U.S. FOOD & DRUG ADMINISTRATION

CLIA Waiver by Application Approval Determination
Decision Summary

I. Document Number

CW240015

II. Parent Document Number

K241652

III. CLIA Waiver Type

Dual 510(k) and CLIA Waiver by Application (Dual Submission)

IV. Applicant

Nuclein, LLC

V. Proprietary and Established Names

DASH SARS-CoV-2 & Flu A/B Test

VI. Measurand (analyte)

SARS-CoV-2 RNA
Influenza A RNA
Influenza B RNA

VII. Sample Type(s)

Anterior nasal swab specimens

VIII. Type of Test

The DASH SARS-CoV-2 & Flu A/B Test is a reverse transcription polymerase chain reaction (RT-PCR) test.

IX. Test System Description

A Overview

The DASH SARS-CoV-2 & Flu A/B Test is a rapid, reverse transcription polymerase chain reaction (RT-PCR) assay performed on the DASH Rapid PCR Instrument (DASH Instrument).

The test combines the technologies of sequence specific capture sample preparation and RT-PCR amplification.

The DASH SARS-CoV-2 & Flu A/B Test cartridge contains all reagents necessary to perform the test to detect RNA from SARS-CoV-2, influenza A and influenza B. An anterior nasal swab (ANS) with a 30-mm breakpoint is used to collect a specimen. After collection, ANS is added directly to the DASH SARS-CoV-2 & Flu A/B Test cartridge sample chamber. The Test cartridge is capped and inserted into the DASH Instrument to initiate the test, and all subsequent test steps are performed automatically by the DASH Instrument.

The Test cartridge sample chamber contains lysis buffer which releases the RNA target from the virus. The target RNA molecules are isolated from specimens by use of biotinylated capture oligomers (capture oligomers) which are in turn captured by streptavidin-coated paramagnetic particles. The biotinylated capture oligomers contain sequences complementary to specific regions of the RNA target molecules. During the hybridization step, the sequence specific regions of the capture oligomers bind to specific regions of the RNA target molecules. The capture oligomer:target complex is then concentrated by the addition of streptavidin-coated paramagnetic particles.

The paramagnetic particles including the bound target molecules are washed to remove residual specimen matrix that may contain amplification reaction inhibitors. After the target capture steps are completed, the target RNA is ready for RT-PCR amplification. Forty amplification cycles are performed, during which the fluorescence is measured with a four-color LED fluorometer. Amplification of any assay targets, Flu A, Flu B, and SARS-CoV-2, leads to fluorescent signal. An algorithm determines whether the result is positive or negative for each viral target assay. Additionally, a process control is included in the cartridge to control for adequate processing of the target virus through the steps of sample purification, nucleic acid amplification and to monitor for the presence of inhibitors in the RT-PCR test. Total run time, from swab insertion into the Test cartridge to display of test results is approximately 15 minutes.

The DASH Instrument contains firmware and software that operate the instrument and analyze the assay data. Test results are displayed on the DASH Instrument screen and can be printed (optional) after the test has been completed. The procedural control will display "Valid" on the results screen in a SARS-CoV-2 & Flu A/B negative sample and in a positive sample (see Table 1 below).

Table 1. DASH SARS CoV-2 & Flu A/B Results Interpretation

Target	Result	Interpretation
SARS-CoV-2	Positive	The target nucleic acids of SARS-CoV-2 are detected.
	Negative	The target nucleic acids of SARS-CoV-2 are not detected.
Flu A	Positive	The target nucleic acids of Flu A are detected.
	Negative	The target nucleic acids of Flu A are not detected.
Flu B	Positive	The target nucleic acids of Flu B are detected.
	Negative	The target nucleic acids of Flu B are not detected.
Procedural Control	Valid	Amplification meets acceptance criteria. Test results may be reported.

	Invalid	Presence or absence of the target nucleic acids cannot be determined. Repeat test with a new cartridge.
Error		Presence or absence of the target nucleic acids cannot be determined. Instrument detected an error and aborted the current test. Repeat test with a new cartridge.

B Test System Components

Materials included within the DASH SARS-CoV-2 & Flu A/B Test consist of the following:

- Ten individually pouched DASH SARS-CoV-2 & Flu A/B Cartridges per kit
- Ten individually packaged anterior nares sample collection swabs (30 mm breakpoint, sterile elongated polyester flock) per kit.
- DASH SARS-CoV-2 & Flu A/B Test Quick Reference Instructions (QRI)

The DASH Instrument, DASH SARS-CoV-2 & Flu A/B Positive Control Swabs Kit, and DASH Negative Control Swabs Kit are provided separately, but are not included within the DASH SARS-CoV-2 & Flu A/B Test kit.

X. Specific Contents for CLIA Waiver

A Demonstrating "Simple":

The DASH SARS-CoV-2 & Flu A/B Test on DASH Instrument was designed to be simple and easy to use. use. The following features are built into the device design to make it simple to use with minimal risk of erroneous results.

Table 2: Demonstration of Simplicity for the DASH SARS-CoV-2 & Flu A/B Test on DASH Instrument

'Simple' Criteria	Device Characteristics
Is a fully automated instrument or a unitized or self-contained test.	The device is a fully automated system and only requires sample collection and insertion into the Test cartridge. After insertion, all test steps are completed by the instrument without further user intervention. Users use a simple touchscreen to operate the instrument.
Uses direct unprocessed specimens, such as capillary blood (fingerstick), venous whole blood, nasal swabs, throat swabs, or urine.	The test uses direct anterior nasal swab specimens that are placed directly into the sample chamber of the Test cartridge.

