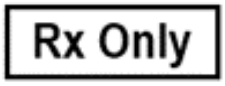
Nuclein

DASH® SARS-CoV-2 & Flu A/B Test Kit- IVD

INSTRUCTIONS FOR USE

DASH® SARS-CoV-2 & Flu A/B Test Kit
Instructions for Use - IVD

SG-0006



For *In Vitro* Diagnostic Use
For Use with DASH Rapid PCR System
CLIA Complexity – Waived
For Use with Direct Anterior Nasal Swab Specimens
For Rx Use Only

Certificate of Waiver is Required to Perform the Test in a Waived Setting

Laboratories with a Certificate of Waiver Must Follow the Manufacturer's Instructions for Performing the Test

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CLIA Complexity: Waived

A Certificate of Waiver is required to perform this test in a CLIA Waived setting. To obtain CLIA waiver information and a Certificate of Waiver, please contact your state health department. Additional CLIA waiver information is available at the Centers for Medicare and Medicaid website at www.cms.hhs.gov/CLIA.

Failure to follow the instructions or modification to the test system instructions will result in the test no longer meeting the requirements for waived classification.

Intended Use

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 and is intended for the simultaneous in vitro qualitative detection and differentiation of SARS-CoV-2, influenza A and influenza B virus ribonucleic acid (RNA) in anterior nasal swab specimens from patients with signs and symptoms of respiratory tract infection. The test is intended to aid in the differential diagnosis of SARS-CoV-2, influenza A, and influenza B in humans in conjunction with other clinical, epidemiologic and laboratory findings.

Positive results of a specific target are indicative of the presence of that viral RNA and may not be the definite cause of disease. Positive results do not rule out co-infection with other pathogens. Negative results do not preclude SARS-CoV-2, influenza A or influenza B infection and should not be used as the sole basis for patient management decisions.

Summary and Explanation

Acute respiratory tract infections (RTIs) are a significant cause of morbidity worldwide. Viral RTIs are a leading cause of hospitalization and clinic visits, especially in vulnerable geriatric and pediatric populations (immunocompromised patients, chronic pulmonary disease patients, and neonates). Many respiratory viruses circulate concurrently seasonally, and present with similar clinical signs and symptoms making diagnosis difficult. A lack of rapid diagnostic tools can also lead to inappropriate antibiotic prescription, contributing to antibiotic resistance.[1,2]

Influenza A and B are enveloped single-stranded negative sense RNA viruses in the *Orthomyxoviridae* family. There are four influenza types but only types A and B are clinically relevant in humans. Influenza A and B mutating strains are responsible for seasonal epidemics worldwide.[3] Influenza viruses are transmitted predominantly by aerosol infection and by direct contact with virus-contaminated surfaces.[4–6] Common symptoms of influenza infection include fever, headache, myalgia, tiredness, general faintness and dry cough. Complications occur particularly in older patients with primary disease.

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is an enveloped, single-stranded, positive sense RNA virus belonging to the *Coronaviridae* family. The virus is highly transmissible through the inhalation of respiratory droplets. SARS-CoV-2 is a novel coronavirus associated with the 2019 global pandemic.[7] The disease it causes, known as COVID-19, is characterized by fevers, coughs, respiratory distress, pneumonia, muscle pain and fatigue, and severe cases can cause lower respiratory tract infections leading to acute respiratory distress syndrome (ARDS).[8–10] The majority of infected individuals experience mild symptoms. However, older adults and individuals with underlying medical conditions are at higher risk for developing ARDS.[11] Despite the introduction of vaccinations and improved treatment strategies, COVID-19 continues to present as a risk to world-wide populations due to quickly mutating SARS-CoV-2 strains.

The seasonality of COVID-19 and influenza overlap and the clinical manifestations of the two diseases can be similar. Rapid and accurate diagnosis and differentiation of SARS-CoV-2 and influenza infections is important in individuals suspected of a respiratory infection to help inform time-critical medical decision-making, facilitate infection control and optimize use of targeted therapies and antimicrobials.

The DASH® SARS-CoV-2 & Flu A/B Test uses reverse transcription polymerase chain reaction (RT-PCR) for rapid qualitative detection and differentiation of SARS-CoV-2, Flu A and Flu B from nasal swabs. The automation, ease of use and small footprint of the DASH platform enable the use of the DASH SARS-CoV-2 & Flu A/B Test at the point-of-care as well as in clinical laboratory settings.[12]

Principle of the Procedure

The DASH SARS-CoV-2 & Flu A/B Test combines the technologies of sequence-specific capture and RT-PCR amplification. The DASH SARS-CoV-2 & Flu A/B Test cartridge (see **Figure 1**) contains all reagents necessary to perform the test.

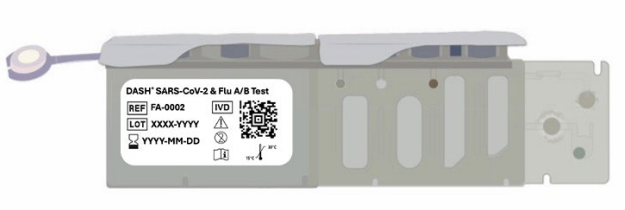


Figure 1: DASH SARS-CoV-2 & Flu A/B Test Cartridge

An anterior nasal swab (ANS) with a 30 mm breakpoint is used to collect a specimen. The nasal swab specimen is added directly to the DASH SARS-CoV-2 & Flu A/B Test cartridge sample chamber containing lysis buffer, which releases the RNA targets from the virus. The operator breaks off the swab at the 30 mm breakpoint into the cartridge, and the cartridge is closed and inserted into the DASH Rapid PCR Instrument to initiate the test. All subsequent test steps are performed automatically by the DASH Rapid PCR Instrument.

The target RNA molecules are isolated from specimens by use of capture oligomers via target capture that utilizes paramagnetic particles. The biotinylated capture oligomers contain sequences complementary to specific regions of the target nucleic acids. During the hybridization step, the sequence-specific regions of the capture oligomers bind to specific regions of the target nucleic acids. The capture oligomer-target complex is then concentrated by the addition of streptavidin-coated paramagnetic particles. The paramagnetic particles, including the bound target nucleic acids, 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 amplification.

The DASH SARS-CoV-2 & Flu A/B RT-PCR assay targets two regions of the Nucleocapsid protein (N) gene that are unique to SARS-CoV-2. The two regions are not differentiated and amplification of either one or both targets leads to fluorescent signal and a “positive” result.

The DASH SARS-CoV-2 & Flu A/B RT-PCR assay targets a well-conserved region of the matrix gene of influenza A. Amplification of the target leads to fluorescent signal and a “positive” result.

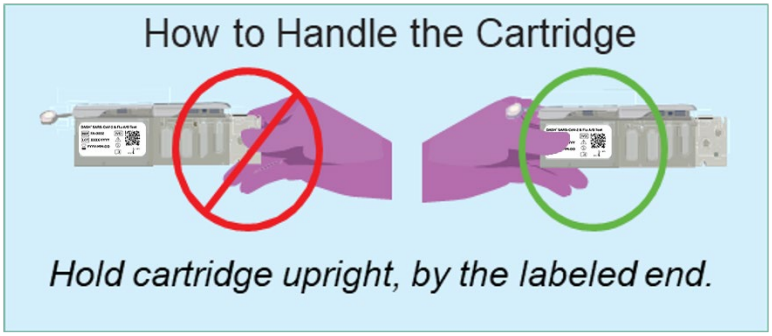
The DASH SARS-CoV-2 & Flu A/B RT-PCR assay targets a well-conserved region of non-structural protein gene of influenza B. Amplification of the target region leads to fluorescent signal and a “positive” result.

A process control is also included to ensure adequate processing of the target virus through the steps of sample purification, nucleic acid amplification and monitoring for the presence of inhibitors in the RT-PCR test.

The DASH® SARS-CoV-2 & Flu A/B Test is used on the DASH Rapid PCR Instrument (DASH Instrument). The DASH Instrument is automated to extract specimens and perform nucleic acid amplification in the test cartridges. Additional details can be found in the DASH Rapid PCR Instrument Manual.

Storage and Handling

Store the DASH SARS-CoV-2 & Flu A/B Test cartridges at 15°C to 30°C until expiration date. When removed from its pouched packaging, the cartridge should be held upright and only handled by the labeled end, as noted in the image that follows:



Materials Provided

- QRG-06-002 | DASH Rapid PCR System SARS-CoV-2 & Flu A/B Test Quick Reference Guide (QRG)
- SG-0006 | DASH SARS-CoV-2 & Flu A/B Test Kit: Qty. 10 Individually Pouched Cartridges per Kit
- SG-0012 (Puritan 25-3606-U BT) | Sample Collection Swabs (30 mm Breakpoint, Sterile Elongated Polyester Flock): Qty. 10 Individually Packaged Swabs

Required But Not Provided

- Personal Protective Equipment (i.e., Powder-free Gloves)
- IFU-06-022 | DASH SARS-CoV-2 & Flu A/B Test Instructions for Use
- IFU-06-023 | DASH Rapid PCR Instrument Manual
- SG-0002 | DASH Rapid PCR Instrument
- SG-0004 | DASH Instrument Accessories Kit
    - Barcode Scanner
    - Power Adaptor
    - Power Cord
    - Printer and Paper
    - Printer Cable
- SG-0008 | DASH SARS-CoV-2 & Flu A/B Positive Control Swabs Kit: Qty. 5 Individually Pouched Swabs
- SG-0010 | DASH Negative Control Swabs Kit: Qty. 5 Individually Pouched Swabs

Warnings and Precautions

1. For in-vitro diagnostic use.
2. For prescription use only.
3. CLIA Complexity: WAIVED. A Certificate of Waiver is required to perform this test in a CLIA Waived setting.
4. Before performing the test, read the DASH® Rapid PCR Instrument Manual (IFU-06-023) and DASH SARS-CoV-2 & Flu A/B Test Instructions for Use (IFU-06-022) completely.
5. Laboratories with a Certificate of Waiver must follow the manufacturer's instructions for performing the test, including use with only the waived specimen type(s), instructions for limitations/intended use, warnings or precautions and performance of QC testing as a failure-alert mechanism.
6. Any modification to the test or the manufacturer's instructions may yield false test results.
7. Device performance has not been established in individuals without signs or symptoms of respiratory infection.
8. **DO NOT** use if packaging is damaged.
9. Leave test cartridge sealed in its foil pouch until just before use. Do not use if pouch is damaged or open.
10. Inspect cartridge prior to use. **DO NOT** use if the test cartridge appears to be damaged.
11. **DO NOT** use a cartridge that is wet or has leaked.
12. **DO NOT** use a cartridge that has been dropped.
13. **DO NOT** use a cartridge if *Cover 1* or *Cover 2* is not intact. See **Figure 2**.

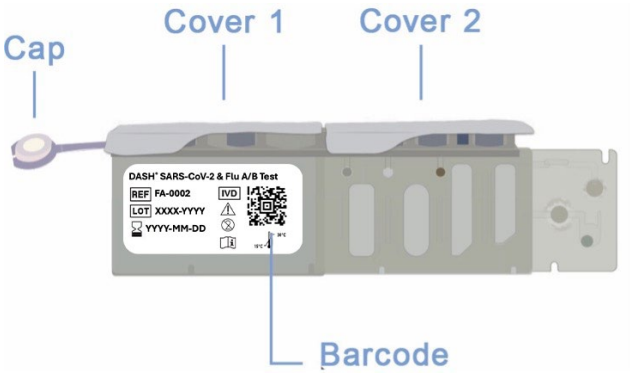


Figure 2: Cartridge Cap & Cover Locations

14. **DO NOT** use a cartridge that has been dropped.
15. **DO NOT** open cartridge *Cover 1* until you are ready to perform sample collection.
16. **DO NOT** open cartridge *Cover 2* until you are ready to insert this cartridge into the DASH Rapid PCR Instrument.
17. **DO NOT** flip a cartridge over after peeling off *Cover 1* or *Cover 2*.
18. **DO NOT** handle the cartridge using the thin films nearest to *Cover 2*.
19. Treat all specimens as potentially infectious. Follow universal precautions when handling samples, the kit, and its contents.

