510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY AND INSTRUMENT

I Background Information:

A 510(k) Number

K242465

B Applicant

Hologic, Inc.

C Proprietary and Established Names

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

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
QOF	Class II	21 CFR 866.3981 - 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	MI - Microbiology
OOI	Class II	21 CFR 862.2570 - Instrumentation for clinical multiplex test systems	CH - Clinical Chemistry

II Submission/Device Overview:

A Purpose for Submission:

To expand the Intended Use of the Panther Fusion SARS-CoV-2/Flu A/B/RSV assay, which was originally FDA-cleared under K222736 for use with nasopharyngeal swab (NPS) specimens in UTM/VTM or eSTM (RespDirect). In K241240, the intended use was expanded to include testing of anterior nasal (AN) swab specimens in UTM/VTM. This current submission includes

testing of AN swab specimens that are collected with the Hologic RespDirect Collection Kit and eluted in eSTM media.

B Measurand:
The Panther Fusion SARS-CoV-2/Flu A/B/RSV assay detects SARS-CoV-2, influenza A, influenza B, and respiratory syncytial virus RNA isolated from NPS specimens and AN swab specimens from individuals with signs and symptoms of a respiratory tract infection.

C Type of Test:
This assay is a multiplexed nucleic acid test that detects and differentiates SARS-CoV-2, influenza A, influenza B, and RSV through nucleic acid extraction, amplification, and detection using real-time RT-PCR. All steps of the assay are automated, after the manual addition of sample into the sample lysis tube (SLT) and performed within the Panther and Panther Fusion system.

III Intended Use/Indications for Use:

A Intended Use(s):
See Indications for Use below.

B Indication(s) for 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.

C Special Conditions for Use Statement(s):
Rx - For Prescription Use Only
For *in vitro* diagnostic use only

D Special Instrument Requirements:
For use with the Panther Fusion System, only.

IV Device/System Characteristics:

A 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 and anterior nasal swab specimens.

The Panther Fusion SARS-CoV-2/Flu A/B/RSV assay cartridge contains the same sample preparation and PCR reaction chemistry as the previously cleared Panther Fusion Flu A/B/RSV assay (K171963). To accommodate addition of the SARS-CoV-2 reagents (primers/probes) to the multiplexed reagents, minor changes were made to the previously cleared analyte primer/probe concentrations and RFU cutoffs. Additionally, the fluorophore for Flu B was changed from ROX to RED647 to accommodate the addition of SARS-CoV-2 to the assay.

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 Specimen Lysis Tube (SLT) containing specimen transport media (STM). Alternatively, samples can be collected with the RespDirect Collection kit which contains enhanced specimen transport media (eSTM). STM and eSTM lyse the cells, release target nucleic acid and protect them from degradation during storage.

b. *Nucleic acid capture and elution* - These steps take 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 each test specimen and controls via the working Panther Fusion Capture Reagent-S (wFCR-S). The IC-S in the reagent is used to monitor specimen processing, amplification, and detection. Magnetic particles with covalently bound oligonucleotides mediate the nucleic acid capture. Capture oligonucleotides hybridize to total nucleic acid in the test specimen. Hybridized nucleic acid is then separated from the lysed specimen in a magnetic field. Wash and aspiration steps remove extraneous components debris from the reaction tube. The elution step elutes purified nucleic acid.

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).

Hologic RespDirect Collection Kit
An ancillary collection kit that consists of the RespDirect Swab, intended for collection of NP swab and AN swab specimens, and the enhanced Direct Load Tube (eDLT), containing enhanced specimen transport media (eSTM). This transport media lyses cells, releasing target nucleic acids and protects them from degradation during storage.

B Principle of Operation:

The assay detects viral nucleic acids that have been extracted from a patient respiratory sample (i.e., NPS or AN swab specimens). A multiplex real-time RT-PCR reaction is carried out under optimized conditions generating amplicons for SARS-CoV-2, influenza A, influenza B, and RSV. The Internal Control-S (IC-S) is added to each test specimen before processing to act as a control for specimen processing, amplification, and detection. Identification of SARS-CoV-2, influenza A, influenza B, RSV, and the IC-S occurs using target-specific primers and fluorescent-labeled probes that hybridize to conserved regions in the viral genomes (**Table 1**).