Needs only basic, non- technique-dependent specimen manipulation, including any for decontamination.	• An untrained operator can conduct the test by performing simple steps without sample manipulation: 1) collect the anterior nasal swab, 2) inserts the swab tip directly into the Test cartridge, 3) inserts the Test cartridge into the DASH Instrument. After insertion, all test steps are completed by the instrument without further user intervention. • No technique-dependent specimen collection is required in order to perform the test. • After the run is completed, the single-use Test cartridge remains sealed to prevent release of the sample and reagents and the entire cartridge can be disposed without further manipulation in accordance with any applicable local, state, and national regulations.
Needs only basic, non- technique-dependent reagent manipulation, such as "mix reagent A and reagent B."	The device consists of a single use, ready-to-use Test cartridge that contains all the reagents required for sample processing and analysis. No manipulation of the reagents by the user is needed.
Needs no operator intervention during the analysis steps.	• The test does not require any operator intervention during the analysis step. • The device performs automated analysis of test results and eliminates subjectivity associated with visual reading of results by the end-user. • Users use a simple touchscreen to operate the instrument. • After insertion of the Test cartridge into the DASH Instrument and the initiation of the test run, the test does not require any operator intervention during the analysis step. • All results are displayed on the integrated LCD Screen and in an associated print-out.
Needs no technical or specialized training with respect to troubleshooting or interpretation of multiple or complex error codes.	Technical or specialized training is not required for operating the device. If an error code is shown, user will follow easy-to-interpret troubleshooting instructions provided in a quick reference guide or operator manual or contact customer service directly.
Needs no electronic or mechanical maintenance beyond simple tasks, e.g., changing a battery or power cord.	• The DASH Instrument does not require service in the field. • There are no required electronic or mechanical maintenance tasks as there are no serviceable parts. • If service is required, the unit is to be returned to the factory and the user is provided a replacement to use for the duration of servicing.
Produces results that require no operator calibration, interpretation, or calculation.	Interpretation of results is automated. Results are displayed on the instrument screen and no additional interpretation or calculations are required.

Produces results that are easy to determine, such as 'positive' or 'negative,' a direct readout of numerical values, the clear presence or absence of a line, or obvious color gradations.	Interpretation of results is automated. Qualitative test results ("Positive" or "Negative", and "Valid" or "Invalid") are automatically displayed on the integrated LCD screen of the DASH Instrument and no additional interpretation or calculations are required.
Includes quick reference instructions (QRI, Operator's Instrument Manual (if applicable), etc.) that are written at no higher than a 7th grade reading level.	A QRI and operator instruction manual are provided as part of the device. These documents are written at no higher than a 7th grade reading level.

B Demonstrating "Insignificant Risk of an Erroneous Result"- Failure Alerts and Fail-Safe Mechanisms

1. Risk Analysis:

   A systematic and comprehensive risk analysis of the DASH SARS-CoV-2 & Flu A/B Test and DASH Instrument was conducted in accordance with *ISO 14971:2019 Medical Devices – Application of risk management to medical devices* to identify all potential sources of error and hazards associated with the use of the device. The detailed analysis included potential system failures and operator errors, to evaluate which of these errors can lead to a risk of erroneous result. Potential sources of errors that could adversely affect system performance were identified and mitigated first through system design and then through the inclusion of warning and caution statements in the labeling. The identified risks which could result in erroneous test results were evaluated in flex studies that stressed the functional limits of the test system (see below).

2. Fail-Safe and Failure Alert Mechanisms:

   Both DASH Instrument and the DASH SARS-CoV-2 & Flu A/B Test include a variety of fail-safe features and failure alert mechanisms to prevent operator and instrument error leading to erroneous results, as summarized in the table below.

Table 3: Summary of Fail-Safe and Failure Alert Mechanisms

Feature	Description
Test Cartridge	• Each Test cassette is packaged in a sealed pouch to maintain the integrity of the device. Each foil pouch includes only one Test cartridge. • Foil pouch incorporates the use of a tear notch to prevent excessive force or dropping of Test cartridge when opening. • The tight fit of a closed Test cartridge cap helps to ensure cartridges are not reused. • Utilization of lyophilized reagent materials contributes to extended stability and prevents user exposure. • Test cartridge channels are sealed preventing fluid leaks during and post-processing preventing user exposure to assay reagents.
Barcode scanner	• Test cartridge has a 2D barcode with the embedded expiration date which is required to be scanned prior to use. If the current date exceeds the expiration date, an error message is displayed, and the run will not start.

