Nuclein, LLC
Chelynne Lee
Associate Director Design Assurance and Regulatory Affairs
8305 Cross Park Dr.
Austin, Texas 78754

Re: K241652
Trade/Device Name: DASH SARS-CoV-2 & Flu A/B Test
Regulation Number: 21 CFR 866.3981
Regulation Name: Device To Detect And Identify Nucleic Acid Targets In Respiratory Specimens From Microbial Agents That Cause The SARS-Cov-2 Respiratory Infection And Other Microbial Agents When In A Multi-Target Test
Regulatory Class: Class II
Product Code: QOF
Dated: September 25, 2024
Received: September 25, 2024

Dear Chelynne Lee:

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

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

Additional information about changes that may require a new premarket notification are provided in the FDA guidance documents entitled "Deciding When to Submit a 510(k) for a Change to an Existing Device"

(https://www.fda.gov/media/99812/download) and "Deciding When to Submit a 510(k) for a Software Change to an Existing Device" (https://www.fda.gov/media/99785/download).

Your device is also subject to, among other requirements, the Quality System (QS) regulation (21 CFR Part 820), which includes, but is not limited to, 21 CFR 820.30, Design controls; 21 CFR 820.90, Nonconforming product; and 21 CFR 820.100, Corrective and preventive action. Please note that regardless of whether a change requires premarket review, the QS regulation requires device manufacturers to review and approve changes to device design and production (21 CFR 820.30 and 21 CFR 820.70) and document changes and approvals in the device master record (21 CFR 820.181).

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

All medical devices, including Class I and unclassified devices and combination product device constituent parts are required to be in compliance with the final Unique Device Identification System rule ("UDI Rule"). The UDI Rule requires, among other things, that a device bear a unique device identifier (UDI) on its label and package (21 CFR 801.20(a)) unless an exception or alternative applies (21 CFR 801.20(b)) and that the dates on the device label be formatted in accordance with 21 CFR 801.18. The UDI Rule (21 CFR 830.300(a) and 830.320(b)) also requires that certain information be submitted to the Global Unique Device Identification Database (GUDID) (21 CFR Part 830 Subpart E). For additional information on these requirements, please see the UDI System webpage at https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance/unique-device-identification-system-udi-system.

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

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

Sincerely

For

Himani Bisht, Ph.D.
Assistant Director
Viral Respiratory and HPV Branch
Division of Microbiology Devices
OHT7: Office of In Vitro Diagnostics Office of Product Evaluation and Quality Center for Devices and Radiological Health

Enclosure

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: 07/31/2026
See PRA Statement below.

510(k) Number *(if known)*
K241652

Device Name
DASH® SARS-CoV-2 & Flu A/B Test

Indications for Use *(Describe)*
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.

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

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

CONTINUE ON A SEPARATE PAGE IF NEEDED.

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510(k) Summary [21 CFR 807.95]

807.92(a)(1), (2), (3)

Date Prepared:	December XX, 2024
Submitter's Name:	Chelynne Lee Nuclein, LLC. 8305 Cross Park Dr. Austin, Tx 78754 +1 512-720-4935
Official Correspondent:	Chelynne Lee Associate Director Design Assurance and Regulatory Affairs +1 512-720-4935
Alternate Correspondent:	Ben Treece VP, Quality Assurance and Regulatory Affairs +1 512-649-3033
Device Trade Name:	DASH® SARS-CoV-2 & Flu A/B Test
Common Name:	DASH SARS-CoV-2 & Flu A/B Test
Review Panel:	Microbiology
Classification Name:	Multi-Target Respiratory Specimen Nucleic Acid Test Including Sars- Cov-2 And Other Microbial Agents
Classification Code:	**QOF**: Multi-Target Respiratory Specimen Nucleic Acid Test Including Sars-Cov-2 And Other Microbial Agents **NSU**: Instrumentation For Clinical Multiplex Test Systems
Predicate Device:	K230719: BioFire SPOTFIRE Respiratory Panel Mini Classification Code: QOF Regulation: 866.3981

Device Description *807.92(a)(4)*:
The DASH® SARS-CoV-2 & Flu A/B Test is a rapid, polymerase chain reaction (PCR) assay performed on the DASH Rapid PCR Instrument (DASH Instrument) with the DASH External Controls. The external control materials and DASH Instrument are sold and distributed separately from the DASH SARS-CoV-2 & Flu A/B Test. The DASH SARS-CoV-2 & Flu A/B Test (for use with the DASH Rapid PCR System components) 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 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. An anterior nares nasal swab 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. The cartridge is capped and inserted into the DASH Rapid PCR Instrument to initiate the test, and all subsequent test steps are performed automatically by the DASH Instrument.