Table 1. Assay Primer and Probe Targets

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-S	Not applicable*	RED677

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

C Instrument Description Information:

1. Instrument Name:
   Panther System and Panther Fusion System, software version 7.2.7 or 7.2.9.

2. Specimen Identification:
   Specimen identification is entered via barcode.

3. Specimen Sampling and Handling:
   NPS and AN swab specimens collected in transport media.

4. Calibration:
   Real Time Fluorometers (RTF) undergo a single calibration during manufacturing. No additional calibration is performed by the end user.

5. Quality Control:
   The assay contains an internal control (IC-S) which is added to each test specimen via the working Panther Fusion Capture Reagent-S (wFCR-S). The IC-S is used to monitor specimen processing, amplification, and detection.

   Two external controls are also included with this assay in a single use vial, the Panther Fusion SARS-CoV-2/Flu A/B/RSV Positive Control and the Panther Fusion Negative Control. The controls were validated in the analytical and clinical studies.

V Substantial Equivalence Information:

A Predicate Device Name(s):

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

B Predicate 510(k) Number(s):

C Comparison with Predicate(s):

Device & Predicate Device(s):	K242465 (Subject)	K241240 (Predicate)
Device Trade Name	Panther Fusion SARS-CoV-2/Flu A/B/RSV assay	Panther Fusion SARS-CoV-2/Flu A/B/RSV assay
General Device Characteristic Similarities		
Regulation Number/Name	Same	21 CFR 866.3981 – 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
Product Code(s)	Same	QOF, OOI
Prescription Use Only	Same	Yes
Platform	Same	Automated nucleic acid amplification platform. Uses Panther Fusion system for all steps including nucleic acid extraction, amplification, detection, and result processing
Technology/Principle of Operation	Same	Multiplexed polymerase chain reaction test
Assay Controls	Same	Internal and external controls
Time to Obtain Test Results	Same	~ 2.5 hours
Patient Population	Same	Individuals with signs and symptoms of respiratory tract infection
Intended User	Same	Professional user
Organisms Detected	Same	SARS-CoV-2, Flu A, Flu B, RSV (RSV A and RSV B)
General Device Characteristic Differences		
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	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

Device & Predicate Device(s):	K242465 (Subject)	K241240 (Predicate)
	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.	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. The Hologic RespDirect Collection Kit is cleared for NP swab specimens only for testing

Device & Predicate Device(s):	K242465 (Subject)	K241240 (Predicate)
		with the Panther Fusion SARS-CoV-2/Flu A/B/RSV assay.
Specimen Type/Transport Media Claims	• NPS in VTM/UTM or AN swab in VTM/UTM • NPS in eSTM (RespDirect) or AN swab in eSTM (RespDirect)	• NPS in VTM/UTM or AN swab in VTM/UTM • NPS in eSTM (RespDirect)
Adaptive Crosstalk Correction Implemented	Same	Yes

VI Standards/Guidance Documents Referenced:

Standards

- CLSI EP12-A2. User Protocol for Evaluation of Qualitative Test Performance; Approved Guideline – Second Edition.
- CLSI EP17-A2. Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline – Second Edition.
- CLSI EP25. Evaluation of Stability of *In Vitro* Medical Laboratory Test Reagents; Second Edition.
- CLSI EP37. Supplemental Tables for Interference Testing in Clinical Chemistry; First Edition.
- CLSI EP05-A3. Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline – Third Edition.
- CLSI MM13. Collection, Transport, Preparation, and Storage of Specimens for Molecular Methods; Second Edition.
- CLSI EP07. Interference Testing in Clinical Chemistry; Third Edition.
- CLSI EP15-A3. User Verification of Precision and Estimation of Bias; Approved Guideline – Third Edition.
- CLSI EP24-A2. Assessment of the Diagnostic Accuracy of Laboratory Testing Using Receiver Operating Characteristic Curves; Approved Guideline – Second Edition.
- CLSI EP25-A. Evaluation of Stability of *In Vitro* Diagnostic Reagents; Approved Guideline.