	• Specimen ID barcode must be scanned to maintain traceability of the results. Test will not initiate without entering a specimen ID. • The DASH instrument has an internal barcode scanner that confirms the unique Test cartridge ID contained in the Test cartridge barcode is identical to the one scanned during the Graphical User Interface (GUI) workflow by the user. If the barcodes do not match, an error message is displayed, and the run will not start, preventing Test cartridge and sample mix-up. • The internal barcode scanner will not scan the Test cartridge if it is inserted in the wrong orientation preventing incorrect cartridge orientation in the instrument.
System software	• Instrument will not receive a Test cartridge for processing until the "cartridge present" sensor is triggered preventing incorrect Test cartridge orientation upon insertion into the instrument. • The software automatically starts a test when the Test cartridge is inserted and the barcode is read preventing Test cartridge/sample mix- up and delay in testing.
DASH Instrument Power On Features	• During Power On, the internal barcode scanners checks for the presence of the Test cartridge and prompts the user to eject. • During Power On, all heaters must achieve default temperature settings within 15 minutes, or user log-in is prevented.
DASH Instrument User Interface	• Workflow instructions are provided on the DASH Instrument touchscreen that the user has to actively engage with to progress through the test workflow (e.g., instruction to close the Cartridge Cap, confirmation that seals are removed from the Test cartridge prior to insertion into the instrument, etc.). • Status of the procedure and/or error codes are displayed on the instrument to keep user informed of test progress.
DASH Instrument Run Errors	• If the Liquid Transfer Capsule (LTC) is not detected as loaded properly during a test, an error is issued and the test is aborted preventing test results when transfer volumes may be inaccurate. • The pump pressure is monitored during a test. If high pressure is detected, an error is issued and the test is aborted. • If any motor encounters an obstruction during a test, an error is issued and the test is aborted preventing test results when test may not have been executed properly. • If the second foil is not removed from the Test cartridge, this will cause an obstruction during a test. An error is issued and the test is aborted preventing results if the test workflow is not followed.

Built-in Procedural Control

The DASH SARS-CoV-2 & Flu A/B Test cartridge includes a built-in Procedural Control (PRC) that undergoes identical processing to the patient specimen which provides confirmation that the test procedure was executed correctly. If the test fails for any reason, the PRC result will be reported as an INVALID and the operator is instructed in the labelling to test the sample again with another test device.

External Controls

External Controls, including a positive control swab (DASH SARS-CoV-2 & Flu A/B Positive Control Swabs Kit) and a negative control swab (DASH Negative Control Swabs Kit), are available separately for use. Both the positive and negative control swabs are tested

using the same procedure as the patient specimens. Each control swab should produce the expected positive or negative results to validate the test performance. The users are instructed to run external controls under the following circumstances:

- Each time a new lot of DASH SARS-CoV-2 & Flu A/B Test cartridges is received.
- Each time a new operator performs the test.
- When the DASH Instrument is used for the first time for a specific test.
- As deemed additionally necessary by internal quality control procedures, and in accordance with local, state and federal regulations or accreditation requirements.

3. Flex Studies:

The operational limits of the DASH SARS-CoV-2 & Flu A/B Test on DASH Instrument were evaluated in a series of experiments under conditions of stress. Contrived samples used for flex study testing were prepared in simulated clinical nasal matrix (SCNM) and consisted of:

- Negative sample: un-spiked SCNM
- Low Positive sample: SCNM co-spiked at 1-2x Limit of Detection (LoD) with the three viral targets using either of the following combination of the strains:
  i. SARS-CoV-2 (hCoV-19/USA/GA-EHC-2811C/2021; Omicron Variant), Flu A (A/Victoria/2570/19) and Flu B (B/Utah/9/14),
  ii. inactivated SARS-CoV-2 (USA-WA1/2020), Flu A (A/Singapore/63/04), Flu B (B/Florida/02/06).

Except where indicated below, for each condition being evaluated in the flex studies, each low positive sample was tested in five replicates and negative sample was tested in three to five replicates. Testing for each condition was conducted in a randomized and blinded fashion. Samples were tested according to the instructions for use protocol, except for the noted deviations dictated by the flex parameter under evaluation. The effect of the following conditions on the performance of the test was evaluated, organized by the potential type of source error:

Environmental conditions

- *Temperature and Humidity:*

  This study evaluated the impact of variations in environmental temperature and humidity on DASH SARS-CoV-2 & Flu A/B Test performance. All pouched Test cartridges and DASH Instruments were placed in environmental chambers with the specified conditions (see table below) and allowed to equilibrate for a minimum of two hours prior to testing. The results from the study are summarized in the table below.

Study Condition	Study Parameters	Agreement (n/N)[1]		Invalid Result	DASH Instrument Errors	Erroneous Results Due to Study Condition
		Negative	Positive			
A	32°C, 90% RH	3/3	5/5	0	0	None
B	40°C, 95% RH	3/5	1/5	0	6	None

C	13°C, 90% RH	3/3	5/5	0	0	None
D	5-10°C, 95%RH	4/5	4/5	0	2	None
E	32°C, <15% RH	3/3	5/5	0	0	None
F	40°C, 5% RH	4/5	2/5	0	4	None
G	13°C, <15% RH	3/3	4/5	1	0	None
H	5-10°C, 5%RH	5/5	5/5	0	0	None

[1]n is number of tests with expected results. N is the total number of tests run.

The labeling specifies optimum conditions for storage and performing the test on DASH Instrument to be room temperature (15°C to 30°C) and 20% - 80% RH (Relative Humidity). All samples with valid results yielded expected results. For condition G, one invalid result was reported. For conditions B, D and F, DASH Instruments displayed run error code (unable to complete the initialization and warm-up sequence or motor movement error during initialization) as fail-safe measures. There were no erroneous results generated, demonstrating that the risk of erroneous results due to extremes of temperature and humidity outside the specified conditions is minimal. These instrument errors are mitigated by clear labeling which instructs the user to operate the DASH SARS-CoV-2 & Flu A/B Test on DASH Instrument at 15°C to 30°C and 20% - 80% RH.

