



**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION  
DECISION SUMMARY  
ASSAY ONLY**

**I Background Information:**

**A 510(k) Number**

K251538

**B Applicant**

Princeton BioMeditech Corp.

**C Proprietary and Established Names**

*Status* COVID-19/Flu A&B

**D Regulatory Information**

Product Code(s)	Classification	Regulation Section	Panel
SCA	Class II	21 CFR 866.3987 - Multi-Analyte Respiratory Virus Antigen Detection Test	MI - Microbiology

**II Submission/Device Overview:**

**A Purpose for Submission:**

To obtain a substantial equivalence determination for the *Status* COVID-19/Flu A&B test.

**B Measurand:**

Influenza A and B nucleoprotein antigens and SARS-CoV-2 nucleocapsid protein antigens

**C Type of Test:**

Qualitative lateral flow immunoassay.

**III Intended Use/Indications for Use:**

## **A Intended Use(s):**

See Indications for Use below.

## **B Indication(s) for Use:**

The *Status* COVID-19/Flu A&B test is a lateral flow immunoassay intended for the qualitative detection and differentiation of influenza A and influenza B nucleoprotein antigens and SARS-CoV-2 nucleocapsid antigen directly from nasopharyngeal (NP) or anterior nasal swab (ANS) specimens from individuals with signs and symptoms of respiratory tract infection. Clinical signs and symptoms of respiratory viral infection due to SARS-CoV-2 and influenza can be similar.

All negative results are presumptive and should be confirmed with a molecular assay, if necessary, for patient management. Negative results do not rule out infection with influenza or SARS-CoV-2 and should not be used as the sole basis for treatment or patient management decisions.

Positive results do not rule out bacterial infection or co-infection with other viruses.

## **C Special Conditions for Use Statement(s):**

Rx - For Prescription Use Only

## **D Special Instrument Requirements:**

Not applicable

# **IV Device/System Characteristics:**

## **A Device Description:**

The *Status* COVID-19/Flu A&B test is a lateral flow immuno-chromatographic assay which utilizes the chemical extraction of viral antigens followed by solid-phase immunoassay technology. The *Status* COVID-19/Flu A&B test is designed to detect antigens from SARS-CoV-2, influenza A, and /or influenza B in nasopharyngeal or anterior nasal swab specimens from individuals with signs and symptoms of respiratory infection. It is intended to aid in the rapid differential diagnosis of SARS-CoV-2, influenza A, and /or influenza B viral infections. The *Status* COVID-19/Flu A&B test is validated for use with direct specimens without transport media.

*Status* COVID-19/Flu A&B kit contains enough reagents and materials for 25 tests. The following components are included in a kit.

- *Status* COVID-19/Flu A&B test devices (25): The test strip in each device contains mouse monoclonal antibodies to nucleoprotein of influenza A, influenza B and nucleocapsid protein of SARS-CoV-2. The device is individually pouched.
- Extraction Reagent in vials (25): For use with swab specimens; 300 µL of phosphate buffer with detergents and preservative
- Sterile Swabs (25): For swab specimen collection

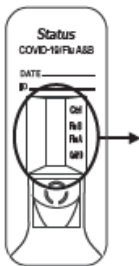








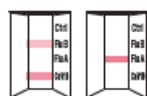
- Positive Control Swab (1): Influenza A, B, and SARS-CoV-2 antigen (non-infective recombinant protein)
- Negative Control Swab (1): Inactivated Group B Streptococcus antigen (non-infective)
- Package Insert/Instructions for use (IFU) (1)
- Quick Reference Instructions (QRI) (1)

## **B Principle of Operation:**

In the test procedure, a nasopharyngeal or anterior nasal swab specimen is collected and placed into extraction reagent in the extraction well of the test device for one minute. During this time the antigen is extracted from disrupted virus particles. The test device is then raised, tapped, and laid back down onto a level surface. Through this action, the solution of extracted specimen flows onto the test strip and migrates through the pads and membrane of the test strip. The pads contain detector antibodies conjugated to gold dye and the membrane contains immobilized capture antibodies. If SARS-CoV-2, influenza A, and/or influenza B antigens are present in the specimen, they will react with anti-SARS-CoV-2 antibody coupled to gold dye particles and/or anti-influenza antibody coupled to gold dye particles, migrate through the membrane as antigen-antibody-dye complexes, bind to the immobilized capture antibody line(s) on the membrane, and generate a colored line in the specific test line position. The rest of the sample and unbound/bound dye complexes continue to migrate to the control line position (Ctrl), where immobilized antibodies to the anti-SARS-CoV-2 and anti-influenza antibodies capture the dye complexes and form the control line. Formation of the control line serves as an internal control to demonstrate that test reagents are functional, antibody-dye conjugates in the dye pad have been hydrated and released and that sufficient sample has been applied to allow for migration through the test and control lines. If the control line does not appear within the designated incubation time, the result is invalid, and the test should be repeated using a new test device and specimen.

*Status* COVID-19/Flu A&B test has three test lines, one for SARS-CoV-2 (CoV19), one for influenza A (Flu A), and one for influenza B (Flu B). The three test lines allow for the separate and differential identification of SARS-CoV-2, influenza A, and/or B from a single specimen. If any test line appears in the test result window, together with the control line, the test result is positive for SARS-CoV-2 and/or influenza.

## C. Interpretation of Results:

INTERPRETATION OF RESULTS				
<p><b>A reddish purple CoV19, Flu A and/or Flu B Line(s) with Ctrl Line is positive.</b></p>				
	 <p>Flu A line: Influenza type A</p>	 <p>Flu B line: Influenza type B</p>	 <p>CoV19 line: COVID-19</p>	
 <p>Flu A &amp; CoV19 lines: Influenza type A &amp; COVID-19*</p>	 <p>Flu B &amp; CoV19 lines: Influenza type B &amp; COVID-19*</p>	 <p>Flu A &amp; Flu B lines: Influenza type A &amp; B*</p>	 <p>Flu A, Flu B &amp; CoV19 lines: Influenza type A, B &amp; COVID-19*</p>	
<p><b>*NOTE: Co-infection with Influenza A, B and/or SARS-CoV-2 is rare. If results are positive for more than one antigen, i.e., Flu A, B and/or COVID-19, the patient specimens should be re-tested.</b></p>				
		<p><b>Ctrl Line only Negative (-)</b></p>  <p><b>Negative Results are presumptive and may need to be confirmed with a molecular assay.</b></p>		<p><b>No Ctrl Line Invalid</b></p>  <p><b>Repeat with new sample and device.</b></p>

## V Substantial Equivalence Information:

### A Predicate Device Name(s):

Healgen Rapid Check COVID-19/Flu A&B Antigen Test

### B Predicate 510(k) Number(s):

DEN240029

### C Comparison with Predicate(s):

Device & Predicate Device(s):	<u>K251538</u>	<u>DEN240029</u>
Device trade name	Status COVID-19/Flu A&B	Healgen Rapid Check COVID-19/Flu A&B Antigen Test
General Device Characteristic Similarities		

Intended use/Indications for use	<p>The <i>Status</i> COVID-19/Flu A&amp;B test is a lateral flow immunoassay intended for the qualitative detection and differentiation of influenza A and influenza B nucleoprotein antigens and SARS-CoV-2 nucleocapsid antigen directly from nasopharyngeal (NP) or anterior nasal swab (ANS) specimens from individuals with signs and symptoms of respiratory tract infection. Clinical signs and symptoms of respiratory viral infection due to SARS-CoV-2 and influenza can be similar.</p> <p>All negative results are presumptive and should be confirmed with a molecular assay, if necessary, for patient management. Negative results do not rule out infection with influenza or SARS-CoV-2 and should not be used as the sole basis for treatment or patient management decisions.</p> <p>Positive results do not rule out bacterial infection or co-infection with other viruses.</p>	<p>The Healgen Rapid Check COVID-19/Flu A&amp;B Antigen Test is a lateral flow immunochromatographic assay intended for the qualitative detection and differentiation of influenza A, and influenza B nucleoprotein antigens and SARS-CoV-2 nucleocapsid antigen directly in anterior nasal swab samples from individuals with signs and symptoms of respiratory tract infection. Symptoms of respiratory infections due to SARS-CoV-2 and influenza can be similar. This test is for non-prescription home use by individuals aged 14 years or older testing themselves, or adults testing individuals aged 2 years or older.</p> <p>All negative results are presumptive and should be confirmed with an FDA-cleared molecular assay when determined to be appropriate by a healthcare provider. Negative results do not rule out infection with influenza, SARS-CoV-2 or other pathogens. Individuals who test negative and experience continued or worsening respiratory symptoms, such as fever, cough and/or shortness of breath, should therefore seek follow-up care from their healthcare provider.</p> <p>Positive results do not rule out co-infection with other respiratory pathogens and therefore do not substitute for a visit to a healthcare provider or appropriate follow-up.</p>
Regulation number	21 CFR 866.3987	Same
Assay principle (Technology)	Lateral flow immune chromatographic assay	Same
Analyte	influenza A, and influenza B nucleoprotein antigens and SARS-CoV-2 nucleocapsid antigen	Same
Test results	Qualitative	Same
Result interpretation	Visually read	Same
Time to results	15-20 minutes	Same
<b>General Device Characteristic Differences</b>		