Special Controls

Class II Special Controls as per 21 CFR 866.3981

FDA Guidance Documents

- Respiratory Viral Panel Multiplex Nucleic Acid Assay – Class II Special Controls Guidance for Industry and FDA Staff, October 9, 2009.
- Guidance for Industry and Food and Drug Administration Staff: The 510(k) Program: Evaluating Substantial Equivalence in Premarket Notifications [510(k)], July 28, 2014.
- Guidance for Industry and FDA Staff: Content of Premarket Submissions for Device Software Functions, June 14, 2023.
- Guidance for Industry and Food and Drug Administration Staff: Electronic Submission Template for Medical Device 510(k) Submissions, October 2, 2023.

- Guidance for Industry and FDA Staff: Cybersecurity in Medical Devices: Quality System Considerations and Content of Premarket Submissions, September 27, 2023.

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

Since the initial clearance of the Panther Fusion SARS-CoV-2/Flu A/B/RSV assay (K222736), an Adaptive Crosstalk Correction (ACC) factor was implemented in the Assay Definition Module (ADM) software (K241240). No further device modifications have been introduced.

For those studies originally performed in K222736 and subsequently in K241240, there have been no device changes. Since there were no modifications to the assay (e.g., test reagent formulation, software, etc.), it was unnecessary to repeat several of the studies performed in support of K222736 or K241240. If a study was not repeated, a reference to where the results can be found (i.e., publicly available K222736 and K241240 Decision Summaries) has been made.

1. Precision/Reproducibility:
   a. Within-Laboratory Precision
      Please refer to the Within-Laboratory Precision Study data presented in the K222736 Decision Summary.

   b. Reproducibility
      Please refer to the Reproducibility Study data presented in the K222736 Decision Summary.

2. Linearity:
   Not applicable; this is a qualitative assay.

3. Analytical Specificity/Interference:

   Analytical Reactivity (Inclusivity)
   a. Wet-Testing
      Please refer to the Inclusivity Wet-Testing Study data presented in the K222736 Decision Summary.

   b. *In silico*
      Please refer to the Inclusivity *in silico* Study data presented in the K222736 Decision Summary.

   Exclusivity Testing
   Please refer to the Exclusivity Study data presented in the K222736 Decision Summary.

   Cross-Reactivity/Microbial Interference

   a. Wet-Testing
      Please refer to the Cross-Reactivity/Microbial Interference Wet-Testing Study data presented in the K222736 Decision Summary.

b. *In silico*
Please refer to the Cross-Reactivity *in silico* Study data presented in the K222736 Decision Summary.

Interfering Substances
Please refer to the Interfering Substance Study data presented in the K222736 Decision Summary.

Competitive Interference
Please refer to the Interfering Substance Study data presented in the K222736 and K241240 Decision Summaries.

4. Assay Reportable Range:
Not applicable; this is a qualitative assay.

5. Traceability, Stability, Expected Values (Controls, Calibrators, or Methods):
   a. Controls
   The assay contains an internal control (IC-S) added to each test specimen and external positive and negative controls. For more information, see **Section IV.C.5. Quality Control**, above.

   b. Sample Stability
   Please refer to the Sample Stability Study data presented in the K222736 Decision Summary.

   c. Kit Stability
   Please refer to the Kit Stability Study data presented in the K222736 Decision Summary.

   d. Shipping Stability
   Please refer to the Shipping Stability Study data presented in the K222736 Decision Summary.

6. Detection Limit:
Please refer to the Limit of Detection Study data presented in the K222736 and K241240 Decision Summaries.

7. Assay Cut-Off:
Please refer to the Assay Cut-Off Study data presented in the K222736 Decision Summary.

8. Accuracy (Instrument):
Not applicable.

9. Carry-Over:
Please refer to the Carry-Over Study data presented in the K222736 Decision Summary.

B Comparison Studies:

1. Method Comparison with Predicate Device:
Not applicable.

2. Matrix Comparison:
Please refer to the Matrix Comparison Study data presented in the K222736 Decision Summary.

C Clinical Studies:

1. Clinical Sensitivity:

Prospective Study to Expand the Intended Use to Include an AN swab specimens collected using RespDirect Collection Kit in eSTM media Specimen Claim
The clinical performance of the Panther Fusion SARS-CoV-2/Flu A/B/RSV assay was established in a multi-center study conducted with two patient-matched paired AN swab specimens that were prospectively collected (i.e., all comers between two time points who meet the inclusion criteria) from individuals with signs and symptoms of respiratory tract infections from January 2023 to May 2023. One AN swab specimen was collected using the RespDirect Collection Kit with eSTM media for candidate device testing and the other AN swab specimen was collected using a synthetic flocked swab by a healthcare professional (HCP) and eluted in UTM/VTM for comparator testing. The collection order for candidate and comparator specimens was randomized. AN swab specimens from nine geographically diverse clinical sites in the U.S. were enrolled and tested fresh (Category I specimens) or frozen (Category II specimens) with the Panther Fusion SARS-CoV-2/Flu A/B/RSV assay at three U.S. testing sites.