- *Environmental Tolerance – Barometric Pressure:*

The DASH SARS-CoV-2 & Flu A/B Test is specified for use at an altitude up to 1,676 meters (5,500 feet). This study examined the effect of altitude on the assay performance. Testing was conducted by placing DASH Instruments inside barometric pressure chambers simulating different altitude conditions (3500, 4500, 5500, 6000, 6500 and 7000 feet). Five replicates of Low Positive and Negative samples were tested in the study. One false negative result for influenza B was observed with Low Positive sample at 3500 feet. Expected results were obtained for this replicate for SARS-CoV-2 and influenza A. One invalid result was obtained for Negative sample at 6000 feet in the study. Two false negative results were obtained when testing was done at 7000 feet. All other samples generated expected results. The study showed that extremes of altitude have an effect on the test results. However, to mitigate the risk of erroneous results, a warning has been added to the labeling that operating the test system outside of specifications may cause incorrect results. The study results are summarized below.

Study Condition	Altitude Tested	Agreement (n/N)[1]		Invalid Result	DASH Instrument Errors	Erroneous Results Due to Study Condition
		Negative	Positive			
A	3500 ft	5/5	3/5	0	1	1[2]
B	4500 ft	5/5	5/5	0	0	None
C	5500 ft	5/5	5/5	0	0	None
D	6000 ft	4/5	5/5	1	0	None
E	6500 ft	5/5	5/5	0	0	None
F	7000 ft	5/5	3/5	0	0	2[3]

[1]n is number of tests with expected results. N is the total number of tests run.

[2]One replicate produced false negative result for influenza B; expected results were obtained for this replicate for SARS-CoV-2 and influenza A analytes.
[3]Two replicates produced false negative result for influenza A; expected results obtained for SARS-CoV-2 and influenza B.

- *Incorrect Placement of Device: Non-Level Surfaces:*

The purpose of this study was to evaluate the effect of non-leveled benchtop or work surface on the test performance of the DASH SARS-CoV-2 & Flu A/B Test on DASH Instrument. Five replicates of each sample (Negative and Low Positive) per condition were tested in the study according to the test procedure. Testing was carried out on DASH instruments inclined at 5° and 10°, in the forward, backward, right or left direction. The results of the study are presented below. A total of three invalid results were obtained for Negative samples tested with DASH Instrument inclined at 5° in forward direction, 10° in forward direction and 10° in backward direction. No erroneous results were observed in this study. The labeling clearly directs users to conduct all testing on a level surface. The study demonstrated that risks of erroneous results due to operating the instrument on non-level surface are low.

Inclination Tested	Study Condition	Agreement (n/N)[1]		Invalid Result	DASH Instrument Errors	Erroneous Results Due to Study Condition
		Negative	Positive			
5°	A (Forward)	4/5	5/5	1	0	None
	B (Backward)	5/5	5/5	0	0	None
	C (Right)	5/5	5/5	0	0	None
	D (Left)	5/5	5/5	0	0	None
10°	A (Forward)	4/5	5/5	1	0	None
	B (Backward)	4/5	5/5	1	0	None
	C (Right)	5/5	5/5	0	0	None
	D (Left)	5/5	5/5	0	0	None

[1]n is number of tests with expected results. N is the total number of tests run.

- *Effect of Sunlight Exposure:*

The purpose of this study was to evaluate the effect of sunlight exposure on the test performance of the DASH SARS-CoV-2 & Flu A/B Test on DASH Instrument. The study was conducted by testing five replicates of each sample (Negative and Low Positive) in a blinded manner. Testing was carried out by simulating the visible light wavelength spectrum similar to that of daytime sunlight (400 nm – 700 nm) by using a combination of halogen and white LED lamps. Both the Test cartridges and the DASH Instrument were exposed to the light during the test. All samples generated expected results demonstrating that the risks of erroneous results due to sunlight exposure during testing are minimal.

- *Effect of Drafty Conditions:*

The purpose of this study was to evaluate the effect of drafty conditions on the test performance of the DASH SARS-CoV-2 & Flu A/B Test on DASH Instrument. The study was conducted by testing five replicates of each sample (Negative and Low Positive) in a blinded manner. To simulate drafty conditions, testing was carried out in the presence of an oscillating fan directed towards both the Test cartridges and the DASH Instrument during testing. All samples generated expected results demonstrating that the risks of erroneous results due to drafty conditions during testing are minimal.

- *Effect of Poor Ventilation:*

  The purpose of this study was to evaluate the effect of poor ventilation on the test performance of the DASH SARS-CoV-2 & Flu A/B Test on DASH Instrument. The study was conducted by testing five replicates of each sample (Negative and Low Positive) in a blinded manner. Poor Ventilation conditions were simulated by carrying out testing with the DASH Instrument positioned against the wall with solid barriers to block air ventilation from both sides as well as the top. All samples generated expected results demonstrating that the risks of erroneous results due to poor ventilation during testing are minimal.

- *Effect of Vibrations:*

  The purpose of this study was to evaluate whether vibration of the test device after sample application will impact the test performance of the DASH SARS-CoV-2 & Flu A/B Test on DASH Instrument. Five replicates of each sample (Negative and Low Positive) were tested in the study wherein the DASH Instrument was operated close to a laboratory vacuum pump operating with a speed of approximately 1800 rpm for the duration of the test. The velocities of vibration created by the vacuum pump was measured by a calibrated vibration meter to be in the range of 1.8 - 2.7 mm/s during the test runs. One Low Positive sample generated a run error code and the test run was aborted issuing no test result. All other nine samples generated expected results. No erroneous results were observed in this study demonstrating that the risks of erroneous results due to vibrations during testing are minimal.

