoD) SARS-CoV-2 & Flu A (H3N2) positive panel, a moderate level (5x LoD) SARS-CoV-2 & Flu A positive panel, a low level (2x LoD) Flu B (Victoria lineage) & RSV B positive panel, and a moderate level (5x LoD) Flu B & RSV B positive panel. Panels were prepared by spiking viruses into processed negative nasopharyngeal (NP) swab VTM/UTM matrix processed with STM (1:1.56 ratio). Two operators tested 2 replicates of each panel in 2 runs per day for each of the 3 reagent lots across 3 Panther Fusion systems for a total of 12 days of testing. In total, 96 measurements were performed for each panel member. All low (2x) and moderate (5x) panel members were 100% positive for the spiked target analytes. The negative panel member was 0.0% positive for Flu A, Flu B, and RSV, while 1.0% positive for SARS-CoV-2 (1/96) An overall total signal variability of ≤2.04% was observed.

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

Panel	Description	Analyte	Agreement/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	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
	Low Pos	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	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
	Low Pos	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	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
	Mod Pos	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	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
	Mod Pos	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 2X LoD.
Mod Pos = Both targets are 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%.

Competitive Interference/Co-Infection

This study evaluated potential competitive interference for paired low/high target combinations of SARS-CoV-2, Flu A, Flu B, RSV A, and RSV B cultured virus in the Panther Fusion SARS-CoV-2/Flu A/B/RSV Assay on the Panther Fusion system. SARS-CoV-2, Flu A H3N2, Flu B Victoria, RSV A, and RSV B cultured viral targets were spiked into processed negative clinical NP VTM/UTM matrix in STM at multiple target combinations of low (3xLoD) and high concentrations (up to $1x10^4$ $TCID_{50}$/mL) and tested at 3 replicates per panel. If less than 100% positivity was observed in the low target, the high target was diluted from $1x10^4$ $TCID_{50}$/mL until 100% positivity was achieved.

SARS-CoV-2 at 3xLoD was 100% positive when paired with Flu A, Flu B, and RSV A at 1 $x10^4$ $TCID_{50}$/mL and RSV B at 30 $TCID_{50}$/mL. Flu A at 3xLoD was 100% positive when paired with Flu B and RSV A at $1x10^4$ $TCID_{50}$/mL, SARS-CoV-2 at 100 $TCID_{50}$/mL and RSV B at 30 $TCID5_0$/mL. Flu B at 3xLoD was 100% positive when paired with SARS-CoV-2, Flu A, RSV A at $1x10^4$ $TCID_{50}$/mL and RSV B at $1x10^3$ $TCID_{50}$/mL. RSV A at 3xLoD was 100% positive when paired with SARS-CoV-2, Flu A, and Flu B at $1x10^4$ $TCID_{50}$/mL. RSV B at 3xLoD was 100% positive when combined with SARS-CoV-2, Flu A, and Flu B at $1x10^4$ $TCID_{50}$/mL.

All targets at 3xLoD maintained positivity when paired with other assay targets at $1x10^4$ $TCID_{50}$/mL with the exception of the following: SARS-CoV-2 and Flu A each in the presence of RSV B at $3x10^1$ $TCID_{50}$/mL, Flu B in the presence of RSV B at $1x10^3$ $TCID_{50}$/mL and Flu A in the presence of SARS-CoV-2 at $1x10^2$ $TCID_{50}$/mL.

Table 8: Competitive Interference

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

Hologic RespDirect Collection Device Equivalency/Swab Equivalency

The purpose of this study was to demonstrate equivalency between the Hologic RespDirect Collection Kit and a control collection device, consisting of a control nasopharyngeal (NP) swab in UTM. A swab equivalency study was also performed to demonstrate equivalency between the Hologic RespDirect swab and a control NP swab for absorption and elution of targets.

Paired individual nasopharyngeal swab specimens were collected with Hologic RespDirect Collection Kits and control collection kits, consisting of Copan Universal Transport Medium (UTM) and Copan NP swab. Paired individual donor NP swabs from 25 symptomatic donors each were co-spiked with SARS-CoV-2, Flu A, Flu B and RSV at 2x and 5x LoD respectively. In addition to this, paired individual donor NP swabs from 25 donors each were spiked to 2x and 5x LoD respectively for SARS-CoV-2 target only.

For the swab equivalency study, Hologic RespDirect swab and Copan NP swab were dipped into eSTM solution that was co-spiked with SARS-CoV-2, Flu A (H3N2), Flu B (Victoria lineage), and RSV B for absorption of targets. The wet swabs were then transferred into an unspiked Hologic RespDirect tube for elution of targets and tested in the Panther Fusion SARS-CoV-2/Flu A/B/RSV assay. The target concentration in the final Hologic RespDirect tube being tested after elution of targets from the swabs was at 4x LoD. A total of 20 swabs were used per swab type. The results from the swab equivalency study demonstrated equivalency between the Hologic RespDirect swab and a control NP swab for absorption and elution of respiratory virus targets and are illustrated in Table 9.