Patient use setting	For prescription use only by healthcare providers in high to moderate complexity labs and CLIA-waived settings	Over the counter use/self-testing
Samples type	Nasopharyngeal swab and anterior nasal swab	Anterior nasal swab

## VI Standards/Guidance Documents Referenced:

Document	Title	Publisher	Applicable study
21 CFR 866.3987	Special controls for multi analyte respiratory virus antigen detection test, an in vitro diagnostic device intended for the detection and/or differentiation of respiratory viruses directly from respiratory clinical specimens. The device is intended to be performed at the site of sample collection, does not involve sample storage and/or transport	FDA/CDRH	All studies
11135:2014	Sterilization of health care products - Ethylene oxide - Requirements for development, validation and routine control of a sterilization process for medical devices	ISO	Sterility
10993-7	Biological Evaluation of Medical Devices – Part 7: Ethylene Oxide Sterilization Residuals	ISO	Sterility
10993-1 Fifth edition 2018-08	Biological Evaluation of Medical Devices – Evaluation testing within risk management process	ISO	Biocompatibility
10993-5: Third Edition 2009-06-01	Biological Evaluation of Medical Devices - Tests for in vitro cytotoxicity	ISO	Biocompatibility
10993-10: 2021 Fourth Edition 2021-11	Biological Evaluation of Medical Devices –Tests for irritation and skin sensitization	ISO	Biocompatibility
14971:2019: Third Edition 2019-12	Biological Evaluation of Medical Devices – Application of risk management to medical devices	ISO	Biocompatibility
14971:2019	Medical devices: Application of risk management to medical devices	ISO	Risk Management

## Performance Characteristics (if/when applicable):

### A Analytical Performance:

1. Precision/Reproducibility:

The precision and reproducibility studies were conducted separately.

**a. Multi-Lot Precision:**

Two precision studies were conducted to evaluate the lot-to-lot variability of the *Status* COVID-19/Flu A&B using contrived samples containing heat-inactivated SARS-CoV-2: omicron variant, live influenza (Flu) A: H3N2/Darwin/9/21, and live Flu B: Yamagata/Phuket/3073/13. Both studies were conducted at a single internal site and are described below separately, and results are summarized in Table 1.

**Study 1** assessed test performance using test samples prepared as follows:

- i. Negative sample (without any analyte)
- ii. Low positive: each analyte at 1x LoD
- iii. Moderate positive: each analyte at 3x LoD

Fifty (50) µL of each coded sample was applied to dry nasal swab and processed per the IFU. Blinded and randomized samples were tested using three (3) device lots by two (2) operators over ten (10) non-consecutive days (2 runs/day, 2 replicates/run), generating 240 total results per analyte.

**Study 2** specifically evaluated lot-to-lot variability using the same viral strains at the following concentrations:

- i. Negative sample (without any analyte)
- ii. Very low positive: each analyte at 0.7x LoD
- iii. Low positive: each analyte at 1x LoD
- iv. Moderate positive: each analyte at 3x LoD

Two (2) operators tested three (3) different lots of the *Status* COVID-19/Flu A&B over three (3) non-consecutive days (2 runs/day, 2 replicates/run), generating 72 results per analyte.

The results from studies 1 and 2 demonstrated that all negative samples and those prepared at 3x LoD exhibited 100% agreement with expected results across the operators, lots, days, and runs. Samples prepared at 1x LoD, demonstrated greater than 95% agreement with the expected result across all test conditions, indicating consistent performance at the limit of detection. As expected, samples prepared at 0.7x LoD exhibited slightly lower precision (<95%), consistent with inherent variability at below the LoD level. However, the performance was consistent across all three lots tested. The results from study 1 and study 2 are summarized below.

**Table 1.** Lot-to-lot Precision Study Results

Sample	Analyte	# of positive result/# of total tested (% positive rate)			Total sample count (% positive rate)
		Lot 1	Lot 2	Lot 3	
Negative	Influenza A	0/104 (0.0%)	0/104 (0.0%)	0/104 (0.0%)	312/312 (0.0%)
	Influenza B	0/104 (0.0%)	0/104 (0.0%)	0/104 (0.0%)	312/312 (0.0%)
	SARS-CoV-2	0/104 (0.0%)	0/104 (0.0%)	0/104 (0.0%)	312/312 (0.0%)

0.7x LoD	Influenza A	9/24 (37.5%)	11/24 (45.8%)	12/24 (50.0%)	32/72 (44.4%)
	Influenza B	22/24 (91.7%)	22/24 (91.7%)	20/24 (83.3%)	64/72 (88.9%)
	SARS-CoV-2	15/24 (62.5%)	14/24 (58.3%)	16/24 (66.7%)	45/72 (62.5%)
1x LoD	Influenza A	102/104 (98.1%)	103/104 (99.0%)	102/104 (98.1%)	307/312 (98.4%)
	Influenza B	101/104 (97.1%)	102/104 (98.1%)	104/104 (100.0%)	307/312 (98.4%)
	SARS-CoV-2	104/104 (100.0%)	102/104 (98.1%)	103/104 (99.0%)	310/312 (99.4%)
3xLoD	Influenza A	104/104 (100.0%)	104/104 (100.0%)	104/104 (100.0%)	312/312 (100.0%)
	Influenza B	104/104 (100.0%)	104/104 (100.0%)	104/104 (100.0%)	312/312 (100.0%)
	SARS-CoV-2	104/104 (100.0%)	104/104 (100.0%)	104/104 (100.0%)	312/312 (100.0%)

**b. Multi-Site Reproducibility Study:**

A multi-site reproducibility study was performed to assess the performance of the candidate device using a contrived sample panel comprised of a true negative, a high negative sample (C5, 95% expected to be negative), a low positive (1x LoD), and a moderate positive (3x LoD) sample for each analyte. The study was conducted by untrained operators in CLIA waived settings over five non-consecutive days.

Contrived swab samples were prepared by spiking pooled human nasal wash using the same panel of SARS-CoV-2, influenza A, and influenza B strains as described above in the precision study. Each diluted sample (50 µL) was directly applied onto the sample collection swab head. True negative swab samples were prepared by applying fifty (50) µL of negative pooled human nasal wash directly onto the sample collection swab head.

The contrived sample swabs were randomized and blinded to each operator at three (3) CLIA-waived sites and one in-house site. Nine (9) untrained operators at the CLIA waived sites and three (3) trained operators at the internal site conducted testing. A total 10 panels were prepared, where each panel consisted of 4 samples at different concentration of each analyte. Each operator tested 10 panels in replicates of 2, 2 runs per day, and on 3 lots of devices for over 5 days.

The results are shown below in Table 2 below. These outcomes all met the predefined acceptance criteria and generated no significant difference between sites.

**Table 2.** Summary of Multi-site Reproducibility Study Results

Sample		# of positive result/# of total tested (% positive rate)				Total sample count (% positive rate)
		Site 1	Site 2	Site 3	Internal Site	
True Negative	Influenza A	0/180 (0.0%)	0/180 (0.0%)	0/180 (0.0%)	0/180 (0.0%)	0/720 (0.0%)
	Influenza B	0/180 (0.0%)	0/180 (0.0%)	0/180 (0.0%)	0/180 (0.0%)	0/720 (0.0%)



	SARS-CoV-2	0/180 (0.0%)	0/180 (0.0%)	0/180 (0.0%)	0/180 (0.0%)	0/720 (0.0%)
High Negative	Influenza A	2/180 (1.1%)	2/180 (1.1%)	1/180 (0.6%)	2/180 (1.1%)	7/720 (0.9%)
	Influenza B	3/180 (1.7%)	1/180 (0.6%)	2/180 (0.6%)	2/180 (1.1%)	8/720 (1.1%)
	SARS-CoV-2	1/180 (0.6%)	2/180 (1.1%)	1/180 (0.6%)	1/180 (0.6%)	5/720 (0.7%)
1x LoD	Influenza A	178/180 (98.9%)	177/180 (98.3%)	178/180 (98.9%)	179/180 (99.4%)	712/720 (98.9%)
	Influenza B	177/180 (98.3%)	178/180 (98.9%)	177/180 (98.3%)	179/180 (99.4%)	711/720 (98.8%)
	SARS-CoV-2	179/180 (99.4%)	179/180 (99.4%)	178/180 (98.9%)	180/180 (100%)	716/720 (99.4%)
3x LoD	Influenza A	180/180 (100%)	180/180 (100%)	180/180 (100%)	180/180 (100%)	720/720 (100%)
	Influenza B	180/180 (100%)	180/180 (100%)	180/180 (100%)	180/180 (100%)	720/720 (100%)
	SARS-CoV-2	180/180 (100%)	180/180 (100%)	180/180 (100%)	180/180 (100%)	720/720 (100%)

2. Linearity:

Not applicable. This is a qualitative assay with binary, visually read results.