Intended Use *807.92(a)(5)*:
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.

Technological Characteristics *807.92(a)(6)*:
The DASH SARS-CoV-2 & Flu A/B Test combines the technologies of sequence-specific capture and RT-PCR amplification. The test cartridge contains all the reagents necessary to perform the DASH SARS-CoV-2 & Flu A/B Test. The assay reagents are lyophilized and rehydrated with buffer as the test is performed by the DASH instrument. There are no user-mediated sample preparation steps; an anterior nares swab (ANS) is placed directly into the cartridge sample chamber containing lysis buffer that is then closed. The cartridge is then placed in the DASH Instrument which performs all subsequent steps automatically.

The target RNA molecules are isolated from specimens using biotinylated target- specific capture oligomers which hybridize to specific regions on the target nucleic acids. After hybridization to target RNA, streptavidin-coated paramagnetic particles bind the biotinylated capture oligomers. 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 amplified. 40 PCR temperature cycles are performed. During the 40 cycles, fluorescence is measured in a single chamber with a four-channel LED fluorometer. A software algorithm determines whether any of the targets are positive. The algorithm includes the requirement for the internal control to have performed

as intended, with its purpose to ensure adequate processing of the target virus through steps of sample purification, nucleic acid amplification, and monitoring for the presence of inhibitors in the RT-PCR test. External controls are sold separately and recommended to be used to confirm that the test cartridge and instrument are functioning as intended. Each control (positive or negative) is run as an individual sample in the exact method and process as a DASH® SARS-CoV-2 & Flu A/B Test patient sample.

Predicate Comparison:

Device & Predicate Device(s):	K241652	K230719
Device Trade Name	DASH® SARS-CoV-2 & Flu A/B Test	BIOFIRE® SPOTFIRE® Respiratory Panel Mini
Regulation Number and Name	SAME	21 CFR 866.3981; Devices to detect and identify nucleic acid targets in respiratory samples from microbial agents that cause the SARS-CoV-2 respiratory infection and other microbial agents when in a multi-analyte test.
Product Code	SAME	QOF
Technology/Detection	SAME, with exception of a single reaction chamber vs array/spot	PCR Magnetic bead purification technology Array/spot format
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.	The BIOFIRE® SPOTFIRE® Respiratory ® Panel Mini (SPOTFIRE R Panel Mini) is a multiplexed polymerase chain reaction (PCR) test intended for use with the BIOFIRE® SPOTFIRE® System for the simultaneous, qualitative detection and identification of multiple respiratory viral nucleic acids in nasopharyngeal swab (NPS) specimens obtained from individuals with signs and symptoms of respiratory tract infection, including COVID-19. The following organism types are identified and differentiated using the SPOTFIRE R Panel Mini: • Coronavirus SARS-CoV-2 • Human rhinovirus • Influenza A virus • Influenza B virus • Respiratory syncytial virus Nucleic acids from the viral organisms identified by this test are generally detectable in NPS specimens during the acute phase of infection. The detection and identification of specific viral nucleic acids from individuals exhibiting signs and/or symptoms of respiratory infection are indicative of the presence of the identified microorganism and aids in diagnosis if used in conjunction with other clinical and epidemiological information, and laboratory findings. The results of this test should not be used as the sole basis for diagnosis, treatment, or other patient management decisions. Negative results in the setting of a respiratory illness may be due to infection with pathogens that are not detected by this test, or lower respiratory tract infection that may not be detected by an NPS specimen. Positive results do not rule out coinfection with other organisms. The agent(s) detected by the SPOTFIRE R Panel Mini may not be the definite cause of disease. Additional laboratory testing (e.g., bacterial and viral culture, immunofluorescence, and radiography) may be necessary when evaluating a patient with possible respiratory tract infection.
Test Format	SAME	Single use
Automated Test Processes	SAME, but also includes sample extraction	Amplification and detection
Results Reported	SAME	Qualitative
Specimen Type	Anterior Nares Swab	Nasopharyngeal swab in transport media
Transport Media	N/A	VTM
Controls	SAME, with the exception of there being a single internal control	Two internal controls: 1 RNA Process control 2 PCR2 control External Controls are provided separately (see K230868)
Instrument Systems	DASH Rapid PCR Instrument	SPOTFIRE System
Time to Result	SAME	~15 minutes