20. Leave test cartridge sealed in its foil pouch until just before use. Do not use if pouch is damaged or open. **DO NOT** use a test cartridge past its expiration date (Figure 3). The DASH Instrument will reject a cartridge that has expired.

Figure 3: Cartridge Expiration Date

21. Discard the test cartridge after use. **DO NOT** reuse a used test cartridge.
22. All components of this kit should be discarded as biohazard waste according to federal, state and local regulatory requirements. Solutions used to make the DASH® SARS-CoV-2 & Flu A/B Positive Control swab are non-infectious; however, patient samples, controls, and test cartridges should be handled as though they could transmit disease. Observe established precautions against microbial hazards during use and disposal.
23. Inadequate or inappropriate sample collection, storage, and transport may yield false test results.
24. Keep the work area clean to prevent contamination. Follow the cleaning instructions provided in the DASH Rapid PCR Instrument Manual (IFU-06-023) for proper cleaning of the DASH Rapid PCR Instrument.
25. Wear appropriate personal protective equipment and gloves when running each test and handling patient specimens. Change gloves between handling each specimen.
26. For use with kit provided swabs. Use only swabs provided with the kit.
27. If infection with SARS-CoV-2 is suspected based on current clinical and epidemiological screening criteria recommended by public health authorities, specimens should be collected with appropriate infection control precautions. Viral culture should not be attempted in cases of positive results for SARS-CoV-2 and/or any similar microbial agents unless a facility with an appropriate level of laboratory biosafety (e.g., BSL 3 and BSL 3+, etc.) is available to receive and culture specimens.
28. If infection with a novel influenza A virus is suspected based on current clinical and epidemiological screening criteria recommended by public health authorities, specimens should be collected with appropriate infection control precautions for novel virulent influenza viruses and sent to a state or local health department for testing. Viral culture should not be attempted in these cases unless a BSL 3+ facility is available to receive and culture specimens.
29. Environmental conditions for operating the DASH SARS-CoV-2 & Flu A/B Test are 15°C to 30°C; 20% - 80% RH; Altitude up to 1,676 meters (5,500 feet). Operating the test system outside of specifications may cause incorrect results.

Chemical Hazards

The DASH® SARS-CoV-2 & Flu A/B Test cartridge overall is not classified as hazardous. For classifications of the individual components, see the Safety Data Sheet.

Sample Collection, Transport and Handling

Specimen Collection and Handling

Use only the swab provided with the kit (SG-0012 | 25-3606-U BT).

Inadequate sample collection or improper sample handling, storage, or transport may yield erroneous results.

Sample collection instructions are provided in the *Running Patient Specimens* section of this IFU, as per the CDC guidance[13].

Specimen Transport and Storage

For the best performance, anterior nasal swab specimens should be tested immediately after collection and should not be stored for testing at a later time.

If the patient sample cannot be tested at the time of collection, place the sample swab into the *Specimen Chamber* of the cartridge without delay. The cartridge may then be run on the DASH instrument within one (1) hour of adding the patient swab into the *Specimen Chamber*. For samples waiting to be tested, store a cartridge with the assembled sample between 15ºC and 30ºC. **DO NOT** store assembled samples outside of the stated parameters.

Quality Control – Internal Controls

The DASH SARS-CoV-2 & Flu A/B Test cartridge contains a positive procedural control (non-infectious MS2 bacteriophage RNA) that is used to verify assay steps including that viral lysis, RNA extraction, nucleic acid amplification and detection were executed properly and that the assay was performed without sample-derived inhibition. The procedural control should have a VALID result with every test performed.

Quality Control – External Controls

External controls must be used to show that the DASH SARS-CoV-2 & Flu A/B Test is working properly. The DASH SARS-CoV-2 & Flu A/B Positive Control and DASH Negative Control swabs are available separately for use as external controls for the test.

The DASH SARS-CoV-2 & Flu A/B Positive Control (SG-0008) and DASH Negative Control (SG-0010) swabs must be stored at room temperature (between 15°C and 30°C). External controls are tested using the same procedure that is used to test a patient specimen.

It is recommended that external controls be tested at the times noted below:

- 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 Rapid PCR Instrument is used for the first time.
- As deemed additionally necessary by internal quality control procedures and in accordance with local, state and federal regulations or accreditation requirements.

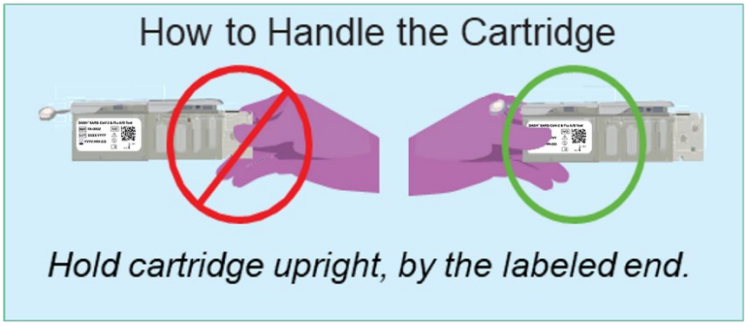
If correct control results are not obtained, repeat the test using a new control swab and a new test cartridge. If the control fails upon repeat testing, contact Customer Support and do not perform additional clinical tests or report results.

Running External Controls

1. **Initialize the DASH® Instrument:**
    - Press the power button to turn on the DASH Instrument.
    - Wait for the instrument to initialize and complete the warm-up process.
    - Select username from the drop-down menu.
    - Enter your 5-digit PIN and touch “Enter.”
    - The instrument is now ready to run a test.

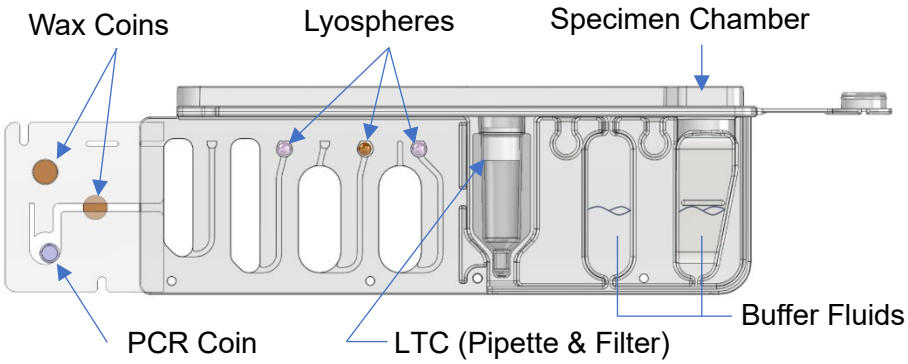
2. **Gather materials needed to run a QC Test:**
    - Select either the DASH Negative Control swab (SG-0010) or the DASH SARS-CoV-2 & Flu A/B Positive Control swab (SG-0008) from the respective kit.
    - Obtain a sealed cartridge package from the DASH SARS-CoV-2 & Flu A/B Test kit (SG-0006).

3. **Prepare Cartridge.**

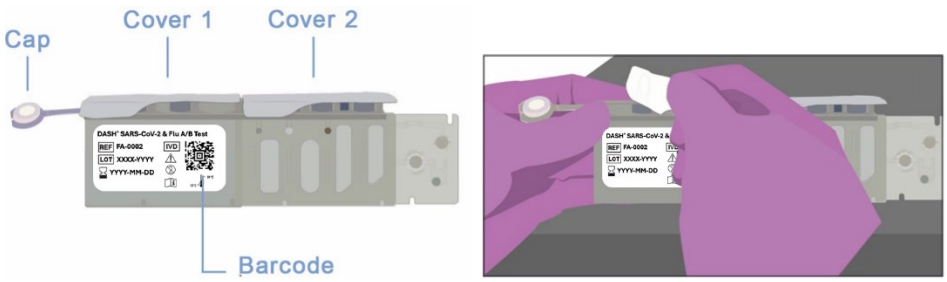


⚠
- Remove the cartridge from the packaging. **DO NOT** handle cartridge by the thin films. Only handle by the labeled end, under *Cover 1*.

⚠
- Inspect cartridge prior to use. **DO NOT** use if the test cartridge appears to be damaged (see intact cartridge image below).



- Hold cartridge upright on flat surface (as shown below), and peel off *Cover 1*. **DO NOT** open *Cover 1* until testing is ready to be performed. **DO NOT** lay the cartridge on its side or flip it over, as liquid materials are enclosed.

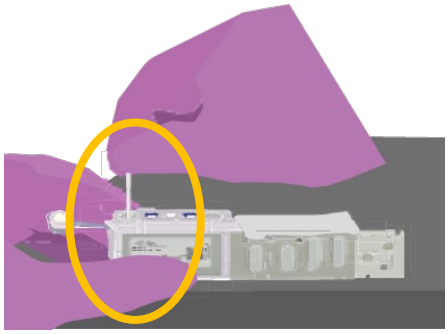


4. Prepare the Control Swab for Testing:

- Open the selected control swab packaging and remove the swab.

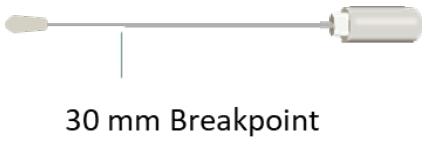
  Note: *If the swab is dropped after opening, use a new swab.*

- Insert the swab into *Specimen Chamber* (opening closest to the *Cap*).

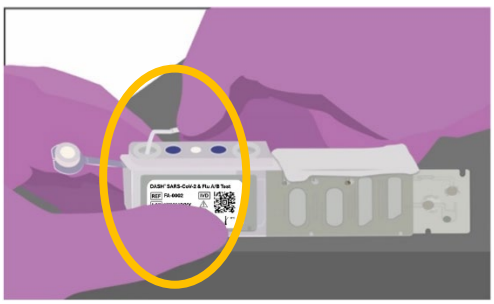


DASH® Negative Control swab:

- The DASH Negative Control swab has a 30 mm Breakpoint (See image below).



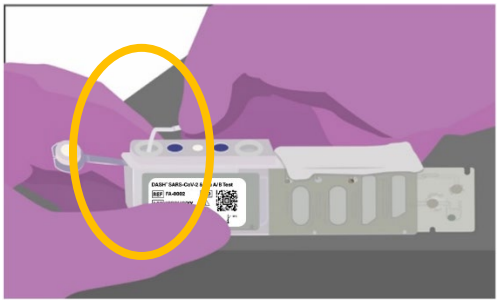
- Break off the stem at the 30 mm breakpoint, then close the cartridge *Cap* by snapping it into place.



***Note**: Discard the remaining unused portion of the swab stick and swab container.*

DASH® SARS-CoV-2 & Flu A/B Positive Control swab:

- Hold the stem and make sure to leave some space (approximately ¼" or 0.5 cm) between the swab tip and the bottom of the *Specimen Chamber*. This is to ensure the *Cap* can be securely closed after the swab is broken off from the stem. Break off the swab stem, then close the cartridge *Cap* by snapping it into place



***Note**: Discard the remaining unused portion of the swab stick and swab container.*

5. Scan Barcodes.

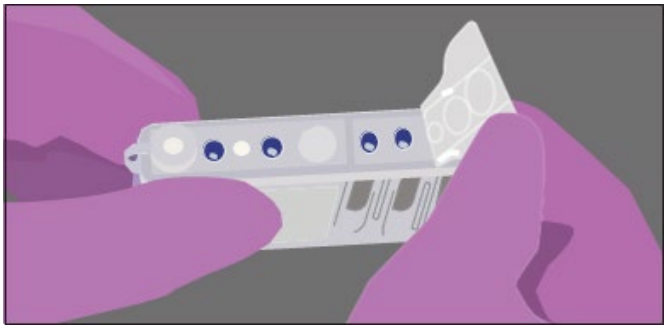
- Scan barcode on outside of control swab packaging.

