May 21, 2024

Centers for Disease Control and Prevention
Melissa Ivey
Lead, Regulatory Affairs Activity (Acting)
1600 Clifton Road, NE
Ms H24-2
Atlanta, Georgia 30329

Re: K241110
Trade/Device Name: CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel, Influenza A Subtyping Kit (Ver4)
Regulation Number: 21 CFR 866.3980
Regulation Name: Respiratory Viral Panel Multiplex Nucleic Acid Assay
Regulatory Class: Class II
Product Code: OZE
Dated: April 22, 2024
Received: April 22, 2024

Dear Melissa Ivey:

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.

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,

Himani Bisht -S

Himani Bisht, Ph.D.
Assistant Director
Viral Respiratory and HPV Branch
Division of Microbiology Devices
OHT7: Office of In Vitro Diagnostics and Radiological health

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)*
K241110

Device Name
CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel, Influenza A Subtyping Kit (Ver4)

Indications for Use *(Describe)*

The Influenza A Subtyping Kit contains reagents and controls of the CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel and is intended for use in real-time RT-PCR (rRT-PCR) assays on an in vitro diagnostic real-time PCR instrument that has been FDA-cleared for use with this kit in conjunction with clinical and epidemiological information:

• For determination of the subtype of seasonal human influenza A viruses as seasonal A(H3) and/or A(H1)pdm09 from viral RNA in upper respiratory tract clinical specimens (including nasopharyngeal swabs [NPS], nasal swabs [NS], throat swabs [TS], nasal aspirates [NA], nasal washes [NW] and dual nasopharyngeal/throat swabs [NPS/TS]) and lower respiratory tract specimens (including bronchoalveolar lavage [BAL], bronchial wash [BW], tracheal aspirate [TA], sputum, and lung tissue) from human patients with signs and symptoms of respiratory infection and/or from viral culture;

• To provide epidemiologic information for surveillance of circulating influenza viruses.

Performance characteristics for influenza were established during a season when seasonal influenza viruses A(H1N1) and A(H3N2) were the predominant influenza A viruses in circulation and during a season when the A(H1N1)pdm09 influenza virus was the predominant influenza A virus in circulation. Performance characteristics may vary with other emerging influenza A viruses.

Negative results do not preclude influenza virus infection and should not be used as the sole basis for treatment or other patient management decisions. Conversely, positive results do not rule out bacterial infection or co- infection with other viruses. The agent detected may not be the definite cause of disease.

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 state or local health department for testing. Viral culture should not be attempted unless a BSL 3E facility is available to receive and culture specimens.

All users, analysts, and any person reporting results from use of this device should be trained to perform and interpret the results from this procedure by a competent instructor prior to use. CDC Influenza Division will limit the distribution of this device to only those users who have successfully completed a training course provided by CDC instructors or designees.

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

I. GENERAL INFORMATION

Submitter
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Date Prepared: April 16, 2024

II. DEVICE INFORMATION

Proprietary Name:	CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel, Influenza A Subtyping Kit (Ver4)
Common Name:	Influenza A Subtyping Kit
Regulation Section:	866.3980-Respiratory viral panel multiplex nucleic acid assay
Device Classification:	Class II
Product Code:	OZE
Subsequent Product Codes:	NSU, NXD, OEP, OOI, OQW
Panel:	Microbiology

III. PREDICATE DEVICE

CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel, Influenza A Subtyping Kit (Ver3) (K200370)

IV. DEVICE DESCRIPTION

The Influenza A Subtyping Kit contains components of the CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel that is used in rRT-PCR assays on an FDA-cleared in vitro diagnostic real-time PCR instrument. The primer and probe sets contained in the Influenza A Subtyping Kit are designed for the detection and characterization of influenza type A viruses that infect humans.

The Influenza A Subtyping Kit consists of oligonucleotide primers and dual-labeled hydrolysis (TaqMan®) probes and controls, which may be used in rRT-PCR assays for the in vitro qualitative detection and characterization of the human influenza virus RNA in respiratory specimens from patients presenting with influenza-like illness (ILI). The oligonucleotide primers and probes for detection of Influenza A and 2009 Influenza A (swine origin) were selected from highly conserved regions of the matrix (M), and the nucleoprotein (NP), respectively. Oligonucleotide primers and probes for characterization and differentiation of seasonal influenza A(H3) and A(H1)pdm09 viruses were selected from highly conserved regions of their respective HA genes. Detection of viral RNA not only aids in the diagnosis of illness caused by seasonal, newly emerging, and novel influenza viruses in patients with ILI, but also provides epidemiological and surveillance information on influenza and aids in the presumptive laboratory identification of specific novel influenza A viruses.