<u>Operator Errors in Performing the Test</u>

- *Using Expired Test Cartridge:*

  As a fail-safe mechanism, DASH SARS-CoV-2 & Flu A/B Test cartridge has a 2D barcode with the embedded expiration date which is required to be scanned prior to use. If the current date exceeds the expiration date, an error message '*Cartridge has expired*' is displayed on the DASH Instrument, and the test run does not start. This study was conducted to demonstrate the functionality of the DASH Instrument to recognize and reject the expired Test cartridges. Five DASH instruments were evaluated using five expired DASH SARS-CoV-2 & Flu A/B Test cartridges with Negative Control swabs. A non-expired Test cartridge was used as a control in the study. All five DASH Instruments successfully recognized the expired cartridges upon scanning the cartridge barcode and were rejected with the error message, demonstrating that the risks of erroneous results due to use of expired Test cartridge is minimal.

- *Delay in Testing Sample Swab After Collection:*

  Labeling of DASH SARS-CoV-2 & Flu A/B Test instructs the user to test nasal swab samples immediately after collection. This study evaluated effect of delay in testing sample swabs after collection. Five replicates of each sample swab (Low Positive and Negative) were held at elevated (40-45°C) and refrigerated (2-8°C) temperatures for 1, 4 and 24 hours, then tested at each timepoint for each condition. All samples yielded expected results after delay in testing up to four hours, when kept at both the elevated and refrigerated conditions. Two Low Positive samples resulted in instrument error and the runs were aborted at the 24-hour timepoint at refrigerated conditions. At the 24-hour timepoint, results showed one false positive call for influenza A at elevated temperature. No other erroneous results were obtained in the study. The study demonstrates that the DASH SARS-CoV-2 & Flu A/B Test performs as expected when the testing is delayed up to 4 hours after collection, with minimal risk of erroneous results.

Study Condition	Study Parameters	Agreement (n/N)[1]		Invalid Result	DASH Instrument Errors	Erroneous Results Due to Study Condition
		Negative	Positive			
A	1 hour at 2-8°C	5/5	5/5	0	0	None
B	4 hour at 2-8°C	5/5	5/5	0	0	None
C	24 hour at 2-8°C	5/5	3/5	0	2	None
D	1 hour at 40-45°C	5/5	5/5	0	0	None
E	4 hour at 40-45°C	5/5	5/5	0	0	None
F	24 hour at 40-45°C	4/5	5/5	0	0	1[2]

[1]n is number of tests with expected results. N is the total number of tests run.
[2]One replicate produced false positive result for influenza A. Expected results were obtained for SARS-CoV-2 and influenza B.

- *Delay in Testing After Sample Swab is Inserted into the Test Cartridge:*

  DASH SARS-CoV-2 & Flu A/B Test labeling instructs users that samples must be inserted into Test cartridge and tested without delay. These studies were conducted to determine the effect of delay in testing and storage conditions after the sample swab is inserted into the Test cartridge.

  1) *Effect of Sample-in-Test cartridge hold time*:
     In this study, the sample swab was loaded into the Test cartridge, then kept at 30°C prior to testing with the DASH SARS-CoV-2 & Flu A/B Test on the DASH Instrument. Twenty replicates of Low Positive samples were tested fresh (T0) and after 1.5 and 3 hours. Negative samples were also included and were tested in three replicates at each time point. All tested samples yielded expected results, indicating that the risk of erroneous results is minimal when testing is delayed up to 3 hours, after sample insertion into the Test cartridge.

  2) *Improper Sample-in-Test Cartridge Storage Temperature Conditions*

This study was conducted to determine the effect of sample-in-Test cartridge hold conditions on sample integrity after the sample swab is loaded into the Test cartridge, stored at elevated (40-45°C) and refrigerated (2-8°C) temperatures for 1, 2, 4 and 8 hours and tested with the DASH SARS-CoV-2 & Flu A/B Test on the DASH Instrument. Five replicates of the Low Positive and Negative samples were tested for each condition in a blinded manner. One Negative sample-in-cartridge stored at 40-45°C for two hours generated an invalid result. All other samples generated the expected results. No erroneous results were observed demonstrating that the risks of erroneous results due to improper sample-in-cartridge storage before testing are minimal.

The DASH SARS-CoV-2 & Flu A/B Test labeling instructs the user that the Test cartridge must be kept at room temperature (15°C to 30°C) and run within one hour of sample insertion. Otherwise, the Test cartridge should be discarded, and a new sample collected for testing.

- *Effect of Dropping the Test Cartridge:*

  This study was conducted to determine the effect of dropping the Test cartridge on performance of the DASH SARS-CoV-2 & Flu A/B Test on the DASH Instrument. Five replicates of the Low Positive and six replicates of Negative samples were tested in a blinded manner. In the study, the Test cartridge was dropped from a minimum of 3-feet height before its use. One Low Positive sample resulted in instrument error and the run was aborted. All other samples generated expected results demonstrating that the risks of erroneous results due to cartridge dropping is minimal. The labeling provides clear instructions to the users for cartridge unpacking and preparation for testing. Additionally, users are warned to not use the cartridge that has been dropped.