Summary of Non-Clinical Test Performance and Safety Testing *807.92(b)(1)*:
The DASH® Rapid PCR System has met the special controls established for product codes QOF and NSU.
The DASH Rapid PCR Instrument was evaluated for performance and found to conform with the following standards:

- IEC 60601-1-2 Ed. 4.1:2020 *Medical electrical equipment - Part 1-2: General requirements for basic safety and essential performance - Collateral Standard: Electromagnetic disturbances - Requirements and tests*
- IEC 61010-1, 3rd edition, May 11, 2012, revision 2023-06-06 *Safety requirements for electrical equipment for measurement, control, and laboratory use - Part 1: general requirements*
- IEC 61010-2-010:2019 *Safety requirements for electrical equipment for measurement, control, and laboratory use - Part 2-010: Particular requirements for laboratory equipment for the heating of materials*
- IEC 61010-2-101:2018 *Safety requirements for electrical equipment for measurement, control, and laboratory use - Part 2-101: Particular requirements for in vitro diagnostic (IVD) medical equipment*

The DASH SARS-CoV-2 & Flu A/B Test was evaluated for performance and found to conform with the following FDA recognized standards:

- CLSI EP12 3rd Edition, Evaluation of Qualitative, Binary Output Examination Performance
- CLSI EP12-A2, User Protocol for Evaluation of Qualitative Test Performance; Approved Guideline - Second Edition.
- CLSI MM03-3rd Edition, Molecular Diagnostic Methods for Infectious Diseases; Approved Guideline
- CLSI EP07 3rd Edition, Interference Testing in Clinical Chemistry
- CLSI EP37 1st Edition, Supplemental Tables for Interference Testing in Clinical Chemistry
- CLSI EP25 2nd Edition, Evaluation of Stability of In Vitro Medical Laboratory Test Reagents
- CLSI EP25-A, Evaluation of Stability of In Vitro Diagnostic Reagents; Approved Guideline

Performance Characteristics

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.

Within-Laboratory Precision

Precision of the DASH SARS-CoV-2 & Flu A/B Test was established and executed 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 tables provide 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, at two (2) runs per day, with three (3) replicates per panel member, totaling over 810 samples (270 replicates per panel member) tested throughout the course of the study.

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, 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) by site for each panel member and a summary by analyte 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 Ct, 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.

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
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

[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

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

Microbial Interference

Microbial Interference was evaluated for the DASH® SARS-CoV-2 & Flu A/B Test on the DASH Instrument by wet testing over 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

Microorganism	Concentration per swab	Concentration per 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
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.
[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 3xLOD. 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 3xLoD, and at SARS-CoV-2 concentrations of >5.65E+06 copies/mL, detection of Flu B at 3xLoD is inhibited. Flu A at concentrations of >1.38E+06 $TCID_{50}$/mL inhibited detection of Flu B at 3xLoD. In addition, Flu B at concentrations >1.01E+03 $TCID_{50}$/mL inhibited detection of both SARS-CoV-2 and Flu A at 3xLoD. The highest coinfection concentration of the competing virus, where the remaining targets are detected for three (3) replicates at 3xLOD, 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 the 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 the 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.

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.

Comparison Studies:

Matrix Equivalency

Equivalency was established between the following matrix materials that were utilized in the evaluation of the DASH SARS-CoV-2 & Flu A/B Test performance:

- Clinical pooled nasal matrix (PNM)
- Simulated clinical nasal matrix (SCNM)

SCNM is formulated in PBS with glycerol and mucins (i.e., 1X PBS, ~1% NaCl, 2.5% Porcine Mucin, 15% glycerol) added to mimic the nasal cavity fluid. The study was performed using co-spiked samples consisting of one SARS-CoV-2 strain (19/USA/GA-EHC-2811C/2021 - Omicron), one influenza A strain (H1N1 Victoria /2570/19), and one influenza B strain (Yamagata - Utah/09/14). Negative (non-analyte) samples were evaluated with only the matrix material.