V. INTENDED USE

The Influenza A Subtyping Kit contains reagents and controls of the CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel and is intended for use in real-time RT-PCR (rRT-PCR) assays on an *in vitro* diagnostic real-time PCR instrument that has been FDA-cleared for use with this kit in conjunction with clinical and epidemiological information:

- For determination of the subtype of seasonal human influenza A viruses as seasonal A(H3) and/or A(H1)pdm09 from viral RNA in upper respiratory tract clinical specimens (including nasopharyngeal swabs [NPS], nasal swabs [NS], throat swabs [TS], nasal aspirates [NA], nasal washes [NW], and dual nasopharyngeal/throat swabs [NPS/TS]) and lower respiratory tract specimens (including bronchoalveolar lavage [BAL], bronchial wash [BW], tracheal aspirate [TA], sputum, and lung tissue) from human patients with signs and symptoms of respiratory infection and/or from viral culture;
- To provide epidemiologic information for surveillance of circulating influenza viruses.

Performance characteristics for influenza were established during a season when seasonal influenza viruses A(H1N1) and A(H3N2) were the predominant influenza A virus in circulation. Performance characteristics may vary with other emerging influenza A viruses.

Negative results do not preclude influenza virus infection and should not be used as the sole basis for treatment or other patient management decisions. Conversely, positive

results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease.

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 influenza viruses and sent to state or local health department for testing. Viral culture should not be attempted unless a BSL 3E facility is available to receive and culture specimens.

All users, analysts, and any person reporting results from use of this device should be trained to perform and interpret the results from this procedure by a competent instructor prior to use. CDC Influenza Division will limit the distribution of this device to only those users who have successfully completed a training course provided by CDC instructors or designees.

VI. TECHNOLOGICAL CHARACTERISTICS

The technological characteristics of the modified CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel, Influenza A Subtyping Kit remain the same as the predicate device.
Modification of the influenza A(H3) primer and probe set was made to address recent evolutionary changes in circulating influenza A(H3) viruses that may impact the reactivity of the current Influenza A Subtyping Kit. Two quencher dyes were included in the analysis.

VII. SUBSTANTIAL EQUIVALENCE COMPARISON

The CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel, Influenza A Subtyping Kit (Ver3) (K200370), will serve as the predicate for the proposed change. See table 8-1 below for a detailed comparison of the modified device to the predicate.

Table 8-1: Device Comparison

	Predicate Device	**Proposed Device**
Item	**CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel, Influenza A Subtyping Kit (Ver3) (K200370)**	**CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel, Influenza A Subtyping Kit (Ver4)**
Intended Use	The Influenza A Subtyping Kit contains reagents and controls of the CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel and is intended for use in real-time RT-PCR (rRT-PCR) assays on an *in vitro* diagnostic real-time PCR instrument that has been FDA-cleared for use with this kit in conjunction with clinical and epidemiological information: • For determination of the subtype of seasonal human influenza A viruses as seasonal A(H3) and/or A(H1)pdm09 from viral RNA in upper respiratory tract clinical specimens (including nasopharyngeal swabs [NPS], nasal swabs [NS], throat swabs [TS], nasal aspirates [NA],	Same

	nasal washes [NW], and dual nasopharyngeal/throat swabs [NPS/TS]) and lower respiratory tract specimens (including bronchoalveolar lavage [BAL], bronchial wash [BW], tracheal aspirate [TA], sputum, and lung tissue) from human patients with signs and symptoms of respiratory infection and/or from viral culture; • To provide epidemiologic information for surveillance of circulating influenza viruses. Performance characteristics for influenza were established during a season when seasonal influenza viruses A(H1N1) and A(H3N2) were the predominant influenza A virus in circulation. Performance characteristics may vary with other emerging influenza A viruses. Negative results do not preclude influenza virus infection and should not be used as the sole basis for treatment or other patient management decisions. Conversely, positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease. 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 influenza viruses and sent to state or local health department for testing. Viral culture should not be attempted unless a BSL 3E facility is available to receive and culture specimens. **All users, analysts, and any person reporting results from use of this device should be trained to perform and interpret the results from this procedure by a competent instructor prior to use. CDC Influenza Division will limit the distribution of this device to only those users who have successfully completed a training course provided by CDC instructors or designees.**	
Organism Detected	Influenza A viruses (animal and human)	Same
Specimen Types	Nasopharyngeal swabs, nasal swabs, throat swabs, nasal aspirates, nasal washes and dual nasopharyngeal/throat swabs, bronchoalveolar lavages, , bronchial washes, tracheal aspirates, sputum, and lung tissue from human patients with signs and symptoms of respiratory infection and/or from viral culture	Same
Technological Characteristics	Real-Time RT-PCR based assay	Same
Nucleic Acid Extraction	• QIAamp® DSP Viral RNA Mini Kit, QIAGEN	• QIAamp® DSP Viral RNA Mini Kit, QIAGEN