3. Analytical Specificity/Interference:

***a. Cross Reactivity and Microbial Interference:***

Cross reactivity and microbial interference studies were conducted to determine potential assay interference from other respiratory pathogens/microbial flora that may be present in nasal swab samples. A comprehensive panel of viruses, bacteria, fungi, and pooled nasal wash was evaluated.

For the cross-reactivity study, organisms were diluted in pooled nasal swab matrix (PNSM) and tested in triplicates in the absence of SARS-CoV-2, influenza A, and influenza B. No cross-reactivity was observed with the organisms tested for any of the 3 analytes (Table 3).

For the microbial interference study, organisms were diluted in PNSM in the presence of low levels (3x LoD) of heat inactivated SARS-CoV-2 (SARS-CoV-2 USA-WA1/2020), live influenza A (H3N2; Darwin/9/21), and live influenza B (Yamagata; Phuket/3073/13) spiked individually and tested in triplicate. No microbial interference was observed for any of the 3 analytes tested (Table below).

**Table 3. Cross Reactivity and Microbial Interference Study Results**

Microorganism	Conc.	Analyte	Test Results	
			Cross reactivity	Microbial interference

			n/N <sup>1</sup>	Result Agreement <sup>2</sup> (%)	n/N <sup>1</sup>	Result Agreement <sup>2</sup> (%)
<b>Adenovirus 1</b>	1.43 X 10 <sup>5</sup> TCID <sub>50</sub> /mL	SARS-CoV-2	0/3	100%	3/3	100%
		Flu A	0/3	100%	3/3	100%
		Flu B	0/3	100%	3/3	100%
<b>Adenovirus 7A</b>	1.43 X 10 <sup>5</sup> TCID <sub>50</sub> /mL	SARS-CoV-2	0/3	100%	3/3	100%
		Flu A	0/3	100%	3/3	100%
		Flu B	0/3	100%	3/3	100%
<b>Enterovirus</b>	1.43 X 10 <sup>5</sup> TCID <sub>50</sub> /mL	SARS-CoV-2	0/3	100%	3/3	100%
		Flu A	0/3	100%	3/3	100%
		Flu B	0/3	100%	3/3	100%
<b>Human coronavirus (OC43)</b>	1.43 X 10 <sup>5</sup> TCID <sub>50</sub> /mL	SARS-CoV-2	0/3	100%	3/3	100%
		Flu A	0/3	100%	3/3	100%
		Flu B	0/3	100%	3/3	100%
<b>Human coronavirus (229E)<sup>1</sup></b>	7.05 X 10 <sup>4</sup> TCID <sub>50</sub> /mL	SARS-CoV-2	0/3	100%	3/3	100%
		Flu A	0/3	100%	3/3	100%
		Flu B	0/3	100%	3/3	100%
<b>Human coronavirus (NL63)</b>	1.43 X 10 <sup>5</sup> TCID <sub>50</sub> /mL	SARS-CoV-2	0/3	100%	3/3	100%
		Flu A	0/3	100%	3/3	100%
		Flu B	0/3	100%	3/3	100%
<b>Human metapneumovirus (hMPV)<sup>1</sup></b>	5.85 X 10 <sup>4</sup> TCID <sub>50</sub> /mL	SARS-CoV-2	0/3	100%	3/3	100%
		Flu A	0/3	100%	3/3	100%
		Flu B	0/3	100%	3/3	100%
<b>Parainfluenza virus type 1</b>	1.43 X 10 <sup>5</sup> TCID <sub>50</sub> /mL	SARS-CoV-2	0/3	100%	3/3	100%
		Flu A	0/3	100%	3/3	100%
		Flu B	0/3	100%	3/3	100%
<b>Parainfluenza virus type 2</b>	1.43 X 10 <sup>5</sup> TCID <sub>50</sub> /mL	SARS-CoV-2	0/3	100%	3/3	100%
		Flu A	0/3	100%	3/3	100%
		Flu B	0/3	100%	3/3	100%
<b>Parainfluenza virus type 3</b>	1.43 X 10 <sup>5</sup> TCID <sub>50</sub> /mL	SARS-CoV-2	0/3	100%	3/3	100%
		Flu A	0/3	100%	3/3	100%
		Flu B	0/3	100%	3/3	100%
<b>Parainfluenza virus type 4<sup>1</sup></b>	7.05 X 10 <sup>4</sup> TCID <sub>50</sub> /mL	SARS-CoV-2	0/3	100%	3/3	100%
		Flu A	0/3	100%	3/3	100%
		Flu B	0/3	100%	3/3	100%
<b>Influenza A, H1N1</b>	1.43 X 10 <sup>5</sup> TCID <sub>50</sub> /mL	SARS-CoV-2	0/3	100%	3/3	100%
		Flu A	3/3	N/A	3/3	N/A
		Flu B	0/3	100%	3/3	100%
<b>Influenza A, H3N2</b>	1.43 X 10 <sup>5</sup> TCID <sub>50</sub> /mL	SARS-CoV-2	0/3	100%	3/3	100%
		Flu A	3/3	N/A	3/3	N/A
		Flu B	0/3	100%	3/3	100%
<b>Influenza B, Victoria</b>	1.43 X 10 <sup>5</sup> TCID <sub>50</sub> /mL	SARS-CoV-2	0/3	100%	3/3	100%
		Flu A	0/3	100%	3/3	100%
		Flu B	3/3	N/A	3/3	N/A

<b>Influenza B, Yamagata</b>	1.43 X 10 <sup>5</sup> TCID <sub>50</sub> /mL	SARS-CoV-2	0/3	100%	3/3	100%
		Flu A	0/3	100%	3/3	100%
		Flu B	3/3	N/A	3/3	N/A
<b>Respiratory Syncytial virus A</b>	1.43 X 10 <sup>5</sup> TCID <sub>50</sub> /mL	SARS-CoV-2	0/3	100%	3/3	100%
		Flu A	0/3	100%	3/3	100%
		Flu B	0/3	100%	3/3	100%
<b>Respiratory Syncytial virus B</b>	1.43 X 10 <sup>5</sup> TCID <sub>50</sub> /mL	SARS-CoV-2	0/3	100%	3/3	100%
		Flu A	0/3	100%	3/3	100%
		Flu B	0/3	100%	3/3	100%
<b>Rhinovirus</b>	1.43 X 10 <sup>5</sup> TCID <sub>50</sub> /mL	SARS-CoV-2	0/3	100%	3/3	100%
		Flu A	0/3	100%	3/3	100%
		Flu B	0/3	100%	3/3	100%
<b>Cytomegalovirus</b>	1.43 X 10 <sup>5</sup> TCID <sub>50</sub> /mL	SARS-CoV-2	0/3	100%	3/3	100%
		Flu A	0/3	100%	3/3	100%
		Flu B	0/3	100%	3/3	100%
<b>Epstein-Barr Virus</b>	1.43 X 10 <sup>5</sup> TCID <sub>50</sub> /mL	SARS-CoV-2	0/3	100%	3/3	100%
		Flu A	0/3	100%	3/3	100%
		Flu B	0/3	100%	3/3	100%
<b>Measles virus</b>	1.43 X 10 <sup>5</sup> TCID <sub>50</sub> /mL	SARS-CoV-2	0/3	100%	3/3	100%
		Flu A	0/3	100%	3/3	100%
		Flu B	0/3	100%	3/3	100%
<b>Mumps virus</b>	1.43 X 10 <sup>5</sup> TCID <sub>50</sub> /mL	SARS-CoV-2	0/3	100%	3/3	100%
		Flu A	0/3	100%	3/3	100%
		Flu B	0/3	100%	3/3	100%
<b>Coxsackievirus A16</b>	1.43 X 10 <sup>5</sup> TCID <sub>50</sub> /mL	SARS-CoV-2	0/3	100%	3/3	100%
		Flu A	0/3	100%	3/3	100%
		Flu B	0/3	100%	3/3	100%
<b>Human Herpes virus</b>	1.43 X 10 <sup>5</sup> TCID <sub>50</sub> /mL	SARS-CoV-2	0/3	100%	3/3	100%
		Flu A	0/3	100%	3/3	100%
		Flu B	0/3	100%	3/3	100%
<b>Human coronavirus HKU1<sup>2</sup></b>	5-Clinical samples	SARS-CoV-2	0/5	100%	5/5	100%
		Flu A	0/5	100%	5/5	100%
		Flu B	0/5	100%	5/5	100%
<b><i>Bordetella pertussis</i></b>	1.0 X 10 <sup>6</sup> CFU/ml	SARS-CoV-2	0/3	100%	3/3	100%
		Flu A	0/3	100%	3/3	100%
		Flu B	0/3	100%	3/3	100%
<b><i>Chlamydophila pneumoniae</i></b>	1.0 X 10 <sup>6</sup> IFU/ml	SARS-CoV-2	0/3	100%	3/3	100%
		Flu A	0/3	100%	3/3	100%
		Flu B	0/3	100%	3/3	100%
<b><i>Haemophilus influenzae</i></b>	1.0 X 10 <sup>6</sup> CFU/ml	SARS-CoV-2	0/3	100%	3/3	100%
		Flu A	0/3	100%	3/3	100%
		Flu B	0/3	100%	3/3	100%
<b><i>Legionella pneumophila</i></b>	1.0 X 10 <sup>6</sup> CFU/ml	SARS-CoV-2	0/3	100%	3/3	100%
		Flu A	0/3	100%	3/3	100%