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 three highly sensitive U.S. FDA EUA SARS-CoV-2 molecular tests. A final CCA result was assigned when two of the three comparator assays were in concordance. The comparator method utilized to establish performance for the Flu A, Flu B, and RSV targets was a U.S. FDA-cleared molecular Flu A/B/RSV assay. All comparator testing was performed in accordance with the respective package inserts at one central laboratory.

A total of 1033 subjects (each providing patient-matched paired AN swab specimens) were acquired and enrolled for the prospective clinical study. Of these 1033 subjects, four were withdrawn because they did not meet the study eligibility criteria, leaving 1029 subjects. Of these 1029 subjects, 7 had their AN swab specimens excluded because they had an invalid candidate device result upon retesting and one specimen was withdrawn because the incorrect sample tube was used. Of the evaluable specimens, 1011 were test fresh (Category I specimens) with the Panther Fusion SARS-CoV-2/Flu A/B/RSV assay and 10 were frozen (Category II specimens) prior to testing. Furthermore, two specimens were excluded for SARS-CoV-2 performance analysis due to invalid or inconclusive CCA results. This left 1019 prospective AN swab specimens (1009 fresh, 10 frozen) with evaluable results for SARS-CoV-2 and 1021 AN swab specimens (1011 fresh, 10 frozen) with evaluable results for Flu A, Flu B, and RSV.

Of the 1033 subjects enrolled in the study, 1032 had their AN swab specimens tested with the candidate device. Eleven (11) of these specimens were invalid by the candidate device during testing, for an initial invalid rate of 1.1% (11/1032). Upon retesting, the invalid rate decreased to 0.7% (7/1032).

Table 2 provides a summary of demographic information for the 1021 evaluable specimens included in the prospective clinical study.

Table 2. Demographic Data for Prospectively Collected, Evaluable AN Swab Specimens in eSTM (RespDirect)

		Overall N (%)
Sex	Male	427 (41.8%)
	Female	594 (58.2%)
Age	<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%)
Total		1021

A summary of the Panther Fusion SARS-CoV-2/Flu A/B/RSV assay prospective clinical study performance is provided in **Table 3**.

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 assay 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 for SARS-CoV-2 underwent additional testing with two U.S. FDA EUA SARS-CoV-2 molecular tests, and discordant results for Flu A, Flu B or RSV underwent additional testing with a U.S. FDA EUA SARS-CoV-2/Flu A/B/RSV molecular test, when sufficient sample volume remained.

Table 3. Panther Fusion SARS-CoV-2/Flu A/B/RSV Assay Performance with Prospectively Collected AN Swab Specimens in eSTM (RespDirect)

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[1]**	**99.0**	**98.1-99.5**
Flu A	Fresh	11/11	100	74.1-100	999/1000	99.9	99.4-99.9
	Frozen	0/0	0	NC	10/10	100	72.3-100
	Overall	**11/11**	**100**	**74.1-100**	**1009/1010[2]**	**99.9**	**99.4-100**

Flu B	Fresh	5/6	83.3	43.6-97.0	1003/1005	99.8	99.3-100
	Frozen	0/0	0	NC	10/10	100	72.3-100
	Overall	**5/6[3]**	**83.3**	**43.6-97.0**	**1013/1015[4]**	**99.8**	**99.3-99.9**
RSV	Fresh	1/1	100	20.7-100	1009/1010	99.9	99.4-100
	Frozen	0/0	0	NC	10/10	100	72.3-100
	Overall	**1/1**	**100**	**20.7-100**	**1019/1020[5]**	**99.9**	**99.4-100**

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

[1]Seven (7) specimens with false positive SARS-CoV-2 results had sufficient sample volume remaining for discordant testing. All were negative for SARS-CoV-2 by two different U.S. FDA EUA SARS-CoV-2 molecular tests.