- Scan the cartridge barcode.

6. Sample and Cartridge Confirmation

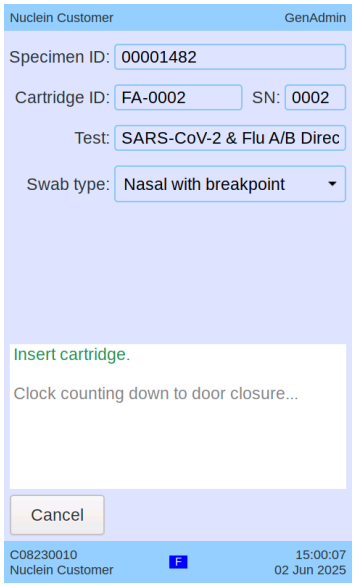
- Confirm *Specimen Chamber* contains swab tip and cartridge *Cap* is closed.
- Hold cartridge upright on flat surface (as shown below).
- **DO NOT** remove *Cover 2* until the cartridge is ready to be inserted into the DASH Instrument.

- Peel off *Cover 2*. Touch the “Open” button on the instrument screen, then touch the “Seal Removed” button.



7. Run Test

- Insert the cartridge.

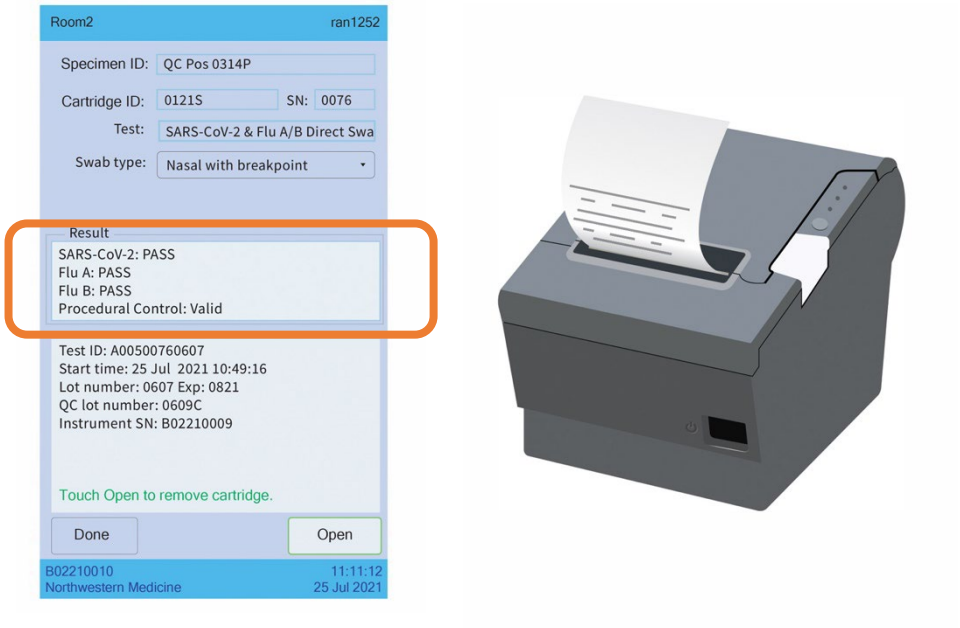


- Test will run automatically. Blue lights on the front of the instrument indicate progress.

Instrument Power Button with “Clock Lights”

8. Complete Test

- After test is complete, results are displayed on the screen and will print automatically.



The QC result will read "PASS" and "Valid" to indicate that the internal and external controls successfully passed.

If the QC result indicates "FAIL" or "Invalid," repeat the test (returning to Step 1 for "Running External Controls.")

- If the QC results fails a second time, contact customer support at customersupport@nuclein.com or 1-888-992-DASH.

- Touch "Open" to remove the cartridge.
- Discard the used cartridge in biohazard waste as per site procedures.

Running Patient Specimens

1. Initialize the DASH® Instrument:

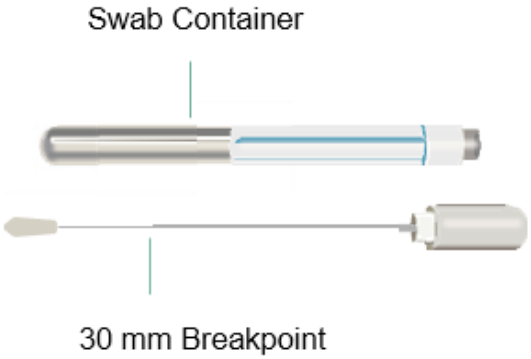
- Press the power button to turn on the DASH Instrument.
- Wait for the instrument to initialize and complete the warm-up process.
- Select username from the drop-down menu.
- Enter your 5-digit PIN and touch "Enter."
- The instrument is now ready to run a test.

2. Collect Patient Specimen.

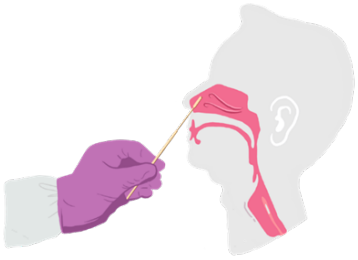
- The anterior nasal sample collection swab has two parts, a swab container and swab with a 30 mm Breakpoint, as pictured below.

⚠ **Note:** Swabs included in the kit (SG-0012; Puritan 25-3606-U BT) have been validated for use with the DASH SARS CoV-2 & Flu A/B Test. **DO NOT** use other swabs.

Sterile Polyester Flock Swab with 30 mm breakpoint.



- The following collection steps are provided, as per the CDC guidance[13]:
  - Insert the entire collection tip of the swab (usually ½ to ¾ of an inch, or 1 to 1.5 cm) inside the nostril.
  - Gently sample the nasal wall by rotating the swab in a circular motion against the nasal wall at least 4 times.
  - Take approximately 15 seconds to collect the specimen. Be sure to collect any nasal drainage that may be present on the swab.
  - Using the same swab, repeat collection with the other nostril.

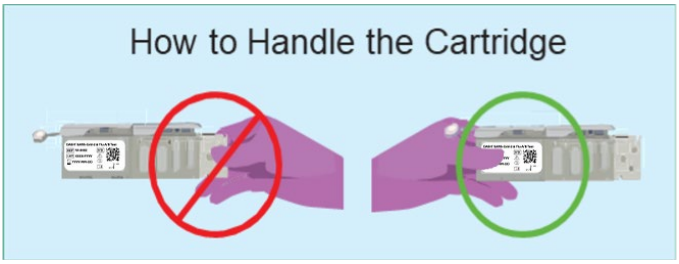


⚠
- Once collection is complete, exercise caution to **NOT** touch the swab tip to surfaces outside of the cartridge *Specimen Chamber.*
- Prepare cartridge and add sample to cartridge without delay.

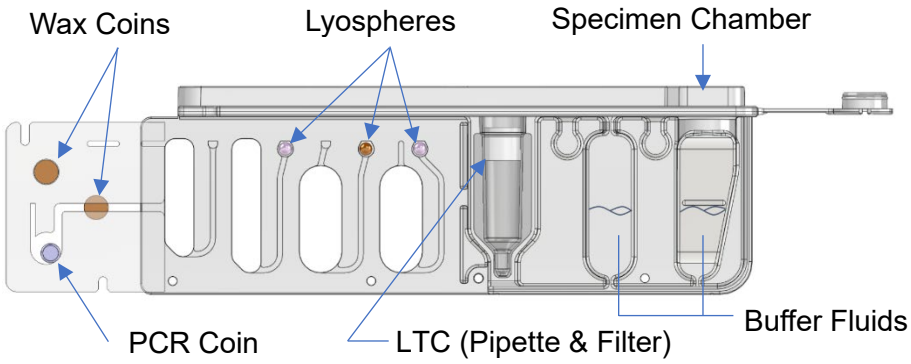
Note: *If the swab is dropped after opening, collect patient specimen with a new swab.*

3. Prepare Cartridge.

- Remove the cartridge from the packaging. **DO NOT** handle cartridge by the thin films. Only handle by the labeled end, under *Cover 1*.

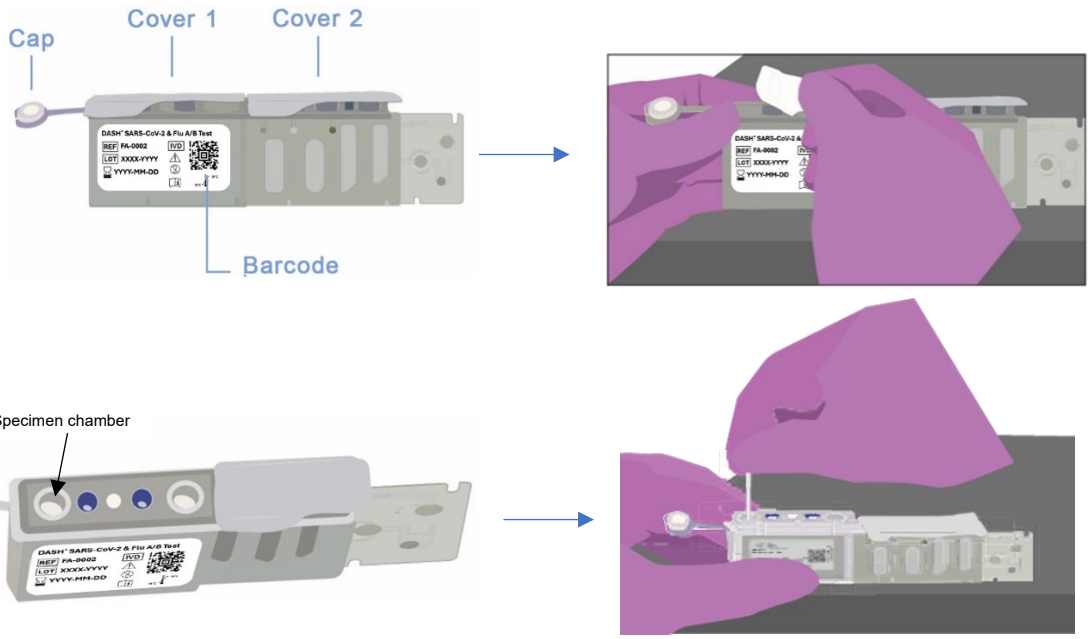


- Inspect cartridge prior to use. **DO NOT** use if the test cartridge appears to be damaged (see intact cartridge image below).



⚠

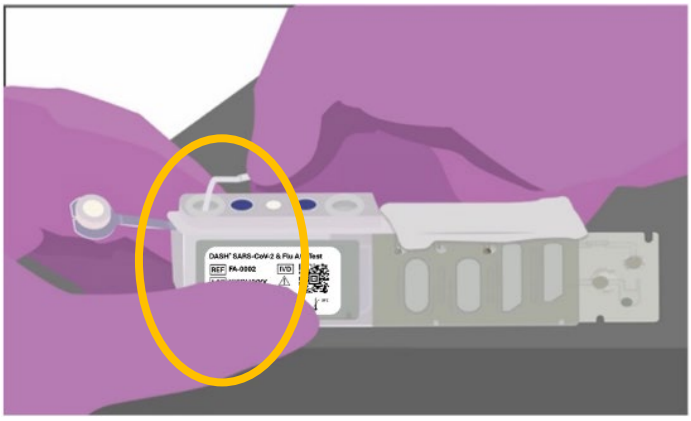
- Hold cartridge upright on flat surface (as shown below), and peel off *Cover 1*. **DO NOT** open *Cover 1* until testing is ready to be performed. **DO NOT** lay the cartridge on its side or flip it over, as liquid materials are enclosed. Without delay, insert the sample swab into the *Specimen Chamber*.