		Flu B	0/3	100%	3/3	100%
<i>Mycoplasma pneumoniae</i>	1.0 X 10 <sup>6</sup> CFU/ml	SARS-CoV-2	0/3	100%	3/3	100%
		Flu A	0/3	100%	3/3	100%
		Flu B	0/3	100%	3/3	100%
<i>Streptococcus pneumoniae</i>	1.0 X 10 <sup>6</sup> CFU/ml	SARS-CoV-2	0/3	100%	3/3	100%
		Flu A	0/3	100%	3/3	100%
		Flu B	0/3	100%	3/3	100%
<i>Streptococcus pyogenes</i>	1.0 X 10 <sup>6</sup> CFU/ml	SARS-CoV-2	0/3	100%	3/3	100%
		Flu A	0/3	100%	3/3	100%
		Flu B	0/3	100%	3/3	100%
<i>Staphylococcus aureus</i>	1.0 X 10 <sup>6</sup> CFU/ml	SARS-CoV-2	0/3	100%	3/3	100%
		Flu A	0/3	100%	3/3	100%
		Flu B	0/3	100%	3/3	100%
<i>Staphylococcus epidermidis</i>	1.0 X 10 <sup>6</sup> CFU/ml	SARS-CoV-2	0/3	100%	3/3	100%
		Flu A	0/3	100%	3/3	100%
		Flu B	0/3	100%	3/3	100%
<i>Candida albicans</i>	1.0 X 10 <sup>6</sup> CFU/ml	SARS-CoV-2	0/3	100%	3/3	100%
		Flu A	0/3	100%	3/3	100%
		Flu B	0/3	100%	3/3	100%
<i>Moraxella catarrhalis</i>	1.0 X 10 <sup>6</sup> CFU/ml	SARS-CoV-2	0/3	100%	3/3	100%
		Flu A	0/3	100%	3/3	100%
		Flu B	0/3	100%	3/3	100%
<i>Neisseria meningitidis</i>	1.0 X 10 <sup>6</sup> CFU/ml	SARS-CoV-2	0/3	100%	3/3	100%
		Flu A	0/3	100%	3/3	100%
		Flu B	0/3	100%	3/3	100%
<i>Neisseria subflava biovarflava</i>	1.0 X 10 <sup>6</sup> CFU/ml	SARS-CoV-2	0/3	100%	3/3	100%
		Flu A	0/3	100%	3/3	100%
		Flu B	0/3	100%	3/3	100%
<i>Corynebacterium diphtheriae</i>	1.0 X 10 <sup>6</sup> CFU/ml	SARS-CoV-2	0/3	100%	3/3	100%
		Flu A	0/3	100%	3/3	100%
		Flu B	0/3	100%	3/3	100%
<i>Escherichia coli</i>	1.0 X 10 <sup>6</sup> CFU/ml	SARS-CoV-2	0/3	100%	3/3	100%
		Flu A	0/3	100%	3/3	100%
		Flu B	0/3	100%	3/3	100%
<i>Mycobacterium tuberculosis</i>	1.0 X 10 <sup>6</sup> CFU/ml	SARS-CoV-2	0/3	100%	3/3	100%
		Flu A	0/3	100%	3/3	100%
		Flu B	0/3	100%	3/3	100%
<i>Lactobacillus acidophilus</i>	1.0 X 10 <sup>6</sup> CFU/ml	SARS-CoV-2	0/3	100%	3/3	100%
		Flu A	0/3	100%	3/3	100%
		Flu B	0/3	100%	3/3	100%
<i>Pneumocystis jirovecii-S. cervisiae (Recombinant)</i>	1.0 X 10 <sup>6</sup> CFU/ml	SARS-CoV-2	0/3	100%	3/3	100%
		Flu A	0/3	100%	3/3	100%
		Flu B	0/3	100%	3/3	100%
<i>Pseudomonas aeruginosa</i>	1.0 X 10 <sup>6</sup> CFU/ml	SARS-CoV-2	0/3	100%	3/3	100%
		Flu A	0/3	100%	3/3	100%

		Flu B	0/3	100%	3/3	100%
<i>Streptococcus salivarius</i>	1.0 X 10 <sup>6</sup> CFU/ml	SARS-CoV-2	0/3	100%	3/3	100%
		Flu A	0/3	100%	3/3	100%
		Flu B	0/3	100%	3/3	100%
<i>Klebsiella pneumoniae</i>	1.0 X 10 <sup>6</sup> CFU/ml	SARS-CoV-2	0/3	100%	3/3	100%
		Flu A	0/3	100%	3/3	100%
		Flu B	0/3	100%	3/3	100%
<b>Pooled human nasal wash</b>	N/A	SARS-CoV-2	0/3	100%	3/3	100%
		Flu A	0/3	100%	3/3	100%
		Flu B	0/3	100%	3/3	100%

1) Recommended testing concentrations were not achievable due to the low vial concentrations.

2) Five (5) Human Coronavirus HKU1 (HCoV-HKU1) clinical samples were tested in the presence and absence of SARS-CoV-2.

3) For MERS and SARS-CoV viruses only In silico analysis was conducted therefore cross reactivity cannot be ruled out

### **b. Competitive Interference:**

A competitive inhibition study was conducted to evaluate the potential for a high concentration of one target analyte to interfere with the detection of another target analyte at a low concentration. Testing was performed in triplicate with different combinations of low (3x LoD) and high concentrations (1000x LoD) of SARS-CoV-2, influenza A, and influenza B. The study used inactivated SARS-CoV-2 and live influenza A and B virus strains. There was no competitive interference observed.

**Table 4.** Competitive Inhibition Study Results

Testing panel	Viral targets in sample			Results (# pos / total reps)		
	Influenza A	Influenza B	SARS-CoV-2	Influenza A	Influenza B	SARS- CoV-2
1	1000x LoD	3x LoD	Negative	3/3	3/3	0/3
2	1000x LoD	Negative	3x LoD	3/3	0/3	3/3
3	1000x LoD	3x LoD	3x LoD	3/3	3/3	3/3
4	3x LoD	1000x LoD	Negative	3/3	3/3	0/3
5	Negative	1000x LoD	3x LoD	0/3	3/3	3/3
6	3x LoD	1000x LoD	3x LoD	3/3	3/3	3/3
7	3x LoD	Negative	300x LoD	3/3	0/3	3/3
8	Negative	3x LoD	300x LoD	0/3	3/3	3/3
9	3x LoD	3x LoD	300x LoD	3/3	3/3	3/3

### **c. Endogenous/Exogenous Substances Interference:**

The *Status* COVID-19/Flu A&B test was evaluated for performance in the presence of a panel of common interfering endogenous and exogenous substances. Potentially interfering substances were prepared and diluted in PNSM to the recommended concentration. Virus negative PNSM specimens were evaluated in triplicate to confirm that the potentially interfering substances were not cross-reactive with the test. Positive samples were also prepared in PNSM at 3x LoD using each analyte individually and were evaluated in the presence of interfering substances in triplicate to confirm that these substances do not interfere with detection of SARS-CoV-2, influenza A, and influenza B. False positive results for both influenza A and influenza B were observed when FluMist was tested at a 15% v/v

concentration. At the 1.5% v/v concentration, false positive results were observed for influenza A only, while no interference was detected for influenza B at this concentration. When tested at  $\leq 0.15\%$  v/v concentration, no cross reactivity or interference was observed for either analyte. All other substances tested demonstrated no cross-reactivity or interference with the assay.