- *Effect of Improper Test Cartridge Handling:*

  This study was conducted to determine the effect of improper Test cartridge handling on performance of the DASH SARS-CoV-2 & Flu A/B Test on the DASH Instrument. Five replicates of the Low Positive and six replicates of Negative samples were tested in a blinded manner. The labeling instructs the user to handle Test cartridge from the labeled end while opening the foil pouch containing the cartridge. In this study, the user handles the cartridge in the incorrect orientation by holding the opposite thin film end of the Test cartridge while opening the pouch prior to transferring of the loaded cartridge to the cartridge holder in the DASH instrument. All samples generated expected results demonstrating that the risks of erroneous results due to cartridge mishandling by holding it from the opposite side are minimal. The labeling provides clear instructions to the users for cartridge unpacking and preparation for testing.

- *Effect of Premature Removal of Test Cartridge Foil Covers:*

  The Test cartridge has two foil covers (Cover 1 and 2) which, according to the test instructions, are removed by the user at different times during the workflow. In this study, the effect of peeling these foil covers prematurely on performance of the DASH SARS-CoV-2 & Flu A/B Test on the DASH Instrument was evaluated. After peeling off

both foil covers, the cartridge was left unused on the workbench for 4 hours before testing. Five replicates of the Low Positive and Negative samples were tested in a blinded manner. All samples generated expected results demonstrating that the risks of erroneous results due to premature removal of the covers during testing are minimal. The labeling provides clear instructions to the users for cartridge unpacking and preparation for testing.

- *Effect of Excessive Shaking of the Test Cartridge*

  This study was conducted to determine the effect of excessive shaking of the Test cartridge on performance of the DASH SARS-CoV-2 & Flu A/B Test on the DASH Instrument. Five replicates of the Low Positive and Negative samples were tested in a blinded manner. During testing, each cartridge was removed from the foil pouch and individually vortexed for 20 seconds. After the sample was loaded, each cartridge was vortexed again for another 20 seconds before loading to the DASH Instrument. All samples generated expected results demonstrating that the risks of erroneous results due to excessive shaking of the Test cartridge are minimal. The labeling provides clear instructions to the users for cartridge unpacking and preparation for testing.

Reagent/Test Material Handling Errors

- *Effect of Incorrect Storage Temperature on Test Cartridge and Positive Control Swabs:*

  Labeling for the DASH SARS-CoV-2 & Flu A/B Test instructs the user to store Test cartridges and DASH SARS-CoV-2 & Flu A/B Positive Control Swabs at ambient temperatures (15-30°C). This study was conducted to determine the effect of storing both cartridge and positive control swabs outside of temperatures defined in the labeling of the DASH SARS-CoV-2 & Flu A/B Test. Test cartridges and control swabs were stored for seven days at refrigerated (2-8°C) or frozen (-20°C) conditions and seven and 14 days at 30°C. No thawing/equilibration time was allowed once the swabs or the Test cartridges were removed after incubation in different temperature conditions for testing. For cartridge evaluation, five replicates of Low Positive or Negative samples were tested for each condition. Testing of the positive control swab was conducted with five replicates using Test cartridges stored per the labeling. Additional replicates of the Test cartridge and the positive control swabs were tested at some of the conditions. The results are summarized below:

Storage Temperature	Test Material	Agreement (n/N)[1]		Invalid Result	DASH Instrument Errors	Erroneous Results Due to Study Condition
		Negative	Positive			
7 Days 2-8°C	Test Cartridge	5/5	5/5	0	0	None
	Positive Control Swab	N/A	5/5	0	0	None
7 Days ≤-20°C	Test Cartridge	5/5	5/5	0	0	None
	Positive Control Swab	N/A	5/5	0	0	None
7 Days	Test Cartridge	5/5	4/5	0	1	None

30°C ±2°C	Positive Control Swab	N/A	5/5	0	0	None
14 Days 30°C ±2°C	Test Cartridge	5/5	3/5	0	2	None
	Positive Control Swab	N/A	4/4	0	1	None

[1]n is number of tests with expected results. N is the total number of tests run.

Instrument error was obtained, and the test run was aborted for Test cartridges at 30°C for seven days (one Low Positive sample) and 14 days (two Low Positive samples). Instrument error was obtained, and the test run was aborted for two positive control swabs stored at 30°C for 14 days. No invalid results were obtained in the study. All other samples generated expected results for all the conditions tested.

- *Effect of Freeze-Thaw on the Test Cartridge:*

  The DASH SARS-CoV-2 & Flu A/B Test labeling states that the Test cartridge should be stored at ambient temperature (15-30°C). To assess the stress condition of inadvertent freezing of the Test cartridge prior to use, the Test cartridge was subjected to a series of two freeze-thaw cycles (freezing at -20°C, followed by thawing at ambient room temperature). The results showed that all samples generated expected results up to one freeze-thaw cycle. However, after two freeze-thaw cycles, a high number of test runs (three Negative and two Low Positive samples) were aborted with no test results, but no erroneous results were reported. Labeling of DASH SARS-CoV-2 & Flu A/B Test cautions the user to not freeze the cartridges.

- *Effect of Incorrect Storage Temperature on Specimen Collection Swabs:*

  Sample collection swabs provided with the DASH SARS-CoV-2 & Flu A/B Test are stored at ambient temperatures. This study was conducted to determine the effect of storing collection swabs outside of temperatures defined in the labeling of the DASH SARS-CoV-2 & Flu A/B Test. Sample collection swabs were stored for either seven or 14 days at refrigerated (2-8°C) or frozen (-20°C) conditions, followed by spiking them with Low Positive or Negative samples and testing using DASH SARS-CoV-2 & Flu A/B Test. Five replicates of each sample were tested at each timepoint for each condition. One Negative sample resulted in instrument error and the run was aborted in frozen condition after seven days. One invalid result was obtained for Negative sample in frozen storage condition after 14 days. All valid samples generated expected results for all the conditions tested demonstrating that the risks of erroneous results due to incorrect sample collection swab storage are minimal.