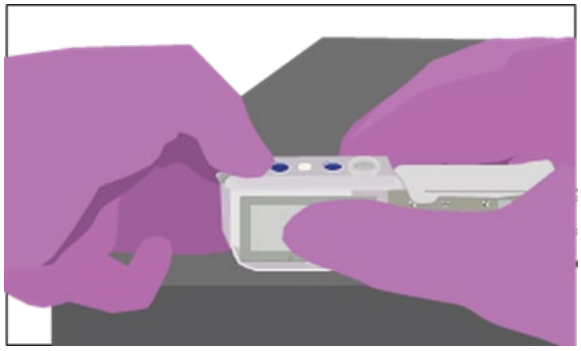
- Break off the stem at the 30 mm breakpoint by using the rim of the *Specimen Chamber* as leverage.

  Note: *If the swab is dropped after opening, use a new swab.*

  - Gently bend the swab stem.
  - Twist the stem until the stem breaks off.
  - Swab tip remains in the *Specimen Chamber*. Discard the stem and swab container.

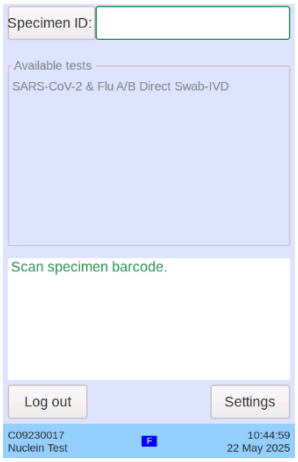


- Close the cartridge *Cap* by snapping it into place and run the cartridge on the DASH® Instrument within an hour after loading the specimen swab.



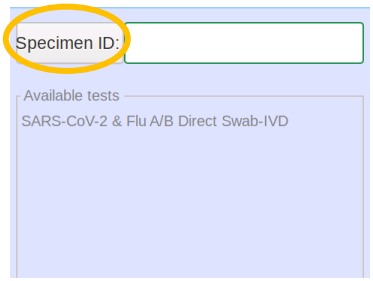
4. Scan Barcodes

- Scan the patient sample barcode.
  - Per site procedures, assign a patient sample barcode ID.
  - Press the button on the barcode reader and aim the reader towards the sample barcode.



  - After scanning the sample barcode, the sample ID is shown in the *Specimen ID* field on the screen.

    - Alternately, to enter the sample ID manually, press the “Specimen ID” button and use the keypad.



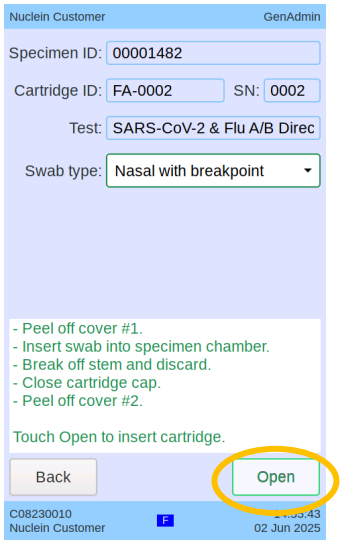
- Scan the cartridge barcode.

5. Sample and Cartridge Confirmation

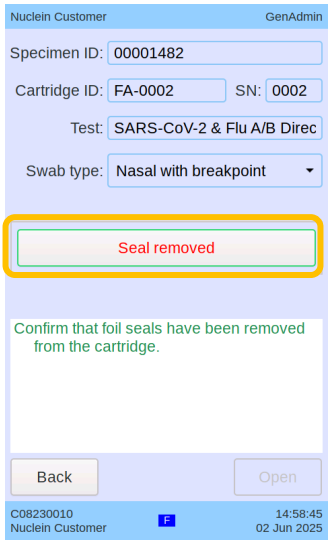
- With *Cover 1* having already been removed, confirm the *Specimen Chamber* contains swab tip and the cartridge *Cap* is closed.
- Hold cartridge upright on flat surface.

⚠

- **DO NOT** remove *Cover 2* until the cartridge is ready to be inserted into the DASH® Instrument.
- Peel off *Cover 2* and touch the "Open" button on the screen to open the instrument door.



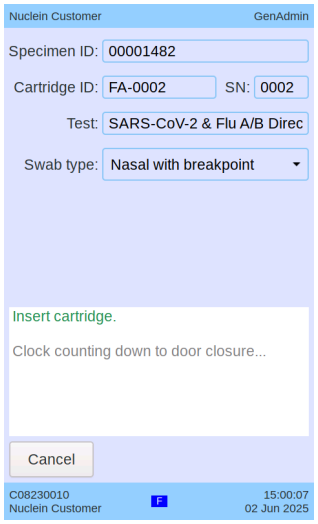
- Touch "Seal removed" button that is now displayed on screen to confirm that *Cover 2* was moved in the previous step.



6. Run Test

- The cartridge receiver extends out through the open door waiting to receive the cartridge.
- Insert the cartridge into the instrument.
- The cartridge receiver slides into the instrument and the door closes.

> ***Note**: If the cartridge receiver closes before a cartridge has been inserted, open the cartridge receiver again by touching "Open".*

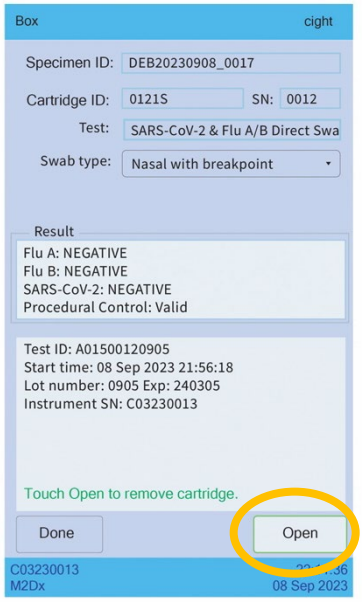


- Test will run automatically.
- Display shows the test start time and starts to perform the test.

- "Clock lights" on the front of the instrument indicate progress.

7. Complete Test

- After test is complete, results are displayed on the screen and will print automatically.
- Touch “Open” to open the instrument door and extend the cartridge receiver with the used cartridge and remove the cartridge.



- Discard the used cartridge in biohazard waste as per site procedures.

Result Interpretation

Test results will be displayed on the touchscreen and printed 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. This indicates that all internal procedural controls passed, and the specimen test result can be reported. The procedural control is "Valid" if it meets the validated acceptance criteria. If the Procedural Control is INVALID, repeat the test with a new sample and a new cartridge.

Screenshots of potential results screens are shown in *Step 7* (pg. 18), and the possible test results are summarized in **Table 1**.

Table 1: Test Results & Interpretations

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. DASH Instrument detected an error and aborted the current test. Repeat test with a new cartridge.*

***** If repeat test also results in an error or invalid, please contact Nuclein Customer Support at customersupport@nuclein.com or 1-888-992-DASH.

Retests

Reasons to Repeat a Patient Test

If an INVALID or ERROR result is returned, repeat the test, once, according to instructions.

Patient Sample Retest Procedure

- Obtain a new DASH® SARS CoV-2 & Flu A/B Test cartridge.
- Prepare and test a new external control swab following the steps in the *Running External Controls* section.
- Collect a new swab specimen from the same patient and repeat the test following the steps in the *Running Patient Samples* section.

Limitations

- DASH® SARS-CoV-2 & Flu A/B Test can be used only with the DASH Rapid PCR Instrument.
- The DASH SARS-CoV-2 & Flu A/B Test is designed as an aid to diagnosis, but should not be used as the sole basis for treatment.
- Test results should be interpreted in conjunction with other clinical and laboratory data available to the clinician.
- Performance of the DASH SARS-Cov-2 & Flu A/B Test was evaluated in accordance with the procedures of this IFU. Any modification to the test or these instructions may yield erroneous results.
- Performance of the DASH SARS-Cov-2 & Flu A/B Test was evaluated with anterior nasal swab specimens. Use with other specimen types has not been evaluated and performance characteristics are unknown.
- Mutations of the target regions of the SARS-CoV-2, Flu A, and Flu B viruses may impact test performance.
- This test should not be used beyond the expiration date listed on the packaging. Use of expired tests can lead to incorrect results.
- Performance characteristics for influenza were established when the influenza A (H1N1)pdm09 and H3N2, were the predominant viruses in circulation. When other influenza A viruses are emerging, performance characteristics may differ.
- The test results should be interpreted in conjunction with other clinical and laboratory data available to the clinician.
- The clinical performance has not been established for all circulating variants of SARS-CoV-2 but is anticipated to be reflective of the prevalent variants in circulation at the time and location of the clinical evaluation. Performance at the time of testing may vary depending on the variants circulating, including newly emerging strains of SARS-CoV-2 and their prevalence, which change over time.
- Positive test results do not rule out co-infections with other pathogens.
- Analyte targets (viral sequences) may persist in vivo, independent of virus viability. Detection of analyte target(s) does not imply that the corresponding virus(es) are infectious, nor are the causative agents for clinical symptoms.
- This test cannot rule out diseases caused by non-target related microorganisms.
- Negative results do not preclude virus infection and should not be used as the sole basis for treatment or other patient management decisions.
- False negative results may occur if the viral load is below the limit of detection. If symptoms of respiratory infection persist, additional follow-up testing may be required.
- Positive and negative predictive values are highly dependent on prevalence.
- The performance of this test has not been validated for specimens from asymptomatic individuals that do not have signs and symptoms of respiratory infection.
- Interfering substances have only been evaluated for the items listed within the labeling (refer to *Analytical Performance* section).
- FluMist was not available for evaluation to assess potential interference.
- Biotin was shown to interfere with low levels of SARS-CoV-2 at a concentration of 4.58 µg/mL.
- Flonase was shown to interfere with low levels of SARS-CoV-2 at a concentration of 5% v/v.
- The assay performance was established during the 2023/2024 season. The performance for some viruses may vary depending on the prevalence and population tested. False positive test results are likely when prevalence of disease is low or non-existent in a community.
- This test does not differentiate influenza A subtypes (i.e., H1N1, H3N2); additional testing is required to differentiate any specific influenza A subtypes or strains, in consultation with local public health departments.

- Interference with detection of SARS-CoV-2 and Flu A (at low concentrations) was observed in the presence of Flu B at concentrations >1.01E+03 $TCID_{50}$/mL. Refer to the *Competitive Interference* section.

Expected Values

The DASH® SARS-CoV-2 & Flu A/B Test clinical evaluation included 795 anterior nasal (AN) specimens from prospectively collected and screened study subjects. All 795 specimens were evaluable for SARS-CoV-2 while 792 specimens were evaluable for Flu A/B. The positivity for SARS-CoV-2, Flu A and Flu B, as determined by DASH SARS-CoV-2 & Flu A/B Test, are shown below, stratified by study site and by age group.