**Table 5. Interfering Substances Study Results**

Potential Interfering substance	Tested Concentration	Analyte	Test results			
			No analyte		With analyte	
			n/N <sup>1</sup>	Result Agreement <sup>2</sup> (%)	n/N <sup>1</sup>	Result Agreement <sup>2</sup> (%)
Human Whole Blood	4% v/v	SARS-CoV-2	0/3	100%	3/3	100%
		Flu A	0/3	100%	3/3	100%
		Flu B	0/3	100%	3/3	100%
Mucin	5.0 mg/mL	SARS-CoV-2	0/3	100%	3/3	100%
		Flu A	0/3	100%	3/3	100%
		Flu B	0/3	100%	3/3	100%
Leukocytes	5x10 <sup>6</sup> cells/mL	SARS-CoV-2	0/3	100%	3/3	100%
		Flu A	0/3	100%	3/3	100%
		Flu B	0/3	100%	3/3	100%
Oral Anesthetic (Benzocaine)	3.0 mg/mL	SARS-CoV-2	0/3	100%	3/3	100%
		Flu A	0/3	100%	3/3	100%
		Flu B	0/3	100%	3/3	100%
Oral Anesthetic (Menthol)	3.0 mg/mL	SARS-CoV-2	0/3	100%	3/3	100%
		Flu A	0/3	100%	3/3	100%
		Flu B	0/3	100%	3/3	100%
Sore Throat Phenol Spray	15% v/v	SARS-CoV-2	0/3	100%	3/3	100%
		Flu A	0/3	100%	3/3	100%
		Flu B	0/3	100%	3/3	100%
Nasal Spray (Phenylephrine)	15% v/v	SARS-CoV-2	0/3	100%	3/3	100%
		Flu A	0/3	100%	3/3	100%
		Flu B	0/3	100%	3/3	100%
Nasal Spray (Cromolyn)	15% v/v	SARS-CoV-2	0/3	100%	3/3	100%
		Flu A	0/3	100%	3/3	100%
		Flu B	0/3	100%	3/3	100%
Nasal Spray (Oxymetazoline)	15% v/v	SARS-CoV-2	0/3	100%	3/3	100%
		Flu A	0/3	100%	3/3	100%
		Flu B	0/3	100%	3/3	100%
Nasal Spray (Sodium chloride with preservatives)	15% v/v	SARS-CoV-2	0/3	100%	3/3	100%
		Flu A	0/3	100%	3/3	100%
		Flu B	0/3	100%	3/3	100%
	15% v/v	SARS-CoV-2	0/3	100%	3/3	100%
		Flu A	0/3	100%	3/3	100%

Normal Saline Solution (Sodium chloride)		Flu B	0/3	100%	3/3	100%
Beclomethasone Dipropionate	15% v/v	SARS-CoV-2	0/3	100%	3/3	100%
		Flu A	0/3	100%	3/3	100%
		Flu B	0/3	100%	3/3	100%
Dexamethasone	15% v/v	SARS-CoV-2	0/3	100%	3/3	100%
		Flu A	0/3	100%	3/3	100%
		Flu B	0/3	100%	3/3	100%
Flunisolide	15% v/v	SARS-CoV-2	0/3	100%	3/3	100%
		Flu A	0/3	100%	3/3	100%
		Flu B	0/3	100%	3/3	100%
Nasal corticosteroids (Triamcinolone acetoneide)	15% v/v	SARS-CoV-2	0/3	100%	3/3	100%
		Flu A	0/3	100%	3/3	100%
		Flu B	0/3	100%	3/3	100%
Nasal corticosteroids (Budesonide)	15% v/v	SARS-CoV-2	0/3	100%	3/3	100%
		Flu A	0/3	100%	3/3	100%
		Flu B	0/3	100%	3/3	100%
Nasal corticosteroids (Mometasone furoate)	15% v/v	SARS-CoV-2	0/3	100%	3/3	100%
		Flu A	0/3	100%	3/3	100%
		Flu B	0/3	100%	3/3	100%
Nasal corticosteroids (Fluticasone Propionate)	15% v/v	SARS-CoV-2	0/3	100%	3/3	100%
		Flu A	0/3	100%	3/3	100%
		Flu B	0/3	100%	3/3	100%
Zicam Nasal Spray (Luffa opperculata, Galphimia glauca, Histaminum hydrochloricum)	15% v/v	SARS-CoV-2	0/3	100%	3/3	100%
		Flu A	0/3	100%	3/3	100%
		Flu B	0/3	100%	3/3	100%
Throat spray (Zinc, Sulphur)	15% v/v	SARS-CoV-2	0/3	100%	3/3	100%
		Flu A	0/3	100%	3/3	100%
		Flu B	0/3	100%	3/3	100%
Nasal Gel	5% w/v	SARS-CoV-2	0/3	100%	3/3	100%
		Flu A	0/3	100%	3/3	100%
		Flu B	0/3	100%	3/3	100%
Homeopathic nasal wash (Alkalol)	15% v/v	SARS-CoV-2	0/3	100%	3/3	100%
		Flu A	0/3	100%	3/3	100%
		Flu B	0/3	100%	3/3	100%
Oseltamivir Phosphate	5 mg/mL	SARS-CoV-2	0/3	100%	3/3	100%
		Flu A	0/3	100%	3/3	100%
		Flu B	0/3	100%	3/3	100%
Remdesivir	10 mg/mL	SARS-CoV-2	0/3	100%	3/3	100%
		Flu A	0/3	100%	3/3	100%

		Flu B	0/3	100%	3/3	100%
Molnupiravir	5 mg/mL	SARS-CoV-2	0/3	100%	3/3	100%
		Flu A	0/3	100%	3/3	100%
		Flu B	0/3	100%	3/3	100%
Zanamivir	5 mg/mL	SARS-CoV-2	0/3	100%	3/3	100%
		Flu A	0/3	100%	3/3	100%
		Flu B	0/3	100%	3/3	100%
Mupirocin	10 mg/mL	SARS-CoV-2	0/3	100%	3/3	100%
		Flu A	0/3	100%	3/3	100%
		Flu B	0/3	100%	3/3	100%
Nirmatrelvir	10 mg/mL	SARS-CoV-2	0/3	100%	3/3	100%
		Flu A	0/3	100%	3/3	100%
		Flu B	0/3	100%	3/3	100%
Ritonavir	10 mg/mL	SARS-CoV-2	0/3	100%	3/3	100%
		Flu A	0/3	100%	3/3	100%
		Flu B	0/3	100%	3/3	100%
Tobramycin	15% v/v	SARS-CoV-2	0/3	100%	3/3	100%
		Flu A	0/3	100%	3/3	100%
		Flu B	0/3	100%	3/3	100%
Body & Hand Lotion	0.5% w/v	SARS-CoV-2	0/3	100%	3/3	100%
		Flu A	0/3	100%	3/3	100%
		Flu B	0/3	100%	3/3	100%
Hand Lotion	5% w/v	SARS-CoV-2	0/3	100%	3/3	100%
		Flu A	0/3	100%	3/3	100%
		Flu B	0/3	100%	3/3	100%
Hand Sanitizer, 70% ethanol	15% w/v	SARS-CoV-2	0/3	100%	3/3	100%
		Flu A	0/3	100%	3/3	100%
		Flu B	0/3	100%	3/3	100%
Hand soap liquid gel	10% w/v	SARS-CoV-2	0/3	100%	3/3	100%
		Flu A	0/3	100%	3/3	100%
		Flu B	0/3	100%	3/3	100%
FluMist	15% v/v <sup>3</sup>	SARS-CoV-2	0/3	100%	3/3	100%
		Flu A	3/3	0%	3/3	100%
		Flu B	3/3	0%	3/3	100%
	1.5% v/v <sup>4</sup>	SARS-CoV-2	0/3	100%	3/3	100%
		Flu A	3/3	0%	3/3	100%
		Flu B	0/3	100%	3/3	100%
	0.15% v/v	SARS-CoV-2	0/3	100%	3/3	100%
		Flu A	0/3	100%	3/3	100%
		Flu B	0/3	100%	3/3	100%
	0.015% v/v	SARS-CoV-2	0/3	100%	3/3	100%
		Flu A	0/3	100%	3/3	100%
		Flu B	0/3	100%	3/3	100%

<sup>1</sup> # of positive results/# of replicates

<sup>2</sup> Agreement with the expected result

<sup>3</sup> Cross reactivity observed at 15%v/v concentration of FluMist for both Flu A and Flu B



<sup>4</sup> Cross reactivity observed at 1.5%v/v concentration of FluMist for Flu A

4. Assay Reportable Range:

Not applicable; the device is a binary qualitative assay that is visually read.

5. Traceability, Stability, Expected Values (Controls, Calibrators, or Methods):

**a. Controls**

*i. Internal Controls:*

The *Status* COVID-19/Flu A&B test contain a built-in internal procedural control. The appearance of the control line 'Ctrl'-Line on the test strip ensures that sufficient flow of the sample occurred during the assay.

*ii. External Controls:*

The *Status* COVID-19/Flu A&B test contains one positive external control swab and one negative external control swab that allows for monitoring of the performance of the assay. The positive control swab contains recombinant nucleocapsid protein for influenza A, influenza B, and SARS-CoV-2 antigens, and the negative control swab (contains inactivated Group B Streptococcus antigen (non-infective)).

**b. Stability**

*i. Specimen Stability:*

Specimen stability study was conducted to evaluate stability of the specimen on Puritan HydraFlock swab under various conditions. Two test samples were prepared: negative samples (pooled negative nasal swab matrix) and contrived positive samples (prepared by spiking heat-inactivated SARS-CoV-2, and live influenza A and influenza B virus at 3x LoD into pooled negative clinical matrix).