Conclusion from Flex Studies

The flex studies carried out in support of this CLIA waiver submission have effectively and comprehensively demonstrated the robustness of the system to generate correct results even when operated under conditions of stress. The combination of the device design and the built-in fail-safe features, along with the clear test instructions which include relevant cautions, render the risks of erroneous results to be minimal. As all identified sources of user errors have been addressed either by fail-safe features, failure alert mechanisms or labeling

mitigations, DASH SARS-CoV-2 & Flu A/B Test was shown to have an insignificant level of risk of generating erroneous results when used according to the test procedure instructions.

C Demonstrating "Insignificant Risk of an Erroneous Result" - Accuracy

Clinical Performance

- *Study Design*

i. Study Sites and Duration:
Clinical performance characteristics of DASH SARS-CoV-2 & Flu A/B Test were evaluated in a multi-site prospective study from January to March 2024, during the 2023-2024 respiratory season within the United States. Seven geographically diverse sites, including emergency departments, out-patient, and urgent care/walk-in clinics participated in the clinical study. All the sites qualified as representative of CLIA waived intended use sites for this device.

ii. Operators:
A total of thirty-two operators representative of intended CLIA waived users participitated in the study across the seven clinical testing sites. The participants consisted of medical staff personnel providing patient care and included medical and research assistants, nurses and office staff. The test operators who participated in the study were untrained in the use of the DASH SARS-CoV-2 & Flu A/B Test and had no hands-on experience with conducting diagnostic testing in a clinical laboratory. Information on the operators' current job title, education and laboratory and relevant work experience was provided. Operators were provided only with the Quick Reference Instructions (QRI) and the instructions for the DASH Instrument.

iii. Subjects:
Specimens for the prospective clinical study were collected under informed consent and according to the inclusion or exclusion criteria in the study protocol. Specimens were collected from patients two years or older. Demographic and clinical data were collected from subjects by interview and medical record review. The subjects enrolled in the study represented the intended use population, i.e., individuals with signs and symptoms of respiratory infection.

iv. Samples:
Two anterior nasal (AN) swabs were collected sequentially from each subject. AN swabs for comparator testing were collected by the healthcare provider from both sides of the nose and placed into transport media for comparator testing. For testing with the DASH SARS-CoV-2 & Flu A/B Test, AN swabs from both sides of the nose were either healthcare provider-collected or adult-collected from individuals below 14 years, or self-collected from individuals aged 14 years or older. The order of swab collection was alternated between the comparator swab and the DASH SARS-CoV-2 & Flu A/B Test.

A total of 819 subjects were enrolled in the study from the seven clinical sites. Of these, two subjects were found ineligible after enrollment and 22 were excluded due to protocol deviations (e.g., specimen shipping issues, comparator sample being unable to be tested

within the stability window of the test Instructions for Use, etc.), leaving 795 specimens to be included in the calculations of estimates of assay performance. All of 795 specimens were included in calculations of estimates of the test performance for SARS-CoV-2. There were three specimens that did not have a comparator result due to comparator testing issues and could not be included in the calculations, resulting in 792 specimens included in the calculations for Flu A and Flu B, when tested on the DASH SARS-CoV-2 & Flu A/B Test.

v. Comparator Method:
The performance of the DASH SARS-CoV-2 & Flu A/B Test with AN swabs was estimated based on the comparison with results obtained with an FDA cleared molecular tests for SARS-CoV-2 and Flu A and B and is presented as positive percent agreement (PPA) and negative percent agreement (NPA) shown below. All discordant results between the DASH SARS-CoV-2 & Flu A/B Test and the comparator were investigated using a third highly sensitive FDA cleared test and are footnoted under the performance table that shortly follows.

- *Clinical Study Results*

The clinical performance of the DASH SARS-CoV-2 & Flu A/B Test, when used by untrained operators, testing AN swabs from patients with symptoms of respiratory tract infection, is shown in table below.

Table 4: Clinical Performance of DASH SARS-CoV-2 & Flu A/B Test with AN Swab Specimens

Test Targets	Positive Percent Agreement		Negative Percent Agreement	
	TP/(TP+FN)	***PPA (95% CI)***	***TN/(TN+FP)***	***NPA (95% CI)***
SARS-CoV-2	160/168[1]	95.2% (90.9% - 97.6%)	624/627	99.5% (98.6% - 99.8%)
Flu A	50/53[2]	94.3% (84.6% - 98.1%)	725/739[3]	98.1% (96.8% - 98.9%)
Flu B	36/37[4]	97.3% (86.2% - 99.9%)	749/755[5]	99.2% (98.3% - 99.6%)

TP-true positive; FN-false negative; TN-true negative; FP-false positive
[1]SARS-CoV-2 (PPA): One (1) sample was negative by another FDA cleared test, agreeing with the DASH.
[2]Flu A PPA: Two (2) samples were negative by another FDA cleared test, agreeing with the DASH.
[3]Flu A NPA: 13 samples were positive by another FDA cleared test, agreeing with the DASH.
[4]Flu B PPA: One (1) sample was negative by another FDA cleared test, agreeing with the DASH.
[5]Flu B NPA: Four (4) samples were positive by another FDA cleared test, agreeing with the DASH.