Overall Positivity Rates Observed during the Clinical Study

Test Target	Overall			Site 1 (N=89)		Site 2 (N=178)		Site 3 (N=152)		Site 4 (N=59)		Site 5 (N=124)		Site 6 (N=93)		Site 7 (N=97)	
	N	#	%	#	%	#	%	#	%	#	%	#	%	#	%	#	%
SARS-CoV-2	***795***	***160***	***20.1%***	10	11.2%	19	10.7%	25	16.4%	6	10.2%	29	23.4%	25	26.9%	46	47.4%
Flu A	***792***	***50***	***6.3%***	5	5.6%	13	7.3%	5	3.3%	2	3.4%	12	9.7%	4	4.3%	9	9.3%
Flu B	***792***	***36***	***4.5%***	9	10.1%	6	3.4%	12	7.9%	2	3.4%	2	1.6%	4	4.3%	1	1.0%

N = Total evaluable specimens
# = Number of Positives
% = % positivity rate

Positivity Rates by Age Group

Test Target	Overall			Subject Age							
				2-<14 years		14-24 years		>24-64 years		≥65 years	
	N	#	%	#	%	#	%	#	%	#	%
SARS-CoV-2	***795***	***160***	***20.1%***	19	11.8%	28	17.5%	97	60.6%	16	10.0%
Flu A	***792***	***50***	***6.3%***	8	16.0%	10	20.0%	29	58.0%	3	6.0%
Flu B	***792***	***36***	***4.5%***	15	41.70%	5	13.9%	16	44.4%	0	0.0%

N = Total evaluable specimens
# = Number of Positives
% = % positivity rate

Performance Characteristics

Clinical Performance & CLIA Waiver Study

Clinical performance characteristics of the DASH SARS-CoV-2 & Flu A/B Test were evaluated for both accuracy and ease of use during the 2023-2024 respiratory season. The predominant subtypes during this season were (H1N1)pdm09 and H3N2 for influenza A, and Victoria lineage for influenza B according to the *Weekly US Influenza Surveillance Report* from May 17, 2024. Seven (7) geographical locations within the United States, participated in the prospective collection of specimens from individuals

showing signs and symptoms of respiratory infection from January to March 2024. Each of these sites were representative of the intended use setting, point-of-care (POC) use, and were CLIA-waived. All testing was performed by untrained operators without any prior hands-on device experience or laboratory training. Operators used only the written test procedure provided with the DASH® SARS-CoV-2 & Flu A/B Test (i.e. quick reference guide) and the DASH Instrument. For testing with the DASH SARS-CoV-2 & Flu A/B Test, anterior nasal 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 table below provides a summary of the subject demographics associated with the specimens collected within the study:

Summary of Subject Demographics

Subject Demographics	Overall (N=795)
Age: Mean (SD)	37.3 (18.3)
Age: Median [Min, Max]	36 [2, 87]
Age Group	
2 - <14 years of age	89 (11.1%)
14 - 24 years of age	113 (14.2%)
>24 - 64 years of age	530 (66.7%)
≥65 years of age	64 (8.1%)
Sex at Birth	
Female	465 (58.5%)
Male	330 (41.5%)
Ethnicity	
Hispanic/Latino	303 (38.1%)
Not Hispanic/Latino	492 (61.9%)
Race	
American Indian or Alaskan Native	3 (0.4%)
Asian	4 (0.5%)
Black or African American	111 (14.0%)
Native Hawaiian/Pacific Islander	4 (0.5%)
White	661 (83.1%)
Unknown/Prefer not to answer	6 (0.8%)
Other (Mixed race/biracial)	6 (0.8%)
Total	795 (100%)

Performance of the DASH SARS-CoV-2 & Flu A/B Test was assessed by a comparison of results with a FDA cleared RT-PCR test for SARS-CoV-2, and a second FDA cleared test for Flu A and Flu B.

Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA), including 95% confidence intervals, were calculated for each target nucleic acid in comparison with the designated comparator test. 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.

Of the 817 subjects enrolled in the study, 795 subjects were evaluable for at least one (1) analyte/target and were included in the data analysis. There were 795 evaluable subjects for SARS-CoV-2 and 792 evaluable subjects for Flu A and Flu B. The most common reason for exclusion was due to the comparator sample being unable to be tested within the stability window of the test IFU. The following table provides a summary of the study results:

Clinical Performance Results

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

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.
Flu A NPA: 13 samples were positive by another FDA cleared test, agreeing with the DASH.
3 Flu B PPA: One (1) sample was negative by another FDA cleared test, agreeing with the DASH.
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%).

Analytical Performance

Analytical Sensitivity (Limit of Detection)

Limit of detection (LoD) was determined for the DASH® SARS-CoV-2 & Flu A/B Test on the DASH Rapid PCR Instrument utilizing five (5) viral strains representing the test targets. All samples were prepared in pooled nasal matrix (PNM) and evaluated to determine the lowest concentration at which ≥95% of replicates yield a positive result. The final LoD results are summarized in the table below:

Limit of Detection Results

Viral Target	Strain	Source/ Product Type	LoD* (copies/swab)	LoD* (copies/mL)
SARS-CoV-2	Isolate hCoV- 19/USA/GA-EHC-2811C/2021 (Lineage B.1.1.529; Omicron Variant)	BEI Resources/ Gamma-Irradiated	1200 copies/swab	3.00E+04 copies/mL
Flu A	H1N1 Victoria/2570/19	Zeptometrics/ Culture Fluid	1.35 $TCID_{50}$/swab	33.75 $TCID_{50}$/mL
	H3N2 /Darwin/9/21	Zeptometrics/ Culture Fluid	0.225 $TCID_{50}$/swab	5.63 $TCID_{50}$/mL
Flu B	Washington/02/19 – Victoria Lineage	Zeptometrics/ Culture Fluid	0.10 $TCID_{50}$/swab	2.50 $TCID_{50}$/mL
	Utah/9/14 - Yamagata Lineage	Zeptometrics/ Culture Fluid	0.675 $TCID_{50}$/swab	16.88 $TCID_{50}$/mL

***** The concentration stated per swab represents microbial material prepared in 40µL pooled nasal matrix and applied to the swab. The per mL concentration is therefore represented as 25x the stated amount per swab.

Analytical Reactivity (Inclusivity)

Inclusivity was established for the DASH® SARS-CoV-2 & Flu A/B Test on the DASH Instrument through wet testing and *in silico* analysis. Wet testing was performed by evaluating seven (7) strains of SARS-CoV-2, 21 strains of Flu A, and 10 strains of Flu B. Each strain was evaluated at concentrations near LoD, prepared in pooled nasal matrix (PNM), and tested individually, with three (3) replicates evaluated per strain. Testing was executed in a blinded fashion with random negatives. Inclusivity was determined if the target had a 100% positive call rate for all three (3) replicates tested. The table that follows summarizes the wet tested analytical reactivity results:

Inclusivity Wet Test Results Summary

Test Target Variant/Subtype	Virus Concentration *(per swab)*	Virus Concentration *(per mL)**
[1]SARS-CoV-2 Variant B.1.1.7 (Isolate:England/204820464/2020)	0.0828 $TCID_{50}$/swab	2.07 $TCID_{50}$/mL
SARS-CoV-2 Variant B.1.351(Isolate:South_Africa/KRISP-K005325/2020)	0.0414 $TCID_{50}$/swab	1.04 $TCID_{50}$/mL
[2]SARS-CoV-2 Lineage B.1.617.2; Delta Variant (USA/PHC658/2021)	0.1656 $TCID_{50}$/swab	4.14 $TCID_{50}$/mL
SARS-CoV-2 Lineage P1;Gamma Variant (Japan/TY7-503/2021)	0.0414 $TCID_{50}$/swab	1.04 $TCID_{50}$/mL
[1]SARS-CoV-2 Lineage B.1.617.1 Kappa Variant (USA/CA-Stanford-15_S02/2021)	0.0828 $TCID_{50}$/swab	2.07 $TCID_{50}$/mL
SARS-CoV-2 Lineage BA 2.3; Omicron Variant	0.0414 $TCID_{50}$/swab	1.04 $TCID_{50}$/mL
Gamma-irradiated SARS-CoV-2, isolate USA-WA1/2020	7200 copies/swab	1.80E+05 copies/mL
Influenza A H1N1 (New Cal/20/99)	4.05 $TCID_{50}$/swab	101.25 $TCID_{50}$/mL
Influenza A H1N1 (PR/8/34)	4.05 $TCID_{50}$/swab	101.25 $TCID_{50}$/mL
Influenza A H1N1 (Brisbane/59/07)	4.05 $TCID_{50}$/swab	101.25 $TCID_{50}$/mL
Influenza A H1N1 (Taiwan/42/06)	4.05 $TCID_{50}$/swab	101.25 $TCID_{50}$/mL
[3]Influenza A H1N1 (Singapore/63/04)	4.05 $TCID_{50}$/swab	101.25 $TCID_{50}$/mL
Influenza A H1N1pdm (California/07/09)	4.05 $TCID_{50}$/swab	101.25 $TCID_{50}$/mL
Influenza A H1N1pdm (New York/18/09)	4.05 $TCID_{50}$/swab	101.25 $TCID_{50}$/mL
Influenza A H1N1pdm Virus (Strain: Guangdong-Maonan/SWL 1536/19)	4.05 $TCID_{50}$/swab	101.25 $TCID_{50}$/mL
Influenza A H1N1pdm (Mexico/4108/09)	4.05 $TCID_{50}$/swab	101.25 $TCID_{50}$/mL
Influenza A H1N1pdm (Victoria/2570/19)	4.05 $TCID_{50}$/swab	101.25 $TCID_{50}$/mL
Influenza A H3N2 (Wisconsin/67/05)	4.05 $TCID_{50}$/swab	101.25 $TCID_{50}$/mL
[1]Influenza A H3N2 Virus (Strain: Hong Kong/2671/19)	8.1 $TCID_{50}$/swab	202.50 $TCID_{50}$/mL
Influenza A H3N2 (Stockholm/6/14)	4.05 $TCID_{50}$/swab	101.25 $TCID_{50}$/mL
Influenza A H3N2 (HK/8/68)	4.05 $TCID_{50}$/swab	101.25 $TCID_{50}$/mL
Influenza A H3N2 (Texas/50/12)	4.05 $TCID_{50}$/swab	101.25 $TCID_{50}$/mL
[1]Influenza A H3N2 (Strain: Kansas/14/17)	8.1 $TCID_{50}$/swab	202.50 $TCID_{50}$/mL
Influenza A H3N2 (California/7/04)	4.05 $TCID_{50}$/swab	101.25 $TCID_{50}$/mL
Influenza A H3N2 (Norway/466/14)	4.05 $TCID_{50}$/swab	101.25 $TCID_{50}$/mL
Influenza A H3N2 (Kumamoto/102/02)	4.05 $TCID_{50}$/swab	101.25 $TCID_{50}$/mL
[1]Influenza A H3N2 (Brisbane/10/07)	8.1 $TCID_{50}$/swab	202.5 $TCID_{50}$/mL
Influenza A H5N3 [Genomic RNA from Kilbourne F3: A/duck/Singapore/645/1997 (H5N3) Mutant, High (Hy) Yield Influenza A Virus]	1.76E+03 ng RNA/swab	4.40E+04 ng RNA/mL
Influenza B (Lee/40)	4.05 $TCID_{50}$/swab	101.25 $TCID_{50}$/mL
Influenza B (Massachusetts/2/12)	2.025 $TCID_{50}$/swab	50.63 $TCID_{50}$/mL
Influenza B Victoria Lineage (Austria/1359417/21)	2.025 $TCID_{50}$/swab	50.63 $TCID_{50}$/mL
Influenza B Victoria Lineage (Victoria/2/87)	2.025 $TCID_{50}$/swab	50.63 $TCID_{50}$/mL
Influenza B Victoria Lineage (Alabama/2/17)	2.025 $TCID_{50}$/swab	50.63 $TCID_{50}$/mL
Influenza B Yamagata Lineage (Panama/45/90)	2.025 $TCID_{50}$/swab	50.63 $TCID_{50}$/mL
Influenza B Yamagata Lineage (Florida/04/06)	2.025 $TCID_{50}$/swab	50.63 $TCID_{50}$/mL
Influenza B Yamagata Lineage (Brisbane/9/14)	2.025 $TCID_{50}$/swab	50.63 $TCID_{50}$/mL

* The concentration stated per swab represents microbial material prepared in 40µL of pooled nasal matrix and applied to the swab. The per mL concentration is therefore represented as 25x the stated amount per swab.
[1]Testing at a lower concentration (i.e., 3x LoD) failed to yield 100% detection. The lowest concentration that yielded 100% detection was 6x LoD.
[2]Testing at a lower concentration (i.e., 3x LoD) failed to yield 100% detection. The lowest concentration that yielded 100% detection was 12x LoD.
[3]Influenza A H1N1 (Singapore/63/04) was detected in 2/3 replicates at 3x LoD. The strain was reprepared at 3x LoD and tested in 7 replicates for 100% detection.