For the collection device equivalency study, comparable positivity was observed between the two collection devices for all concentrations and targets tested. The results from this study demonstrated equivalency between the Hologic RespDirect Collection Kit and a control collection device for use in the Panther Fusion SARS-CoV-2/Flu A/B/RSV assay and are illustrated in Table 10.

Table 9: Positivity Results Summary from the Swab Device Equivalency Study

Condition	SARS-CoV-2 (ROX)		Flu A (FAM)		Flu B (Red647)		RSV (HEX)	
	N Pos. / N Valid	% Pos	N Pos. / N Valid	% Pos	N Pos. / N Valid	% Pos	N Pos. / N Valid	% Pos
Copan NP Swab	20/20	100.0%	20/20	100.0%	20/20	100.0%	20/20	100.0%
RespDirect Swab	20/20	100.0%	20/20	100.0%	20/20	100.0%	20/20	100.0%

Table 10: Results of negative and contrived panels composed of paired individual donor NP clinical specimens, collected with each collection device spiked with SARS-CoV-2, Flu A, Flu B, and RSV

Analyte	Sample Concentration	N per Collection Device	VTM/UTM % Positive	Hologic 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

Carry-over/Cross-contamination

The purpose of this study was to demonstrate that no carry-over or cross-contamination is observed from specimens collected in eSTM using the Hologic RespDirect Collection Kit when tested with the Panther Fusion SARS-CoV-2/Flu A/B/RSV assay.

Unique pools of negative clinical eSTM were assembled. Positive panels were prepared by spiking Flu A H3N2 at high titer ($1x10^4$ $TCID_{50}$/mL) into one pool. Multiple identical positive and negative panels were prepared. 100 replicates of negative panel were tested per baseline run. One baseline run and five "checkerboard" runs were completed on each of two Panther Fusion instruments. Checkerboard runs were set up by alternating 30 positive and 30 negative panel in the sample racks.

Results showed 100% Flu A positivity for all positive panels, and 0% Flu A positivity in the negative panels. The Panther Fusion SARS-CoV-2/Flu A/B/RSV Assay using the Hologic RespDirect sample collection kit does not show carry-over or cross-contamination between samples on the same or adjacent sample racks.

Panther Fusion System Assay Performance

Clinical Performance Study

Prospective Clinical Study

This study was performed to demonstrate clinical performance characteristics for the Panther Fusion SARS-CoV-2/Flu A/B/RSV assay. 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 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 shown in Table 11.

Table 11: Summary of Subject Demographics for Evaluable Prospectively Collected 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)
	5-21 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 specimens is summarized in Table 12. 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 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 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 12: Panther Fusion SARS-CoV-2/Flu A/B/RSV Assay Performance with Prospectively Collected 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***

TP – true positive; FN – false negative; TN – true negative; FP – false positive; NC – Not calculable

[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 the 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

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. 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 samples were distributed uniformly across all three clinical testing sites. Demographic information for the 95 evaluable retrospective specimens is provided in Table 13.

Table 13: Summary of Subject Demographics for Evaluable Retrospective Specimens

		N (%)
Total		95 (100)
	Female	51 (53.7)
Sex	Male	44 (46.3)
	<5 years	16 (16.8)
	5-21 years	12 (12.6)
Age Group	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 were calculated against 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 14 shows the PPA for specimens that were confirmed positive for at least one target analyte.

Table 14: Panther Fusion SARS-CoV-2/ Flu A/B/RSV Assay Clinical Performance in Retrospective 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

TP = true positive; FN = false negative; TN = true negative; FP = false 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 15 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 15: Panther Fusion SARS-CoV-2/ Flu A/B/RSV Assay Clinical Performance with Confirmed Negative 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

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.

Reproducibility Study

Panther Fusion SARS-CoV-2/Flu A/B/RSV assay reproducibility was evaluated at three US sites using five panel members. At each of the three sites, two operators at each site completed 5 valid runs each, for a total of 10 valid runs per site, using one reagent kit lot. Samples were tested over approximately 5 days at each site.

Overall, 150 samples were tested at each of the 3 sites, for a total of approximately 450 samples tested. Each panel member had 90 results per panel member component across all sites.