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

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

[4]Both specimens with false positive Flu B results were negative for Flu B by a U.S. FDA EUA SARS-CoV-2/Flu A/B/RSV molecular test.

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

The Panther Fusion SARS-CoV-2/Flu A/B/RSV assay reported one prospective, evaluable AN swab specimen in eSTM (RespDirect) with a co-infection (0.1% of all prospective specimens, 1/1021). The co-infection contained two pathogens, SARS-CoV-2 and Flu B. The sample was negative for all analytes by the comparator methods.

Data for prospective clinical study conducted with NP swab specimens and AN swab specimens in UTM/VTM are presented in the Decision Summaries for K222736 and K241240.

Supplemental Clinical Data for Low Prevalence Analytes (Category III Specimens)

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 media. To supplement the results of the prospective clinical study, an enrichment phase of the study was initiated which enrolled only symptomatic individuals who had obtained a Flu A, Flu B and/or RSV positive molecular standard of care (SOC) test within five days of enrollment. Enrichment occurred from October 2023- February 2024. One AN swab specimen was collected using the RespDirect Collection Kit with eSTM media for candidate device testing and the other AN swab specimen was collected using a synthetic flocked swab by a healthcare professional (HCP) and eluted in UTM/VTM for comparator testing. The collection order for candidate and comparator specimens was randomized. AN swab specimens from six geographically diverse clinical sites in the U.S. were enrolled and frozen prior to testing with the Panther Fusion SARS-CoV-2/Flu A/B/RSV assay at one U.S. testing site. The same U.S. FDA-cleared molecular Flu A/B/RSV assay was used as the comparator method; SARS-CoV-2 was not evaluated during the enrichment phase of the study.

A total of 210 subjects (each providing patient-matched paired AN swab specimens) were acquired and enrolled for the enrichment phase of the clinical study. Of these 210 subjects, five were withdrawn because they did not meet the study eligibility criteria, and one additional subject chose to withdraw from the study leaving a total of 204 evaluable AN swab specimens in eSTM (RespDirect) from the enrichment phase.

Of the 210 subjects enrolled in the study, 205 had their AN swab specimens tested with the candidate device. 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, the invalid rate decreased to 0% (0/205).

Table 4 provides a summary of demographic information for the 204 evaluable specimens included in the enrichment phase of the clinical study.

Table 4. Demographic Data for Evaluable AN Swab Specimens in eSTM (RespDirect) from Enrichment Phase

		Overall
Sex	Male	85 (41.7%)
	Female	119 (58.3%)
Age	<5 years	43 (21.1%)
	5-21 years	54 (26.5%)
	22-40 years	34 (16.7%)
	41-60 years	37 (18.1%)
	>60 years	36 (17.6%)
Total		204

A summary of the Panther Fusion SARS-CoV-2/Flu A/B/RSV assay performance from the enrichment phase of the clinical study is provided in **Table 5**.

Table 5. Panther Fusion SARS-CoV-2/Flu A/B/RSV Assay Performance with AN Swab Specimens in eSTM (RespDirect) from the Enrichment Phase

Analyte	**Positive Percent Agreement**			**Negative Percent Agreement[1]**		
	TP/ (TP+FN)	**%**	**95% CI**	**TN/ (TN+FP)**	**%**	**95% CI**
Flu A	69/71[2]	97.2	90.3-99.2	124/133[3]	93.2	87.6-96.4
Flu B	44/45[4]	97.8	88.4-99.6	158/159[5]	99.4	96.5-99.9
RSV	60/61[6]	98.4	91.3-99.7	137/143[7]	95.8	91.2-98.1

TP – true positive; FN – false negative; TN – true negative; FP – false positive

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

[2]Both specimens with false negative Flu A results were positive for Flu A by a U.S. FDA EUA SARS-CoV-2/Flu A/B/RSV molecular test.

[3]One (1) of the 9 samples with a false positive Flu A results was positive for Flu A by a U.S. FDA EUA SARS-CoV-2/Flu A/B/RSV molecular test and 8 of the 9 samples were negative for Flu A.