In Silico Analysis for SARS-CoV-2, Flu A, and Flu B:

The inclusivity of the DASH® SARS-CoV-2 & Flu A/B Test was also evaluated using *in silico* analysis of the sample preparation capture oligos and the PCR primers and probes for SARS-CoV-2 and influenza A and B in relation to sequences available in the NCBI SARS-CoV-2 Data Hub and the NCBI Influenza Virus Database.

Genomic sequences for SARS-CoV-2 were retrieved from the NCBI SARS-CoV2 Data Hub on May 8, 2024, and included all lineages and variants of concern (VOC) or variants of interest (VOI). The influenza genomic sequences were retrieved from the NCBI Influenza Virus Database on May 13, 2024. Influenza A sequences were limited to areas of interest, including subtype H1N1 (excluding Swine flu) or H3N2, based upon completeness. Influenza B sequences were required to be full-length, with no subtype limitations applied. All genomic sequences containing ambiguous nucleotides were excluded from the analysis.

The query oligos for the specific virus were aligned to the filtered sets of viral genome sequences using the Burrows-Wheeler Aligner (BWA) program, invoking parameters that guarantee any difference would be a mismatch or an insertion/deletion.

The genomic sequences were ranked according to the number of capture oligos and PCR oligos and probes containing zero (0), one (1), and two (2) or more mismatches. Melting temperature (Tm) analysis was performed for any oligo:genome pairing that contained a mismatch, and the pairings that passed the Tm analysis criteria were combined with pairings containing no mismatches, then grouped into amplifiable sets. The resulting oligo:genome pairings were counted to determine the number of sequences predicted to be captured and, if captured, predicted to be detected. The results of this predicted-to-detect analysis are shown in the table below:

Sequences Predicted to Be Detected by PCR According to *in silico* Analysis

Target	Sequences Predicted to be Detected
Influenza A	≥99.97% (18112 of 18117)
Influenza B	≥98.27% (8136 of 8279)
SARS-CoV-2	≥99.99% (994778 of 994846)

DASH SARS-CoV-2 & Flu A/B Test in silico inclusivity analysis for Flu A subtypes H7N7 and H5N3 was also performed. As influenza A H7N7 and H5N3 did not have reference genomes available for analysis, an alternative method was used for assessment involving a GISAID (Global Initiative on Sharing All Influenza Data) database query to compare oligonucleotide sequences of the detection limiting region of the DASH SARS-CoV-2 & Flu A/B Test. The resulting data, placed through a BLAST analysis, demonstrated that the subtype H5N3 and H7N7 of Flu A are expected to be detected by DASH SARS-CoV-2 & Flu A/B Test. Wet testing for H5N3 strain of Flu A was also performed, confirming inclusivity of DASH SARS-CoV-2 & Flu A/B Test with the H5N3 strain of Flu A (see Inclusivity Wet Test Results Summary table above). The Flu A H7N7 strain was not available for wet testing confirmation.

Additional influenza A, *in silico* inclusivity analysis was performed for all human host influenza A subtypes (H5N1, H5N6, H9N2, H10N3, and H10N5) available from the GISAID database, and collected from November 5, 2023 to November 5, 2024, wherein all possible combinations of hemagglutinin and neuraminidase were considered. Each sequence of the limiting region was required to be of full-length, with no subtype limitations applied, and all sequences containing ambiguous nucleotides were excluded from the analysis. The resulting oligo:genome pairings were counted to determine the number of sequences predicted to be captured and, if captured, predicted to be detected. Based on *in silico*

inclusivity analysis of the influenza A oligos, it is predicted that the test will detect ~97.3% (n = 37) of all human host influenza A sequences collected between November 5, 2023 and November 5 2024. The performance characteristics of DASH® SARS-CoV-2 & Flu A/B Test with these Flu A subtypes have not been confirmed by wet testing analysis.

Analytical Specificity (Cross-Reactivity & Microbial Interference)

***In silico* Analysis**

In silico analysis was completed on 1,217 non-target organisms. Any microorganism showing ≥80% homology to a single primer, probe, or capture oligonucleotide was further evaluated for homology for the other target primers and probes for amplification. DASH SARS-CoV-2 & Flu A/B Test primers for the SARS-CoV-2 targets exhibited >80% homology to SARS-coronavirus isolate Tor2 and although amplification may be possible, it will not be detected by the DASH SARS-CoV-2 & Flu A/B Test. Performance of DASH SARS-CoV-2 & Flu A/B Test with SARS-coronavirus Tor2 has not been confirmed by wet testing analysis.

Cross-Reactivity Wet Testing

Cross-Reactivity was established for the DASH® SARS-CoV-2 & Flu A/B Test on the DASH Instrument by wet testing of 50 different viruses, bacteria, and fungi that are common causes of respiratory infections. All samples were prepared in simulated clinical nasal matrix (SCNM) and tested individually, without the presence of the test viral targets. Cross-reactivity was not present if the target had a 0% positive call rate for all three (3) replicates tested.

The table that follows provides a summary of the wet tested results. None of the evaluated organisms demonstrated cross-reactivity with the assay at the tested concentrations as shown in the table below:

Cross-reactivity Viral Wet Testing Results Summary

Viruses/Viral RNA	Concentration per swab	Concentration per mL*
Adenovirus type C1	1.44E+04 $TCID_{50}$/swab	3.60E+05 $TCID_{50}$/mL
Cytomegalovirus (also known as HHV-5)	2.82E+03 $TCID_{50}$/swab	7.05E+04 $TCID_{50}$/mL
Epstein-Barr virus (also known as HHV-4)	1.51E+04 $TCID_{50}$/swab	3.78E+05 $TCID_{50}$/mL
Human coronavirus 229E	4.00E+03 $TCID_{50}$/swab	1.00E+05 $TCID_{50}$/mL
Human coronavirus OC43	4.75E+03 $TCID_{50}$/swab	2.19E+05 $TCID_{50}$/mL
Human coronavirus HKU1	1.10E+04 Copies/swab	2.75E+05 Copies/mL
Human coronavirus NL63	4.00E+03 $TCID_{50}$/swab	1.00E+05 $TCID_{50}$/mL
Human Metapneumovirus	1.19E+04 $TCID_{50}$/swab	2.98E+05 $TCID_{50}$/mL
Measles virus	1.54E+04 $TCID_{50}$/swab	3.85E+05 $TCID_{50}$/mL
Middle East Respiratory Syndrome coronavirus (MERS-CoV)	[1]N/A	
Genomic RNA Middle East Respiratory Syndrome coronavirus (MERS-CoV)	1.35 ng RNA/swab	33.8 ng RNA/mL
Mumps virus	1.19E+04 $TCID_{50}$/swab	2.98E+05 $TCID_{50}$/mL
Parainfluenza virus 1	4.75E+03 $TCID_{50}$/swab	1.19E+05 $TCID_{50}$/mL

Viruses/Viral RNA	Concentration per swab	Concentration per mL*
Parainfluenza virus 2	4.00E+03 $TCID_{50}$/swab	1.00E+05 $TCID_{50}$/mL
Parainfluenza virus 3	1.44E+04 $TCID_{50}$/swab	3.60E+05 $TCID_{50}$/mL
Parainfluenza virus 4	2.82E+03 $TCID_{50}$/swab	7.05E+04 $TCID_{50}$/mL
Respiratory syncytial virus	2.82E+03 $TCID_{50}$/swab	7.05E+04 $TCID_{50}$/mL
Rhinovirus type 1A	2.82E+03 $TCID_{50}$/swab	7.05E+04 $TCID_{50}$/mL
Enterovirus type 68	3.40E+03 $TCID_{50}$/swab	8.50E+04 $TCID_{50}$/mL
Severe Acute Respiratory Syndrome coronavirus (2003 RNA)	9.80E+03 Copies/swab	2.45E+05 Copies/mL

* The concentration stated per swab represents microbial material prepared in 40µL of pooled nasal matrix and applied to the swab. The per mL concentration is therefore represented as 25x the stated amount per swab.
[1] Concentration not available from vendor, sample is qualitative non-infectious purified intact viral particles.

Cross-reactivity Bacterial Wet Testing Results Summary

Bacteria	Concentration per swab	Concentration per mL*
Bordetella pertussis	5.00E+04 CFU/swab	1.25E+06 CFU/mL
Bordetella parapertussis	5.00E+04 CFU/swab	1.25E+06 CFU/mL
Chlamydia pneumoniae	5.00E+04 CFU/swab	1.25E+06 CFU/mL
Corynebacterium diphtheriae	5.00E+04 CFU/swab	1.25E+06 CFU/mL
Escherichia coli	5.00E+04 CFU/swab	1.25E+06 CFU/mL
Haemophilus influenzae	5.00E+04 CFU/swab	1.25E+06 CFU/mL
Fusobacterium necrophorum	5.00E+04 CFU/swab	1.25E+06 CFU/mL
Lactobacillus acidophilus	5.00E+04 CFU/swab	1.25E+06 CFU/mL
Legionella pneumophila	5.00E+04 CFU/swab	1.25E+06 CFU/mL
Moraxella catarrhalis	5.00E+04 CFU/swab	1.25E+06 CFU/mL
Mycobacterium tuberculosis (heat-inactivated)	5.00E+04 CFU/swab	1.25E+06 CFU/mL
Mycoplasma pneumoniae	5.00E+04 CFU/swab	1.25E+06 CFU/mL
Mycoplasma genitalium	5.00E+04 CFU/swab	1.25E+06 CFU/mL
Neisseria meningitidis	5.00E+04 CFU/swab	1.25E+06 CFU/mL
Neisseria elongata subsp. glycolytica	5.00E+04 CFU/swab	1.25E+06 CFU/mL
Neisseria mucosa	5.00E+04 CFU/swab	1.25E+06 CFU/mL
Nocardia asteroides	1.74E+05 CFU/swab	4.35E+06 CFU/mL
Pseudomonas aeruginosa	5.00E+04 CFU/swab	1.25E+06 CFU/mL
Staphylococcus aureus	5.00E+04 CFU/swab	1.25E+06 CFU/mL
Staphylococcus epidermidis	5.00E+04 CFU/swab	1.25E+06 CFU/mL
Streptococcus pneumoniae	5.00E+04 CFU/swab	1.25E+06 CFU/mL
Streptococcus pyogenes	5.00E+04 CFU/swab	1.25E+06 CFU/mL
Streptococcus salivarius	5.00E+04 CFU/swab	1.25E+06 CFU/mL
Streptococcus mitis	5.00E+04 CFU/swab	1.25E+06 CFU/mL
Streptococcus mutans	5.00E+04 CFU/swab	1.25E+06 CFU/mL

* The concentration stated per swab represents microbial material prepared in 40µL of pooled nasal matrix and applied to the swab. The per mL concentration is therefore represented as 25x the stated amount per swab.

Cross-reactivity Fungal Wet Testing Results Summary

Fungi	Concentration per swab	Concentration per mL*
Aspergillus niger	5.00E+04 CFU/swab	1.25E+06 CFU/mL
Candida albicans	5.00E+04 CFU/swab	1.25E+06 CFU/mL
Cryptococcus neoformans	5.00E+04 CFU/swab	1.25E+06 CFU/mL
Pneumocystis jirovecii	5.00E+04 CFU/swab	1.25E+06 CFU/mL
Eikenella corrodens	5.00E+04 CFU/swab	1.25E+06 CFU/mL

* The concentration stated per swab represents microbial material prepared in 40µL of pooled nasal matrix and applied to the swab. The per mL concentration is therefore represented as 25x the stated amount per swab.