Co-spiked samples with all viral targets were prepared in both matrices at 2x and 5x LoD. For both matrices, all replicates tested at 5x LoD were positive at a 100% rate and, as expected, replicates tested at 2x LoD varied and were positive at a rate of ≥95%. All negative samples were negative for both matrices. For both matrices, 10 replicates of the negative samples, 40 replicates of the 2x LOD positive samples and 10 replicates of the 5x LOD positive samples were tested.

Results provide evidence that performance of the DASH SARS-CoV-2 & Flu A/B Test is equivalent with viral materials prepared in either clinical pooled nasal matrix (PNM) or in simulated clinical nasal matrix, and further supports the use of SCNM in select analytical studies.

Fresh vs. Frozen Contrived Samples

This study was performed to establish equivalency between freshly prepared samples and those that were subjected to two (2) successive freeze/thaw cycles for materials used in the evaluation of the DASH SARS-CoV-2 & Flu A/B Test performance. Co-spiked samples were prepared in SCNM at 2x and 5x LoD concentrations containing the following viral materials: one SARS-Cov-2 strain (19/USA/GA-EHC-2811C/2021 - Omicron), one influenza A strain (H1N1 Victoria/2570/19), and one influenza B strain (Yamagata - Utah/9/14). Negative (no analyte) samples prepared with only the matrix material were also evaluated.

For both conditions evaluated, all replicates tested at 5x LoD were positive at a 100% rate and, as expected, replicates tested at 2x LoD varied and were positive at a rate of ≥95%. All negative samples were negative for both conditions. Positive samples co-spiked at 5x LoD and negative samples were both tested in replicates of 10 for each condition, and positive samples prepared at 2x LoD were tested in replicates of 40 for each condition.

Results demonstrate equivalency between fresh and frozen contrived sample performance on the DASH SARS-CoV-2 & Flu A/B Test, and supports the use of frozen contrived samples within select analytical studies.

Summary of Clinical Tests *807.92(b)(2)*:

Clinical Performance:

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 nares 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 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. 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%).

Conclusion *807.92(b)(3)*:

The **DASH SARS-CoV-2 & Flu A/B Test; DASH Rapid PCR Instrument; DASH SARS-CoV-2 & Flu A|B Positive Control and DASH Negative Control**, as part of the **DASH Rapid PCR System**, are substantially equivalent to the predicate and reference devices (BioFire SPOTFIRE Respiratory Panel Mini with SPOTFIRE System and SPOTFIRE RSP Pos & Neg Controls external controls). The subject device meets requirements as described in FDA Guidance Class II Special Controls established for product codes QOF and NSU.

The subject device and the predicates all achieve their intended use as a system; assay, instrument, external controls. They share the same intended use, patient population, fundamental technology, and are both for the qualitative detection and differentiation of SARS-CoV-2, influenza A and influenza B in upper respiratory swab samples. The subject device and primary predicate utilize similar reagents, have an internal control, and provide external QC controls that are sold separately.

The risks identified in the special controls have been mitigated by the subject device through

design control and evidence is demonstrated by performance studies and in the labeling. Both the subject device and primary predicate device completed testing as described in the special controls guidance including sensitivity, specificity, interfering substances testing, and method comparison. Demonstration that the risks are mitigated by the subject device through the completion of performance studies and through labeling.

The results of performance testing demonstrate that the device system functions as intended, and differences between DASH® SARS-CoV-2 & Flu A/B Test (for use with the DASH Rapid PCR System components) and the predicate(s) do not raise new or different concerns of safety or effectiveness when used as intended. The DASH SARS- CoV-2 & Flu A/B Test, as part of the DASH Rapid PCR System, is substantially equivalent to the primary predicate, BIOFIRE® SPOTFIRE® Respiratory (R) Panel Mini and the reference devices, BioFire SPOTFIRE System and SPOTFIRE RSP Pos & Neg Controls, SPOTFIRE RSP Positive Control, SPOTFIRE RSP Negative Control.