[4]The sample with a false negative Flu B result was negative for Flu B by a U.S. FDA EUA SARS-CoV-2/Flu A/B/RSV molecular test.

[5]The sample with a false positive Flu B result was positive for Flu B by a by a U.S. FDA EUA SARS-CoV-2/Flu A/B/RSV molecular test.

[6]The sample with a false negative RSV result was negative for RSV by a U.S. FDA EUA SARS-CoV-2/Flu A/B/RSV molecular test.

[7]One (1) of the 6 samples with a false positive RSV results was positive for RSV by a U.S. FDA EUA SARS-CoV-2/Flu A/B/RSV molecular test and 5 of the 6 samples were negative for RSV.

As shown in **Table 6**, the Panther Fusion SARS-CoV-2/Flu A/B/RSV assay reported 7 evaluable AN swab specimens in eSTM (RespDirect) in the enrichment phase with co-infections (3.4% of all enriched specimens, 7/204). All co-infections contained two pathogens, 4 contained Flu A and RSV, one contained SARS-CoV-2 and Flu A, one

contained SARS-CoV-2 and RSV and one contained Flu B and RSV. For the candidate results that were positive for Flu A and RSV, the comparator method did not detect RSV in two of the samples. 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.

Table 6. Co-infections Detected by the Panther Fusion SARS-CoV-2/ Flu A/B/RSV Assay in AN Swab Specimens during Enrichment Phase

Co-Infection Combination	Number of Specimens	Discrepant Co-Infections[1]
Flu A + RSV	4	2
SARS-CoV-2 + Flu A	1	ND[2]
SARS-CoV-2 + RSV	1	ND[2]
Flu B + RSV	1	0
Total	7	2

ND- Not determined.
[1]A discrepant co-infection was defined as a specimen that contains at least one pathogen detected by the Panther Fusion SARS-CoV-2/Flu A/B/RSV assay which was not detected by the comparator method.
[2]The comparator method does not detect SARS-CoV-2. Coinfections detected by the candidate assay that include SARS-CoV-2 cannot be confirmed.

2. Clinical Specificity:
   See section "Clinical Sensitivity" above.

3. Other Clinical Supportive Data (When 1. and 2. Are Not Applicable):
   Not applicable.

D Clinical Cut-Off:

Not applicable.

E Expected Values/Reference Range:

The Panther Fusion SARS-CoV-2/Flu A/B/RSV assay prospective clinical study included a total of 1033 prospectively collected AN swab specimens in eSTM (RespDirect), of which 1019 were evaluable for SARS-CoV-2 and 1021 were evaluable for Flu A, Flu B, and RSV. The number and percentage of cases positive for SARS-CoV-2, influenza A, influenza B, and RSV, as determined by the Panther Fusion SARS-CoV-2/Flu A/B/RSV assay, are presented in **Table 7**, stratified by collection site.

Table 7. Panther Fusion SARS-CoV-2/Flu A/B/RSV Assay – Expected Values by Specimen Collection Site for AN Swab Specimens in eSTM (RespDirect)

Site	SARS-CoV-2	Flu A	Flu B	RSV
Total	11.6% (118/1019)	1.2% (12/1021)	0.7% (7/1021)	0.2% (2/1021)
Site 1	50.0% (1/2)	0% (0/2)	0% (0/2)	0% (0/2)
Site 2	4.2% (1/24)	0% (0/24)	0% (0/24)	0% (0/24)

Site 3	2.2% (3/136)	0.7% (1/136)	0% (1/136)	0% (1/136)
Site 4	15.3% (42/275)	0.4% (1/276)	0% (0/276)	0% (0/276)
Site 5	18.6% (34/183)	0.5% (1/183)	0% (1/183)	0.5% (1/183)
Site 6	7.7% (2/26)	0% (0/26)	0% (0/26)	0% (0/26)
Site 7	19.3% (16/83)	1.2% (1/84)	2.4% (2/84)	0% (0/84)
Site 8	2.5% (4/160)	3.1% (5/160)	1.9% (3/160)	0.6% (1/160)
Site 9	11.5% (15/130)	2.3% (3/130)	1.5% (2/130)	0% (0/130)

F Other Supportive Instrument Performance Characteristics Data:
Not applicable.

VIII Proposed Labeling:

The labeling supports the finding of substantial equivalence for this device.

IX Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.