Of the 833 samples tested on DASH SARS-CoV-2 & Flu A/B Test, including 16 repeat samples, there were 24 instances of invalid results (2.9%; 95% CI: 1.9% - 4.3%).

Device Performance with Analyte Concentrations Near the Cutoff

The performance of the DASH SARS-CoV-2 & Flu A/B Test with samples at analyte concentrations near the assay cutoff was evaluated in a study conducted at three external sites representative of CLIA waived sites, with a total of nine untrained operators. The test samples

were contrived in simulated clinical nasal matrix, co-spiked with SARS-CoV-2, Flu A, and Flu B viruses to target concentrations. Three panel members were prepared: true negative (unspiked), low positive (2x LoD), and moderate positive (5x LoD). The test samples were prepared by applying the contrived viral dilutions onto the swabs. Blinded operators tested a randomized coded panel of contrived samples, with three replicates per level tested by each operator over five days. Testing was performed according to the test instructions in the QRI.

In the study, a total of 15 samples generated invalid results on the first attempt; 14 samples generated valid results upon repeat and were included in the data analysis. The percent agreement with the expected results for each panel member, stratified by site, is summarized in the table below.

Table 5: Summary of Results Testing Samples with Analyte Concentrations Near the Cutoff with the DASH SARS-CoV-2 & Flu A/B Test

Analyte	Sample Concentration	% Agreement with Expected Results (n/N[1]) (95% Confidence Interval)			
		Site 1	Site 2	Site 3	Overall
SARS-CoV-2	Low Positive	100.0% (90/90) (95.9% -100.0%)	97.8% (88/90) (92.3% - 99.4%)	100.0% (90/90) (95.9% -100.0%)	99.3% (268/270) (97.3% -99.8%)
	Moderate Positive	98.9% (89/90) (94.0% - 99.9%)	100.0% (89/89)[2] (95.9% -100.0%)	100.0% (90/90) (95.9% -100.0%)	99.6% (268/269) (97.9% -100.0%)
	Negative	100.0% (90/90) (95.9% -100.0%)	100.0% (90/90) (95.9% -100.0%)	100.0% (90/90) (95.9% -100.0%)	100.0% (270/270) (98.6% -100.0%)
Flu A	Low Positive	100.0% (90/90) (95.9% -100.0%)	97.8% (88/90) (92.3% - 99.4%)	100.0% (90/90) (95.9% -100.0%)	99.3% (268/270) (97.3% -99.8%)
	Moderate Positive	100.0% (90/90) (95.9% -100.0%)	100.0% (89/89)[2] (95.9% -100.0%)	100.0% (90/90) (95.9% -100.0%)	100.0% (269/269) (98.6% -100.0%)
	Negative	100.0% (90/90) (95.9% -100.0%)	100.0% (90/90) (95.9% -100.0%)	100.0% (90/90) (95.9% -100.0%)	100.0% (270/270) (98.6% -100.0%)
Flu B	Low Positive	100.0% (90/90) (95.9% -100.0%)	97.8% (88/90) (92.3% - 99.4%)	100.0% (90/90) (95.9% -100.0%)	99.3% (268/270) (97.3% -99.8%)
	Moderate Positive	100.0% (90/90) (95.9% -100.0%)	100.0% (89/89)[2] (95.9% -100.0%)	100.0% (90/90) (95.9% -100.0%)	100.0% (269/269) (98.6% -100.0%)
	Negative	100.0% (90/90) (95.9% -100.0%)	100.0% (90/90) (95.9% -100.0%)	100.0% (90/90) (95.9% -100.0%)	100.0% (270/270) (98.6% -100.0%)

[1]n is number of tests with expected results. N is the total number of valid tests

[2]One moderate positive sample produced an error/no result and was excluded from the analysis, yielding a total of 89 results for this panel member at Site 2.

No significant differences between sites, or between operators were observed in the study. The study results demonstrated that users untrained in the test procedure of the DASH SARS-CoV-2

& Flu A/B Test were able to perform the test correctly and the test provided the expected results for samples with organism concentration near the assay cutoff.

Operator Questionnaire

Following completion of the study, operators at each site were asked to complete a questionnaire to help assess whether participants understood how to use the DASH SARS-CoV-2 & Flu A/B Test correctly. The questionnaire consisted of a series of questions pertaining to the ease of use of the test with answers rated on a scale from one to five where one indicated the greatest ease of use and five indicated the least ease of use. Additionally, the questionnaire also assessed the operators' proficiency of interpreting the test cartridge expiration date and quality control and test results interpretation. Thirty-three operators provided responses to the questionnaire. Participants graded the overall ease of use at a rating ranging from one to two. They found the test to be easy to use and the instructions easy to understand. Based on the operators' feedback, the results were easy to interpret.

D Labeling for Waived Devices

The labeling consists of:

1. Product Insert
2. Quick Reference Instructions (QRI)
3. DASH Instrument System Manual

The following elements are appropriately present:

- The QRI is written in simple language and, where appropriate, contains graphic representation of system components and procedure steps.
- The labeling identifies the test for prescription use only.
- The labeling contains a statement that a Certificate of Waiver is required to perform the test in a waived setting.
- The labeling identifies the sample type that may be used in Waived Settings.
- The labeling contains a statement that laboratories with a Certificate of Waiver must follow the manufacturer's instructions for performing the test.
- The QRI provides instructions for conducting quality control procedures.
- The labeling is sufficient and satisfies the requirements of 21 CFR Part 809.10.

XI. Conclusion

The submitted information in this CLIA waiver application supports a CLIA waiver approval decision.