Microbial Interference

Microbial Interference was evaluated for the DASH® SARS-CoV-2 & Flu A/B Test on the DASH Instrument by wet testing 50 different viruses, bacteria, and fungi that are common causes of respiratory infections. All samples were prepared in simulated clinical nasal matrix (SCNM) and tested with individual microorganisms or with pooled groups of up to four (4) microorganisms in the presence of the test viral targets at 3x their individual LoDs. Microbial non-interference was determined if the target had a 100% positive call rate for all three (3) replicates tested.

The following tables provide a summary of the wet tested results. None of the evaluated microorganisms demonstrated interference with the assay at the tested concentrations as shown in the table below:

Microbial Interference Wet Testing Results Summary

Microorganism	Concentration per swab	Concentration per mL*
Adenovirus C1	1.44E+04 $TCID_{50}$/swab	3.60E+05 $TCID_{50}$/mL
Cytomegalovirus (also known as HHV-5)	2.48E+03 $TCID_{50}$/swab	6.20E+04 $TCID_{50}$/mL
Epstein-Barr virus (also known as HHV-4)	1.51E+04 $TCID_{50}$/swab	3.78E+05 $TCID_{50}$/mL
Human coronavirus 229E	4.00E+03 $TCID_{50}$/swab	1.00E+05 $TCID_{50}$/mL
Human coronavirus OC43	4.75E+03 $TCID_{50}$/swab	2.19E+05 $TCID_{50}$/mL
Human coronavirus HKU1	9.68E+03 Copies/swab	2.41E+05 Copies/mL
[1]Human coronavirus NL63	4.00E+03 $TCID_{50}$/swab	1.00E+05 $TCID_{50}$/mL
[1]Measles virus	1.54E+04 $TCID_{50}$/swab	3.85E+05 $TCID_{50}$/mL
Genomic RNA Middle East Respiratory Syndrome coronavirus (MERS-CoV)	1.35 ng RNA/swab	33.8 ng RNA/mL
Middle East Respiratory Syndrome coronavirus (MERS-CoV)	[2]N/A	
Mumps virus	1.19E+04 $TCID_{50}$/swab	2.98E+05 $TCID_{50}$/mL
Human Metapneumovirus	1.19E+04 $TCID_{50}$/swab	2.98E+05 $TCID_{50}$/mL
Parainfluenza virus 1	4.75E+03 $TCID_{50}$/swab	1.19E+05 $TCID_{50}$/mL
Parainfluenza virus 2	4.00E+03 $TCID_{50}$/swab	1.00E+05 $TCID_{50}$/mL
Parainfluenza virus 3	1.44E+04 $TCID_{50}$/swab	3.60E+05 $TCID_{50}$/mL
Parainfluenza virus 4	2.48E+03 $TCID_{50}$/swab	6.20E+04 $TCID_{50}$/mL
Respiratory syncytial virus	2.48E+03 $TCID_{50}$/swab	6.20E+04 $TCID_{50}$/mL
Rhinovirus type 1A	2.48E+03 $TCID_{50}$/swab	6.20E+04 $TCID_{50}$/mL
Enterovirus type 68	2.99E+03 $TCID_{50}$/swab	7.48E+04 $TCID_{50}$/mL
Severe Acute Respiratory Syndrome coronavirus (SARS-CoV)	8.62E+03 Copies/swab	2.16E+05 Copies/mL
Bordetella pertussis	5.00E+04 CFU/swab	1.25E+06 CFU/mL
Bordetella parapertussis	5.00E+04 CFU/swab	1.25E+06 CFU/mL
Candida albicans	5.00E+04 CFU/swab	1.25E+06 CFU/mL
Chlamydia pneumoniae	5.00E+04 CFU/swab	1.25E+06 CFU/mL
Corynebacterium diphtheriae	5.00E+04 CFU/swab	1.25E+06 CFU/mL
Escherichia coli	5.00E+04 CFU/swab	1.25E+06 CFU/mL
Haemophilus influenzae	5.00E+04 CFU/swab	1.25E+06 CFU/mL
Fusobacterium necrophorum	5.00E+04 CFU/swab	1.25E+06 CFU/mL
Lactobacillus acidophilus	5.00E+04 CFU/swab	1.25E+06 CFU/mL

Microorganism	Concentration per swab	Concentration per mL*
Legionella pneumophila	5.00E+04 CFU/swab	1.25E+06 CFU/mL
Moraxella catarrhalis	5.00E+04 CFU/swab	1.25E+06 CFU/mL
Mycobacterium tuberculosis (heat-inactivated)	5.00E+04 CFU/swab	1.25E+06 CFU/mL
Mycoplasma pneumoniae	5.00E+04 CFU/swab	1.25E+06 CFU/mL
Mycoplasma genitalium	5.00E+04 CFU/swab	1.25E+06 CFU/mL
Neisseria meningitidis	5.00E+04 CFU/swab	1.25E+06 CFU/mL
Neisseria elongata subsp. glycolytica	5.00E+04 CFU/swab	1.25E+06 CFU/mL
Pseudomonas aeruginosa	5.00E+04 CFU/swab	1.25E+06 CFU/mL
Staphylococcus aureus	5.00E+04 CFU/swab	1.25E+06 CFU/mL
Staphylococcus epidermidis	5.00E+04 CFU/swab	1.25E+06 CFU/mL
Streptococcus pneumoniae	5.00E+04 CFU/swab	1.25E+06 CFU/mL
Streptococcus pyogenes	5.00E+04 CFU/swab	1.25E+06 CFU/mL
Streptococcus salivarius	5.00E+04 CFU/swab	1.25E+06 CFU/mL
Aspergillus niger	5.00E+04 CFU/swab	1.25E+06 CFU/mL
Cryptococcus neoformans	5.00E+04 CFU/swab	1.25E+06 CFU/mL
Pneumocystis jirovecii	5.00E+04 CFU/swab	1.25E+06 CFU/mL
Eikenella corrodens	5.00E+04 CFU/swab	1.25E+06 CFU/mL
Neisseria mucosa	5.00E+04 CFU/swab	1.25E+06 CFU/mL
Streptococcus mitis	5.00E+04 CFU/swab	1.25E+06 CFU/mL
Streptococcus mutans	5.00E+04 CFU/swab	1.25E+06 CFU/mL
Nocardia asteroides	1.74E+05 CFU/swab	4.35E+06 CFU/mL

Note: Thicker outer border indicates groups of pooled microorganisms that were tested.

* The concentration stated per swab represents microbial material prepared in 40µL of pooled nasal matrix and applied to the swab. The per mL concentration is therefore represented as 25x the stated amount per swab.

[1]Tested individually as interference was observed when tested as a group.

[2]Concentration not available from vendor, sample is qualitative non-infectious purified intact viral particles.

Competitive Interference

Competitive interference can occur when there is a co-infection and one viral target concentration is near the LoD while another viral target is present at high concentration.

Competitive Interference was evaluated for the DASH® SARS-CoV-2 & Flu A/B Test on the DASH Rapid PCR Instrument. One (1) viral target was tested at a high concentration while the other two viral targets were held at 3x LoD. Replicates of three (3) were tested. If results reported less than 3/3 positive results for all targets, the concentration of the competing virus was reduced until positive results were achieved for all targets.

Study results demonstrate that SARS-CoV-2, at concentrations >1.41E+06 copies/mL, inhibit detection of Flu A at 3x LoD, and at SARS-CoV-2 concentrations of >5.65E+06 copies/mL, detection of Flu B at 3x LoD is inhibited. Flu A, at concentrations of >1.38E+06 $TCID_{50}$/mL, inhibited detection of Flu B at 3x LoD. In addition, Flu B, at concentrations >1.01E+03 $TCID_{50}$/mL, inhibited detection of both SARS-CoV-2 and Flu A at 3x LoD. The highest co-infection concentration of the competing virus, where the remaining targets are detected for three (3) replicates at 3x LoD, are reported in the table below.

Competitive Interference Summary

	Concentration* at which All Three (3) Targets Achieved Positive Calls		
Competing Virus	**SARS-CoV-2** SARS-Cov-2, Isolate hCoV- 19/USA/GA-EHC-2811C/2021 (Lineage B.1.1.529; Omicron Variant)	**Flu A** Flu A H1N1 A/Victoria/2570/2019	**Flu B** Flu B Utah/9/14 – Yamagata Lineage
SARS-CoV-2	5.65E+04 copies/swab 1.41E+06 copies/mL ~47x LoD	3x LoD	3x LoD
Flu A	3x LoD	5.52E+04 $TCID_{50}$/swab 1.38E+06 $TCID_{50}$/mL ~3405x LoD	3x LoD
Flu B	3x LoD	3x LoD	40.40 $TCID_{50}$/swab 1.01E+03 $TCID_{50}$/mL ~60x LoD

* The concentration stated per swab represents microbial material prepared in 40µL of simulated clinical nasal matrix and applied to the swab. The per mL concentration is therefore represented as 25x the stated amount per swab.

Exogenous/Endogenous Interfering Substances

Medically relevant substances found within clinical respiratory specimens (known as endogenous materials) and substances not commonly found in clinical respiratory specimens (exogenous substances) were evaluated for their potential impact on the DASH SARS-CoV-2 & Flu A/B Test, and its ability to detect SARS-CoV-2, Flu A, and Flu B.

All samples were prepared in simulated clinical nasal matrix (SCNM). Each positive sample was composed of a single exogenous or endogenous interfering substance and combined with a contrived co-spiked sample of SARS-CoV-2, Flu A, and Flu B at 3X LoD concentrations. Negative (no analyte) samples were prepared with simulated clinical nasal matrix (SCNM). Each interferent was tested in triplicate.

Interference with SARS-CoV-2 assay was observed in the presence of Biotin (vitamin B-7) at 4.58 µg/mL. When diluted by 2-fold, no further interference was observed at 2.29 µg/mL for each of the three (3) replicate samples.

Interference with SARS-CoV-2 assay was observed in the presence of Flonase® (5% v/v, active ingredient Fluticasone Propionate). Subsequent confirmatory testing was performed at a lower Flonase concentration (2.5% v/v) and provided objective evidence of no observed interference, wherein 3/3 replicates resulted in positive results for all viral targets.

No interference was observed for any of the substances tested at the concentrations noted in the table below.

Exogenous/Endogenous Interfering Substances Study Results Summary

Interfering Substance	Concentration
Afrin® Original Nasal Spray	15% v/v
Chloroseptic® Sore Throat spray	20% v/v
Flonase®	2.5% v/v*
Relenza®	0.3 mg/ml
Tobramycin	4 µg/mL
Mupirocin	10 mg/mL
Mucin	0.1% w/v
Blood	2% v/v
Biotin	2.29 µg/mL*
Viral Transport Medium	50% v/v
Neo-Synephrine® Nasal Spray	15% v/v
Walgreens Saline Nasal Spray	15% v/v
Beclomethasone Dipropionate, USP (Micronized)	0.068 mg/mL
Flunisolide Nasal Solution, USP 0.025%	0.04 mg/mL
Nasacort® Nasal Spray	0.04 mg/mL
Walgreens 24 Hour Budesonide Nasal Spray	0.051 mg/mL
Nasonex	0.04 mg/mL
Zicam® Allergy Relief Nasal Spray	20% v/v
Snuff	0.1% (w/v)
Latex glove powder	1% (w/v)
Mucinex® Insta Sooth Kickstart Sore Throat	1.7 mg/mL
Zinc gluconate hydrate	0.1 µg/mL

*Interference potential was observed at 2x higher concentrations.