For each analyte, 50 µL of either three (3) positive or three (3) negative samples were applied to dry Puritan HydraFlock swabs. Each swab was placed into a sterile, empty tube and stored under one of three temperature conditions for specified durations as described below:

- Refrigerated (2-8 °C): Samples were stored at for 0, 2, 4, 8, and 24 hours.
- Ambient conditions (15 °C, and 30 °C): Samples were stored separately at 15 °C, and 30 °C for 0, 1, 2, 4, 6, 8, and 24 hours.
- Frozen (below -20 °C): Samples were stored below -20 °C for 0, 1, 3, 7, and 10 days.

Subsequently, all exposed sample swabs were tested at each storage condition and timepoint. All negative samples produced negative results at all conditions and timepoints tested. All 3x LoD positive samples yielded expected positive results at all conditions and timepoints tested, except for storage at 30°C for 8 hours for influenza A and 24 hours for both influenza A and influenza B, where 1/3 replicates yielded false negative result. The samples are recommended to be tested immediately after collection.

*ii. Real Time Stability:*

A real-time stability study was conducted to evaluate stability and determine the shelf-life of the unopened kit. To validate shelf life for 2-30 °C, three (3) unopened *Status* COVID-19/Flu A&B kit lots were stored at 2-8 °C and 15-30 °C. At defined intervals, an assessment of each lot was conducted with the following panel of test samples: negative clinical matrix and individually spiked positive samples with inactivated SARS-CoV-2 (3x and 5x LoD) and live Flu A (3x and 10x LoD) and Flu B (3x and 10x LoD) viruses.

Fifty (50) µL of each sample were applied to the swab and tested according to the IFU. Five replicates of each sample were tested per lot for each time point.

Baseline testing was performed within one month of each manufactured lot. Subsequent time testing was conducted every month for up to 31 months. At the time of clearance, all study data have met the protocol defined acceptance criteria, and support storage of the test kits at 2-30°C for up to 29 months.

### *iii. Shipping Stability:*

Transport stability under simulated summer and winter shipping conditions was tested to evaluate worst-case shipping and handling conditions. Unopened test kits were stored at each condition and performance of unopened test kits was assessed by comparing pre- (T0) and every day post-distribution (Td) results for up to 7 days using the individually spiked samples prepared at 2x LoD for each analyte and a negative sample. Samples were tested in replicates of five (5) for each of three (3) device lots. Candidate test kits were stored at the designated temperature profiles described below and then tested with the test panel to evaluate performance. The following temperature profiles were assessed:

- To mimic summer shipping conditions, test kits were stored at 45 °C for 8 hours then moved to 25 °C for 16 hours.
- To mimic winter shipping conditions, test kits were stored at -10 °C for 8 hours and then moved to 18 °C for 16 hours.

All results were as expected for all time points and support shipping at high and low temperatures experienced during summer and winter months, respectively.

## 6. Detection Limit:

### *a. Single Analyte Limit of Detection (LoD):*

An LoD study was conducted to determine the lowest detectable concentration of two (2) strains of SARS-CoV-2 (USA-WA1/2020, and BA.5 omicron; both heat-inactivated), three (3) strains of live influenza A (2 strains of H1N1 and 1 strain of H3N2), and two (2) strains of live influenza B (1 strain of Victoria and 1 strains of Yamagata) at which at least 95% of all true positive replicates return a positive result. Testing was conducted on three (3) lots of test devices.

A preliminary LoD was first determined by testing serial 10-fold dilutions of virus stocks diluted in PNSM in three (3) replicates per device lot for a total of 9 replicates per dilution. A 50 µL sample of each virus diluted in PNSM was pipetted onto the dry swab. The swab was then tested per the IFU. The preliminary LoD of each virus was confirmed by testing an additional twenty samples/lot for each viral stock at the preliminary LoD concentration. If the preliminary LoD yielded 20-positive test results/lot from each 1:10 dilution, it was further evaluated using a 3-fold dilution series, in 20 replicates/lot for each level, to refine the LoD.

As per the acceptance criteria for confirmation of the LoD, at least 95% of the replicates ( $\geq 19/20$ ) should be positive to be considered as the confirmed LoD. The confirmed LoDs observed were identical for the three lots tested for each virus strain.

**Table 6.** Single Analyte LoD

Virus strains	Sources	LoD	#Positive/#Total	% Positive
SARS-COV-2 USA-WA1/2020	Zeptomatrix, 0810587CFHI	$3.39 \times 10^4$ TCID <sub>50</sub> /mL	58/60	96.6%
SARS-CoV-2 Lineage BA.5, Omicron	Zeptomatrix, 0810658CFHI	$2.8 \times 10^3$ TCID <sub>50</sub> /mL	57/60	95.0%
Influenza A, H1N1/Victoria/2570/ 19	Zeptomatrix, Cat# 0810663CF	$1.56 \times 10^1$ TCID <sub>50</sub> /mL	59/60	98.3%
Influenza A, H1N1/Victoria/4897/ 22	Zeptomatrix, 0810684CFHI	$3.89 \times 10^1$ TCID <sub>50</sub> /mL	59/60	98.3%
Influenza A, H3N2/Darwin/9/21	Zeptomatrix, Cat# 0810650CF	$1.25 \times 10^1$ CEID <sub>50</sub> /mL	57/60	95.0%
Influenza B/Victoria/Austria/13 59417/21	Zeptomatrix, 0810654CF	$9.40 \times 10^2$ TCID <sub>50</sub> /mL	58/60	96.6%
Influenza B/Yamagata, Phuket/3073/13	Zeptomatrix, 0810515CF	$1.30 \times 10^1$ TCID <sub>50</sub> /mL	60/60	100.0%

***b. Co-Spiked Multi-Analyte LoD:***

After single analyte LoDs were determined, co-spike equivalency testing was conducted to characterize the performance of samples that contained all analytes at their respective 1x LoD concentrations. Based on individual analyte LoDs, 1x LoD of each single analyte was evaluated in co-spiked samples prepared by mixing viruses (SARS-CoV-2 BA.5 (omicron), influenza A, H3N2/Darwin/9/21, and influenza B, Yamagata/Phuket/3073/13) in negative nasal matrix.

Overall, 20 replicates were evaluated using one lot of devices by pipetting 50  $\mu$ L of co-spiked sample at defined LoD concentration onto the dry swab and testing swabs with the device according to the IFU.

The *Status* COVID-19/Flu A&B test demonstrated co-spike equivalency for SARS-CoV-2, influenza A and influenza B at 1x single analyte LoD. This study supports the use of co-spiked samples in subsequent analytical studies.

**Table 7.** Summary of Co-spike Equivalency LoD Study Results

Test samples	# of positive results / of total tested		
	Flu A	FluB	COVID-19
Negative	0/5	0/5	0/5
Flu A only (1x LoD)	19/20	0/20	0/20
Flu B only (1x LoD)	0/20	20/20	0/20
COVID-19 only (1x LoD)	0/20	0/20	20/20
Flu A, Flu B, COVID-19 (1x LoD)	20/20	20/20	20/20

**c. International Standard Material NIBSC code: 21/368 – Limit of Detection:**

The LoD of the *Status* COVID-19/Flu A&B test was also determined by evaluating different dilutions of the International Standard for SARS-CoV-2 antigen (NIBSC code: 32/368) in negative pooled nasal swab matrix. The International Standard for SARS-CoV-2 containing lyophilized SARS-CoV-2 antigen was reconstituted in ultra-pure water (for a final concentration of 20,000 IU/mL). The LoD was determined as the lowest virus concentration that was detected  $\geq 95\%$  of the time (i.e., concentration at which at least 19/20 replicates tested positive).

Five (5)-fold first dilution and subsequent 2-fold serial dilutions were made from the International Standard for SARS-CoV-2 antigen into negative clinical matrix (pooled nasal swab matrix). Three (3) replicates were tested on two (2) lots of the test device for each dilution to determine the preliminary LoD concentration of the device. For each replicate, 50  $\mu$ L of virus dilution was applied to a swab and the swab was processed according to the IFU. The lowest concentration with all concordant positive results was considered the preliminary LoD.

The preliminary LoD concentration was tested with an additional 20 replicates to confirm the LoD. Concentrations above and below the preliminary LoD were also tested with 20 replicates to further refine the LoD. Samples were prepared as for the preliminary LoD study above. To confirm the LoD, at least 19 of 20 replicates should be positive per lot. The results are summarized in Table below.