One (1) panel member was negative for SARS-CoV-2, Flu A, Flu B, and RSV (i.e., NP specimen in UTM/VTM processed into STM matrix only), 2 panel members were dual-positive for Flu A and SARS-CoV-2and 2 panel members were dual-positive for Flu B and RSV. Each dual positive panel was generated at 2 concentrations, a low positive (1-2x LoD) and a moderate positive (3-5x LoD). The positive panel members were prepared by spiking viral strain stocks into a negative matrix of pooled negative clinical NP specimens in VTM/UTM processed into STM. The negative panel member contained matrix only.

Agreement values (see Table 16) were 100% for all panel member components except the following:

- True negative: 98.9% - One negative panel member had a positive Flu B result.
- Flu A low positive: 98.9% - One low positive panel member had a negative Flu A result.

Table 16: Reproducibility Study: Overall Agreement of Panther Fusion SARS-CoV-2/Flu A/B/RSV Assay Results with Expected Results

Panel Member	Description	Expected Result	Agreed/n	Agreement (%) 95% CI
1	True Negative*	Negative	89/90	98.9 (94.0-99.8)
2	SARS-CoV-2 Low Positive	Positive	90/90	100 (95.9-100)
4	SARS-CoV-2 Moderate Positive	Positive	90/90	100 (95.9-100)
2	Flu A Low Positive	Positive	89/90	98.9 (94.0-99.8)
4	Flu A Moderate Positive	Positive	90/90	100 (95.9-100)
3	Flu B Low Positive	Positive	90/90	100 (95.9-100)
5	Flu B Moderate Positive	Positive	90/90	100 (95.9-100)
3	RSV Low Positive	Positive	90/90	100 (95.9-100)
5	RSV Moderate Positive	Positive	90/90	100 (95.9-100)

*There was one false positive Flu B result on the negative panel.

All moderate positive panel member components had total %CV less than or equal to 1.53 and total SD values less than or equal to 0.51. Low positive panel member components for SARS-CoV-2, Flu B, and RSV had total %CV less than or equal to 1.82 and total SD values less than or equal to 0.65. The total %CV and SD for the Flu A low positive panel member component were 10.92% and 3.77, respectively, due to the false negative result for 1 sample. For all positive panel member components, the within-run factor (i.e., random error) was the largest contributor to total variability (see Table 17).

Table 17: Reproducibility Study: Signal Variability of the Panther Fusion SARS-CoV-2/Flu A/B/RSV Assay by Positive Panel Component

					Contribution to Total Variance SD (%CV)				
Target Virus	Panel Member	Description	N	Mean Ct	Between Sites	Between Operators/Runs[1]	Between Days	Within Runs	Total Variance SD (%CV)
SARS-CoV-2	2	Low Pos	90	35.53	0.24 (0.68)	0.18 (0.50)	0.19 (0.52)	0.49 (1.38)	0.60 (1.70)
	4	Mod Pos	90	34.15	0.11 (0.32)	0.00 (0.00)	0.00 (0.00)	0.40 (1.16)	0.41 (1.20)
Flu A	2	Low Pos	90	34.55	0.57 (1.66)	0.62 (1.81)	0.00 (0.00)	3.68 (10.64)	3.77 (10.92)
	4	Mod Pos	90	33.55	0.09 (0.27)	0.03 (0.10)	0.17 (0.49)	0.48 (1.42)	0.51 (1.53)
Flu B	3	Low Pos	90	35.80	0.12 (0.35)	0.00 (0.00)	0.22 (0.60)	0.39 (1.10)	0.47 (1.30)

	5	Mod Pos	90	34.56	0.00 (0.00)	0.10 (0.29)	0.00 (0.00)	0.29 (0.83)	0.30 (0.88)
RSV	3	Low Pos	90	35.78	0.07 (0.20)	0.23 (0.65)	0.14 (0.39)	0.59 (1.64)	0.65 (1.82)
	5	Mod Pos	90	34.41	0.05 (0.14)	0.00 (0.00)	0.00 (0.00)	0.43 (1.25)	0.43 (1.26)

Ct = cycle threshold; CV = coefficient of variation; Mod = moderate; Pos = positive.
Note: Variability from some factors may be numerically negative; this can occur if the variability due to those factors is very small. In these cases, SD and %CV are displayed as 0.
[1]Between-Operator may be confounded with Between-Run; therefore, Between-Operator and Between-Run estimates are combined in Between-Operator/Run.

These results indicate that the repeatability and reproducibility of the PF SARS/Flu/RSV assay on the Panther Fusion system are robust in NP samples. These findings support the proposed intended use.

VIII. CONCLUSIONS

The analytical and clinical study results demonstrate that the Panther Fusion SARS-CoV-2/Flu A/B/RSV assay on the Panther Fusion system is substantially equivalent to the predicate device that is currently marketed for the same intended use. Hardware and software verification and validation demonstrate that the Panther Fusion SARS-CoV-2/Flu A/B/RSV assay on the Panther Fusion system will perform as intended.