Carry-over and Cross-contamination Study

A study was conducted to evaluate the risk of contamination from one test run to another by alternating the testing of a series of single high titer (≥1x10^5 copies/mL or ≥1x10^5 $TCID_{50}$/mL) viral target (either SARS-CoV-2, Flu A or Flu B) samples with negative samples, when run consecutively on the same instrument. Contrived samples of single viral targets of either SARS-CoV-2, Flu A, or Flu B were spiked into simulated clinical nasal matrix (SCNM) and applied to a sample swab. A total of eight (8) viral targets were used, including 2 SARS-CoV-2 strains and 3 strains each for Flu A and Flu B. Negative samples were composed only of SCNM. To demonstrate robustness of the DASH SARS-CoV-2 & Flu A/B Test when run on the DASH Instrument, each viral target was evaluated in replicates of five (5) and run on five (5) DASH Rapid PCR Instruments.

No false positive calls were observed among the negative samples run immediately after high titer positive runs. Likewise, no erroneous results were issued from the positive samples. The data show that 100% call accuracy was attained for both the positive target samples (40/40 positive calls) and the

negative samples (40/40 negative calls); and no carry-over or cross contamination occurred when using the DASH® SARS-CoV-2 & Flu A/B Test on the DASH Rapid PCR Instrument.

Precision

Precision of the DASH SARS-CoV-2 & Flu A/B Test was evaluated over a 12-day period, conducted by two (2) operators at a single site, and resulted in 96 replicates per concentration of the SARS-CoV-2, Flu A and Flu B targets.

Triple positive samples (SARS-CoV-2, Flu A, and Flu B) were prepared in simulated clinical nasal matrix (SCNM) at 2x and 5x LoD concentrations, and negative (no analyte) samples were SCNM without viral targets. Results of replicate samples tested by each operator at 2x and 5x LoD achieved 100% positivity for each target. Negative samples also showed the expected result of 0% positivity for each target. A percent positive agreement of 100% was achieved between operators as well as between testing days for each target at 2x and 5x LoD. Data analysis included a statistical two-way nested ANOVA for each target across different sources of variability (e.g., operator, day, run). The following table provides a summary of results:

Summary of Precision Study Results

Viral Strain	Concentration	Results (Call Accuracy/Agreement)
SARS Cov-2 19/USA/GA-EHC-2811C/2021 (Omicron)	2x LoD	96/96 (100%)
	5x LoD	96/96 (100%)
Flu A H1N1 Victoria /2570/19	2x LoD	96/96 (100%)
	5x LoD	96/96 (100%)
Flu B Yamagata (Utah/09/14)	2x LoD	96/96 (100%)
	5x LoD	96/96 (100%)
Negative	N/A	96/96 (100%)

Mean, Standard Deviation (SD), and Percent Coefficient of Variation (%CV) for Cq*

Sample	Sample Type	N	Mean Cq*	Between Operator		Between Day (Within Operator)		Between Runs (Within Day)		Within Run/ Repeatability		Total	
				SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Flu A	Low Positive	96	24.0	0.4	1.5	0.3	1.3	0.0	0.0	1.2	5.2	1.3	5.5
	Moderate Positive	96	22.7	0.3	1.2	0.3	1.1	0.2	0.9	0.5	2.3	0.7	3.0
Flu B	Low Positive	96	25.9	0.0	0.0	0.0	0.0	0.0	0.0	0.9	3.6	0.9	3.6
	Moderate Positive	96	24.8	0.0	0.0	0.4	1.5	0.0	0.0	0.6	2.2	0.7	2.7
SARS-CoV-2	Low Positive	96	29.4	0.0	0.0	0.0	0.0	0.0	0.0	0.8	2.7	0.8	2.7
	Moderate Positive	96	28.2	0.0	0.0	0.2	0.9	0.2	0.6	0.5	1.8	0.6	2.0

Note: SD and CV% were estimated using ANOVA variance component analysis using the R Package VCA.
*DASH software identifies the cycle at which an amplification curve meets a predefined mathematical criterion as Cq.

Reproducibility

A multi-site study was conducted to demonstrate the reproducibility of the DASH® SARS-CoV-2 & Flu A/B Test with analyte concentrations near the detection limit of the assay, using contrived nasal swabs and a 3-member panel consisting of a true negative (no analyte), low positive (2x LoD), and a moderate positive (5x LoD) panel member. The study was conducted using three (3) lots of DASH SARS-CoV-2 & Flu A/B Test cartridges by nine (9) untrained operators across three (3) sites, and performed over five (5) non-consecutive days, with two (2) runs per day and three (3) replicates per panel member, totaling 810 samples (270 replicates per panel member) tested throughout the course of the study and accounting for invalid repeat testing.

Positive samples were contrived using co-spiked inactivated SARS-Cov-2, Isolate hCoV- 19/USA/GA-EHC-2811C/2021, Lineage B.1.1.529; Omicron Variant, and cultured Flu A H1N1 Victoria/2570/19, and Flu B Utah/9/14 - Yamagata Lineage.

The DASH SARS-CoV-2 & Flu A/B Test reported the expected positive results for panel members in 97.8%-100% of the samples and the expected negative results in 100% of samples. A summary of the results (percent (%) agreement with the expected positive or negative result) for each panel member by analyte and site are provided below:

Qualitative Results by Analyte Concentration and Site

Analyte*	Sample Concentration	% Agreement with Expected Results (n Agreement/N Tested) (95% CI)#			
		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)^ (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)^ (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)^ (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%)

*All samples were co-spiked with all analytes, so results are presented three times, including the Negative results.
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.
^One Moderate Positive sample produced an error/no result and was excluded from the analysis.

For each panel member, statistical analysis of mean Cq, standard deviation (SD), and percent coefficient of variation (%CV) was evaluated for between sites, between lot, between day, between operator, between run, and within run, as presented in the table below:

Cq Variability Analysis Results

Analyte	Sample Type	n/N	Mean Cq*	Between Site		Between Lot		Between Day		Between Operator		Between Run		Within-Run		Total	
				SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
SARS-CoV-2	Low Positive	268/270	29.6	0.2	0.7	0.6	2.1	0	0	0.0	0.0	0.0	0.0	2.7	9.2	2.8	9.4
	Moderate Positive	268/269	28.4	0.0	0.0	0.5	1.7	0	0	0.4	1.5	0.0	0.0	1.9	6.5	2.0	6.9
Flu A	Low Positive	268/270	24.2	0.0	0.2	0.0	0.0	0	0	0.0	0.0	0.4	1.6	2.3	9.5	2.3	9.6
	Moderate Positive	269/269	23.1	0.1	0.3	0.1	0.6	0	0	0.3	1.3	0.0	0.0	0.9	3.7	0.9	4.0
Flu B	Low Positive	268/270	26.1	0.0	0.0	0.3	1.1	0	0	0.3	1.2	0.0	0.0	2.3	8.9	2.4	9.1
	Moderate Positive	269/269	25.3	0.2	0.8	0.2	0.7	0	0	0.2	0.7	0.2	0.8	0.7	2.9	0.8	3.3

Note: SD and CV% were estimated using REML variance component analysis using the R Package VCA.
*DASH software identifies the cycle at which an amplification curve meets a predefined mathematical criterion as Cq.

CLIA Waiver

Clinical Study

Clinical performance characteristics of the DASH® SARS-CoV-2 & Flu A/B Test were evaluated for the differential detection of SARS-CoV-2, influenza A, and influenza B, using anterior nasal samples collected during the 2023 – 2024 respiratory season. Seven (7) CLIA-waived sites participated in the study, including emergency departments, out-patient, and urgent care/walk-in clinics. Each site was representative of the intended use setting associated with point-of-care (POC) use. Across all sites, testing was performed by a total of 32 untrained operators who did not have any prior hands-on device experience or laboratory training. Testing with the DASH SARS-CoV-2 & Flu A/B Test was conducted using anterior nasal 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.

A total of 817 subjects were enrolled in the study, and 795 of them were evaluable for at least one (1) analyte/target and were included in the data analysis. There were 795 evaluable subjects for SARS-CoV-2 and 792 evaluable subjects for Flu A and Flu B. The most common reason for exclusion was due to the comparator sample being unable to be tested within the stability window of the test IFU.

Performance of the DASH SARS-CoV-2 & Flu A/B Test was assessed by a comparison of results with an FDA cleared RT-PCR test for SARS-CoV-2, and a second FDA cleared test for Flu A and Flu B. Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA), including 95% confidence intervals, were calculated for each target nucleic acid in comparison with the designated comparator test. 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. Additional details regarding the performance results associated with the DASH SARS-CoV-2 & Flu A/B Test are provided within the above *Performance Characteristics* section.

Samples Near the Limit of Detection Study

A multi-site study was conducted to demonstrate the reproducibility of the DASH® SARS-CoV-2 & Flu A/B Test with analyte concentrations near the detection limit of the assay, using negative (no analyte) swabs and contrived nasal swabs at low positive (2x LoD). The study was conducted by nine (9)

untrained operators across three (3) sites and performed over five (5) non-consecutive days with 540 tests in total.

The DASH® SARS-CoV-2 & Flu A/B Test reported the expected positive results for panel members in 97.8%-100% of the samples and the expected negative results in 100% of samples. A summary of the results [percent (%) agreement with the expected positive or negative result] by analyte and site are provided in the table below:

Qualitative Results by Analyte Concentration and Site

Analyte*	Sample Concentration	% Agreement with Expected Results (n Agreement/N Tested) (95% CI)^			
		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%)
	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%)
	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%)
	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%)

* All samples were co-spiked with all analytes, so results are presented three times, including the Negative results.
^ A total of 10 samples generated invalid results on the first attempt. All 10 samples generated valid results upon repeat and were included in the data analysis.

The standard deviation (SD) and percent coefficient of variance (%CV) between sites is presented in the table below:

Analyte	Sample Type	n/N	Mean Ct*	Between Site	
				SD	%CV
SARS- CoV-2	Low Positive	268/270	29.6	0.2	0.7
Flu A	Low Positive	268/270	24.2	0.0	0.2
Flu B	Low Positive	268/270	26.1	0.0	0.0

Note: SD and CV% were estimated using REML variance component analysis using the R Package VCA.
*DASH software identifies the cycle at which an amplification curve meets a predefined mathematical criterion as Cq.

Flex Studies

Using risk analysis as a guide, analytical flex studies were conducted on DASH SARS-CoV-2 & Flu A/B Test on the DASH Rapid PCR Instrument. The testing evaluated numerous sources of potential human errors and environmental factors that could affect the accuracy of results, including those related to

sample handling, reagent handling, and extremes of operational conditions. The studies demonstrated that the test is robust to usage variation and environmental factors that may be encountered.

Nuclein LLC. Headquarters Location

Nuclein LLC.
8305 Cross Park Drive
Austin, Texas 78754
1-888-992-DASH
www.nuclein.com

Technical Assistance

Before contacting Nuclein Customer Support, please collect the following information:

- Product name
- Cartridge Lot Code (LOT: LLLL-CCCC)
    - LLLL is the cartridge lot number
    - CCCC is the cartridge serial number
- Serial number of the instrument
- Error message(s) (if any)

Telephone | US + 1.888.992.DASH
Email | customersupport@nuclein.com

Contact information for all Nuclein Customer Support offices is available on our website: www.nuclein.com

Medical Device Reporting

MedWatch is an FDA program that supports voluntary reports of FDA-regulated products. The MedWatch contact information is provided below:

Phone:1-800-FDA-1088
Fax: 1-800-FDA-0178
Website: www.fda.gov/medwatch

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Table of Symbols

REF	Catalog Number		Manufacturer	RX Only	Prescription Device
LOT	Batch Code or Lot Number		Use by or Expiration Date		Do Not Reuse
	Temperature Limitations	UDI	Unique Device Identifier	Σ	Contains Sufficient Materials for <n> Tests
IVD	*In vitro* Diagnostic Medical Device		Fragile, Handle with Care		Caution; Consult Accompanying Documents
	Consult Instructions for Use				

Revision History

Revision Date	Description of Change
Jan. 2025	Initial commercial release.
June 2025	Insertion of additional detail regarding sample collection.