**Table 8.** Summary of LoD Study for the International Standard

Conc. (IU/mL)	# Positive replicates			
	Preliminary LoD		Confirmatory LoD	
	Lot 1	Lo2	Lot 1	Lo2
4000	3/3	3/3	-	-
2000	3/3	3/3	20/20	
1000	3/3	3/3	19/20	
500	3/3	3/3	20/20	20/20
<b>250</b>	<b>3/3</b>	<b>3/3</b>	<b>20/20</b>	<b>20/20</b>
125	2/3	2/3	3/20	2/20

The LoD for the *Status* COVID-19/Flu A&B test using the 1<sup>st</sup> International Standard for SARS-CoV-2 antigen (NIBSC code: 21/368) in nasal matrix was determined to be 250 IU/mL.

**d. High Dose Hook Effect:**

A high-dose hook effect study was conducted to evaluate whether high levels of any of the target analytes in a sample could result in a false negative test result. Individually spiked samples were prepared; where each sample was prepared at a high, or "stock," viral concentration. Fifty (50)  $\mu$ L sample was spiked onto swabs and swabs were processed in accordance with the IFU. Analytes were tested individually spiked, and all samples were

tested in three (3)-replicates. No evidence of a high-dose hook effect was observed with the virus stocks and concentrations tested.

**Table 9.** High-Dose Hook Effect Study Results

Virus strain	Testing concentration	Test results (# of positives/ # total replicates)		
		SARS-CoV-2	Influenza A	Influenza B
SARS-CoV-2				
USA-WA1/2020	3.39 x 10 <sup>7</sup> TCID <sub>50</sub> /mL	3/3	0/3	0/3
B.1.1.529, Omicron	2.53 x 10 <sup>6</sup> TCID <sub>50</sub> /mL	3/3	0/3	0/3
Influenza A				
H1N1, A/Baltimore/JH-22377/2022 pdm09	1.6 x 10 <sup>9</sup> TCID <sub>50</sub> /mL	0/3	3/3	0/3
H1N1, Victoria/2570/19	4.7 x 10 <sup>4</sup> TCID <sub>50</sub> /mL	0/3	3/3	0/3
H3N2, A/Baltimore/JH-0440/2022	2.8 x 10 <sup>7</sup> TCID <sub>50</sub> /mL	0/3	3/3	0/3
H3N2, A/Darwin/9/21	3.7 x 10 <sup>4</sup> TCID <sub>50</sub> /mL	0/3	3/3	0/3
Influenza B				
Victoria/Austria/1359417/21	2.8 x 10 <sup>6</sup> TCID <sub>50</sub> /mL	0/3	0/3	3/3
Yamagata/Texas/6/11	3.8 x 10 <sup>6</sup> TCID <sub>50</sub> /mL	0/3	0/3	3/3
Yamagata/Phuket/3073/13	3.9 x 10 <sup>4</sup> TCID <sub>50</sub> /mL	0/3	0/3	3/3

***e. Inclusivity:***

Analytical reactivity testing for the *Status* COVID-19/Flu A&B test was conducted to ensure that the device can adequately detect a variety of strains for SARS-CoV-2, influenza A, and influenza B viruses. A selection of temporally, geographically, and genetically diverse SARS-CoV-2 and influenza strains were tested for inclusivity, including 3 SARS-CoV-2 strains, 21 Influenza A strains (9 H1N1, 10 H3N2, and 2 H5N1 (1 live and 1 gamma irradiated)), and 12 Influenza B strains (5 Yamagata and 7 Victoria lineages). A series of ten-fold dilutions of each virus strain was spiked into PNSM and tested to determine an approximate LoD of the test for each virus. The lowest concentration with 100% positive replicates was identified and additional 3-fold dilutions below that approximate LoD were tested to demonstrate inclusivity. Based on the dilution series, the minimum detectable concentration was defined as the lowest concentration for which all three (3) replicates were detected. Results are summarized below and demonstrate that the test tests can detect the analytes across a range of viral strains.

**Table 10.** Inclusivity Results

Analyte	Subtype/lineage	Strain/Isolate	Lowest concentration with 100% detection
SARS-CoV-2 (Omicron)	B.1.1.529	USA/MD-HP20874/2021	5.01×10 <sup>2</sup> TCID <sub>50</sub> /mL
	BA.2.3	USA/MD-HP245560	8.16×10 <sup>2</sup> TCID <sub>50</sub> /mL
	JN.1	USA/New York/PV96109/2023	3.49×10 <sup>1</sup> TCID <sub>50</sub> /mL
Influenza A (H1N1)	H1N1	A/Brisbane/02/18	4.41×10 <sup>2</sup> TCID <sub>50</sub> /mL
	H1N1	A/Baltimore/JH-22377/2022	5.33×10 <sup>6</sup> TCID <sub>50</sub> /mL

	H1N1	A/Guangdong-Maonan/SWL 1536/19	3.16×10 <sup>3</sup> TCID <sub>50</sub> /mL
	H1N1	A/Michigan/45/15	2.70×10 <sup>2</sup> TCID <sub>50</sub> /mL
	H1N1	A/Wisconsin/588/19	4.20×10 <sup>3</sup> TCID <sub>50</sub> /mL
	H1N1	A/Wisconsin/67/22	1.40×10 <sup>3</sup> TCID <sub>50</sub> /mL
	H1N1	A/California/07/09	2.43×10 <sup>4</sup> TCID <sub>50</sub> /mL
	H1N1	A/Virginia/ATCC3/2009	6.00×10 <sup>4</sup> PFU/mL
	H1N1	A/Connecticut/11/2023	2.80×10 <sup>4</sup> TCID <sub>50</sub> /mL
Influenza A (H3N2)	H3N2	A/Kansas/14/17	5.03×10 <sup>4</sup> TCID <sub>50</sub> /mL
	H3N2	A/Baltimore/JH-0440/2022	9.33×10 <sup>4</sup> TCID <sub>50</sub> /mL
	H3N2	A/Hong Kong/2671/19	1.05×10 <sup>3</sup> TCID <sub>50</sub> /mL
	H3N2	A/Singapore/INFIMH-16- 0019/16	3.16×10 <sup>3</sup> TCID <sub>50</sub> /mL
	H3N2	A/Norway/466/14	4.63×10 <sup>2</sup> TCID <sub>50</sub> /mL
	H3N2	A/Switzerland/9715293/13	1.52×10 <sup>3</sup> TCID <sub>50</sub> /mL
	H3N2	A/Texas/50/12	1.26×10 <sup>3</sup> TCID <sub>50</sub> /mL
	H3N2	A/Tasmania/503/20	4.70×10 <sup>3</sup> TCID <sub>50</sub> /mL
	H3N2	A/Cambodia/E0826360/20	3.90×10 <sup>2</sup> TCID <sub>50</sub> /mL
	H3N2	A/Michigan/173/20	3.50×10 <sup>3</sup> TCID <sub>50</sub> /mL
Influenza A (H5N1)	H5N1 <sup>1)</sup>	A/bovine/Ohio/B24OSU- 439/2024	3.88×10 <sup>4</sup> TCID <sub>50</sub> /mL
	H5N1 <sup>2)</sup>	A/bovine/Ohio/B24OSU-439- 2024	3.1×10 <sup>3</sup> TCID <sub>50</sub> /mL
Influenza B (Victoria)	Victoria	B/Alabama/2/17	3.90×10 <sup>1</sup> TCID <sub>50</sub> /mL
	Victoria	B/Victoria/705/18 Wild-Type	1.40×10 <sup>3</sup> TCID <sub>50</sub> /mL
	Victoria	B/Texas/2/13	1.67×10 <sup>1</sup> TCID <sub>50</sub> /mL
	Victoria	B/Michigan/01/21	1.17×10 <sup>4</sup> TCID <sub>50</sub> /mL
	Victoria	B/Washington/02/19	6.27×10 <sup>2</sup> TCID <sub>50</sub> /mL
	Victoria	B/Hong Kong/574/19 Wild Type	1.39×10 <sup>2</sup> TCID <sub>50</sub> /mL
	Victoria	B/Brisbane/35/18	1.15×10 <sup>3</sup> TCID <sub>50</sub> /mL
Influenza B (Yamagata)	Yamagata	B/Victoria/504/00	5.20×10 <sup>0</sup> TCID <sub>50</sub> /mL
	Yamagata	B/Utah/9/14	1.39×10 <sup>2</sup> TCID <sub>50</sub> /mL
	Yamagata	B/Texas/6/11	3.80×10 <sup>2</sup> TCID <sub>50</sub> /mL
	Yamagata	B/Florida/04/06	1.17×10 <sup>2</sup> TCID <sub>50</sub> /mL
	Yamagata	B/Massachusetts/2/12	4.20×10 <sup>2</sup> TCID <sub>50</sub> /mL

7. Assay Cut-Off:

Not applicable as this is a qualitative visually read assay without numeric data.

**B Comparison Studies:**

1. Method Comparison with Predicate Device:

See section C (clinical studies) below.

## 2. Matrix Comparison:

The *Status* COVID-19/Flu A&B test is intended for use with direct anterior nasal swab and nasopharyngeal swab specimens. As both sample types are evaluated in clinical study, a matrix comparison study is not applicable.

## C Clinical Studies:

### 1. *Anterior Nasal (AN) Swab Specimen:*

A prospective clinical study was conducted to evaluate the performance of the *Status* COVID-19/Flu A&B test using AN swab specimens. A total of four hundred fifty-five (455) AN swab specimens were prospectively collected from six (6) CLIA-waived clinical sites between September 2023 and October 2024. Data from nine (9) patients were excluded from the analysis because they did not meet inclusion criteria. Therefore, the final performance evaluation was based on four hundred forty-six (446) AN swab specimens. Results from the candidate device were compared to NP swab specimen results when tested with an FDA cleared RT-PCR assay for both SARS-CoV-2 and influenza A and B to demonstrate performance.

#### ***Patient Demographics***

Demographic information was collected for all four hundred forty-six (446) patients included in the study.

**Table 11.** Demographic Characteristics of the Study Population

Characteristics of the study population		N=446	Percent (%)
Sex	Male	160	35.9%
	Female	284	63.7%
	Prefer not to say	2	0.4%
Age	<2	3	0.7%
	2-4	6	1.3%
	5-7	18	4.0%
	8-10	19	4.3%
	11-13	24	5.4%
	14-17	23	5.2%
	18-25	92	20.6%
	26-35	89	20.0%
	36-65	142	31.8%
	>65	30	6.7%
	Prefer not to say	0	0.0%
Ethnicity	Hispanic or Latino	60	13.5%
	Not Hispanic or Latino	361	80.9%
	Prefer not to say	25	5.6%
Race	Asian	7	1.6%
	Black or African American	27	6.1%

	White or Caucasian	376	84.3%
	Native Hawaiian or Other Pacific Islander	3	0.7%
	American Indian or Alaska Native	2	0.4%
	Other (Mixed race)	11	2.5%
	Prefer not to say	20	4.5%

**Table 12.** SARS-CoV-2 Performance of the *Status* COVID-19/Flu A&B Test with AN Swab Specimens Compared to RT-PCR

SARS-CoV-2		Comparator test		
		Positive	Negative	Total
<i>Status</i> COVID-19 /Flu A&B	Positive	114	0	114
	Negative	3	329	332
	Total	117	329	446
	Positive Percent Agreement (PPA) = 97.4% (95% CI: 92.7% to 99.1%)			
	Negative Percent Agreement (NPA) = 100.0% (95% CI: 98.9% to 100.0%)			

**Table 13.** SARS-CoV-2 Test Performance by Days Post-Symptom Onset (DPSO) with AN Swab Specimens

DPSO	Specimens tested	<i>Status</i> positive	RT-PCR positive	PPA (95% CI)
Day 0	14	5	6	83.3% (43.7%-97.0%)
Day 1	109	32	32	100.0% (89.3%-100.0%)
Day 2	165	45	46	97.8% (88.7%-99.6%)
Day 3	92	20	21	95.2% (77.3%-99.2%)
Day 4	50	11	11	100.0% (74.1%-100.0%)
Day 5	16	1	1	100.0% (20.7%-100.0%)
Total	446	114	117	97.4% (92.7%-99.1%)

**Table 14.** Influenza A Performance of the *Status* COVID-19/Flu A&B Test with AN Swab Specimens Compared to RT-PCR

Influenza A		Comparator test		
		Positive	Negative	Total
<i>Status</i> COVID-19 /Flu A&B	Positive	43	2	45
	Negative	4	397	401
	Total	47	399	446
	Positive Percent Agreement (PPA) = 91.5% (95% CI: 80.1% to 96.6%)			
	Negative Percent Agreement (NPA) = 99.5% (95% CI: 98.2% to 99.9%)			

**Table 15.** Influenza B Performance of the *Status* COVID-19/Flu A&B Test with AN Swab Specimens Compared to RT-PCR



Influenza B		Comparator test		
		Positive	Negative	Total
<b>Status COVID-19 /Flu A&amp;B</b>	<b>Positive</b>	37	1	38
	<b>Negative</b>	4	404	408
	<b>Total</b>	41	405	446
	Positive Percent Agreement (PPA) = 90.2% (95% CI: 77.5% to 96.1%)			
	Negative Percent Agreement (NPA) = 99.8% (95% CI: 98.6% to 100.0%)			

## 2. **Nasopharyngeal (NP) Swab Specimen:**

In the clinical study, NP swab specimens were also collected and tested using the *Status* COVID-19/Flu A&B assay. A total of five hundred fifty (550) NP specimens were obtained from six (6) CLIA Waived clinical sites between September 2023 and October 2024. Thirteen (13) specimens were excluded from the final analysis because they did not meet inclusion criteria. Accordingly, the performance evaluation of the *Status* COVID-19/Flu A&B assay was conducted using five hundred thirty-seven (537) prospectively collected NP swab specimens. Results from the candidate device were compared to NP swab specimen results when tested with an FDA cleared RT-PCR assay for both SARS-CoV-2 and influenza A and B to demonstrate performance.

### ***Patient Demographics***

Patient demographic data were available for all five hundred thirty-seven (537) patients included in the NP swab specimen study population.

**Table 16.** Patient Demographics – NP Swab Specimens

Characteristics of the study population		N=537	Percent (%)
Sex	Male	191	35.6
	Female	346	64.4
	Prefer not to say	0	0.0
Age	<2	0	0.0
	2-4	3	0.6
	5-7	11	2.0
	8-10	16	3.0
	11-13	21	3.9
	14-17	33	6.1
	18-25	106	19.7
	26-35	108	20.1
	36-65	198	36.9
	>65	41	7.6
	Prefer not to say	0	0.0
Ethnicity	Hispanic or Latino	22	4.1

	Not Hispanic or Latino	494	92.0
	Prefer not to say	21	3.9
Race	Asian	3	0.6
	Black or African American	13	2.4
	White or Caucasian	500	93.1
	Native Hawaiian or Other Pacific Islander	2	0.4
	Other (Mixed race)	4	0.7
	Prefer not to say	15	2.8

**Table 17.** SARS-CoV-2 Performance of the *Status* COVID-19/Flu A&B Test with NP Swab Specimens Compared to RT-PCR

SARS-CoV-2		Comparator test		
		Positive	Negative	Total
<i>Status</i> COVID-19 /Flu A&B	Positive	171	1	172
	Negative	8	357	365
	Total	179	358	537
	Positive Percent Agreement (PPA) = 95.5% (95% CI: 91.4% to 97.7%)			
	Negative Percent Agreement (NPA) = 99.7% (95% CI: 98.4% to 99.9%)			

**Table 18.** SARS-CoV-2 Test Performance by DPSO with NP Swab Specimens

DPSO	Specimens tested	<i>Status</i> positive	PCR positive	PPA (95% CI)
Day 0	15	7	7	100.0% (64.6%-100.0%)
Day 1	163	63	64	98.4% (91.7%-99.7%)
Day 2	197	61	64	95.3% (87.1%-98.4%)
Day 3	106	25*	28	89.3% (72.8%-96.3%)
Day 4	40	9	10	90.0% (59.6%-98.2%)
Day 5	16	6	6	100.0% (61.0%-100.0%)
Total	537	171*	179	95.5% (91.4%-97.7%)

\* One false positive result was excluded from the analysis. Total includes only true positives based on PCR comparator.

**Table 19.** Influenza A Performance of the *Status* COVID-19/Flu A&B Test with NP Swab Specimens Compared to RT-PCR

Influenza A		Comparator test		
		Positive	Negative	Total
<i>Status</i> COVID-19 /Flu A&B	Positive	48	3	51
	Negative	3	483	486
	Total	51	486	537

	Positive Percent Agreement (PPA) = 94.1% (95% CI: 84.1%-98.0%)
	Negative Percent Agreement (NPA) = 99.4% (95% CI: 98.2%-99.8%)

**Table 20.** Influenza B Performance of the *Status* COVID-19/Flu A&B Test with NP Swab Specimens Compared to RT-PCR

Influenza B		Comparator Test		
		Positive	Negative	Total
<b><i>Status</i> COVID- 19 /Flu A&amp;B</b>	<b>Positive</b>	51	0	51
	<b>Negative</b>	4	482	486
	<b>Total</b>	55	482	537
	Positive Percent Agreement (PPA) = 92.7% (95% CI: 82.7% to 97.1%)			
	Negative Percent Agreement (NPA) = 100.0% (95% CI: 99.2% to 100.0%)			

3. Clinical Sensitivity:

Please refer to Section VI.C (Clinical Studies) above for the clinical validation.

4. Clinical Specificity:

Please refer to Section VI.C (Clinical Studies) above for the clinical validation.

**D Clinical Cut-Off:**

The test is a qualitative test with a binary positive/negative signal and there is no clinical cut-off for the test.

**E Expected Values/Reference Range:**

A patient sample is expected to be negative for SARS-CoV-2, influenza A, and influenza B.

**VII Proposed Labeling:**

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

**VIII Conclusion:**

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