	• MagNA Pure Compact –Nucleic Acid Isolation Kit I, Roche • MagNA Pure Compact – RNA Isolation Kit, Roche • MagNA Pure LC – Total Nucleic Acid Kit, Roche • QIAcube – QIAamp® DSP Viral RNA Mini Kit, QIAGEN • NucliSENS® easyMAG®, bioMérieux • EMAG®, bioMérieux • EZ1 Advanced XL – EZ1 DSP Virus Kit and EZ1 RNA Tissue Mini Kit, QIAGEN • MagNA Pure 96 - DNA and Viral NA Small Volume Kit, Roche	• MagNA Pure LC – Total Nucleic Acid Kit, Roche • QIAcube – QIAamp® DSP Viral RNA Mini Kit, QIAGEN • NucliSENS® easyMAG®, bioMérieux • EMAG®, bioMérieux • EZ1 Advanced XL – EZ1 DSP Virus Kit and EZ1 RNA Tissue Mini Kit, QIAGEN • MagNA Pure 96 - DNA and Viral NA Small Volume Kit, Roche *MagNa Pure Compact has been removed from the labeling after discontinuation by the manufacturer
Enzyme Master Mix	• Invitrogen SuperScript™ III Platinum® One-Step Quantitative RT-PCR Kit (with or without ROX) • Quanta BioSciences qScript™ One-Step qRT-PCR Kit, Low ROX	Same

VIII. Analytical Performance Evaluation

Analytical Sensitivity- Limit of Detection (LoD)

Analytical sensitivity of the modified influenza A(H3) primers and probe was determined in limit of detection (LoD) studies using both the modified (H3_v2 with either ZEN or BHQ) and current (H3_IVD) versions of the CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel, Influenza A Subtyping assay. All LoD studies were performed using RNA extracted by the Qiagen EZ1 Advanced XL DSP Virus Kit, on the ABI™ 7500 Fast Dx using two enzymes: the Invitrogen Superscript™ III Platinum and Quanta BioSciences qScript™. Serial 3-fold dilutions of representative strains of influenza A(H3) with and without the 3' mutation (Table 8-2) were tested in triplicate with each assay. An LoD comparison study was conducted where the lowest concentration of virus that was positive for 3 out of 3 replicates for each representative strain was compared across all assays. The lowest concentration of virus that tested positive for 3 out of 3 replicates in the LoD comparison study was considered the estimated LoD. Results of the LoD comparison study are in Tables 8-3 and 8-4.

Table 8-2: Viruses Used in LoD Studies

Virus	Type	3' Mutation	Stock Titer (EID_{50}/mL)	Assays Tested
A/Darwin/09/2021	Influenza A/H3	Yes	$10^{8.35}$	H3_v2 (ZEN) H3_v2 (BHQ) H3_IVD
A/HongKong/4801/2014	Influenza A/H3	No	$10^{8.94}$	

Table 8-3: LoD Comparison- A/Darwin/09/2021

Log (EID_{50}/mL)	Invitrogen Platinum III SuperScript™			Quanta qScript™		
	H3_v2 (ZEN)	H3_v2 (BHQ)	H3_IVD	H3_v2 (ZEN)	H3_v2 (BHQ)	H3_IVD
$10^{0.70}$	0/3	0/3	0/3	0/3	0/3	0/3
$10^{1.18}$	0/3	0/3	2/3	2/3	1/3	0/3
$10^{1.66}$	3/3	3/3	3/3	3/3	3/3	2/3
$10^{2.13}$	3/3	3/3	3/3	3/3	3/3	2/3
$10^{2.61}$	3/3	3/3	3/3	3/3	3/3	3/3
$10^{3.09}$	3/3	3/3	3/3	3/3	3/3	3/3
$10^{3.57}$	3/3	3/3	3/3	3/3	3/3	3/3
$10^{4.05}$	3/3	3/3	3/3	3/3	3/3	3/3

Table 8-4: LoD Comparison- A/HongKong/4801/2014

Log (EID_{50}/mL)	Invitrogen Platinum III SuperScript™			Quanta qScript™		
	H3_v2 (ZEN)	H3_v2 (BHQ)	H3_IVD	H3_v2 (ZEN)	H3_v2 (BHQ)	H3_IVD
$10^{0.60}$	0/3	0/3	0/3	0/3	1/3	0/3
$10^{1.17}$	0/3	1/3	0/3	0/3	1/3	0/3
$10^{1.65}$	1/3	0/3	0/3	0/3	2/3	0/3
$10^{2.12}$	2/3	0/3	0/3	3/3	2/3	0/3
$10^{2.60}$	2/3	3/3	2/3	3/3	3/3	3/3
$10^{3.08}$	3/3	3/3	3/3	3/3	3/3	3/3
$10^{3.56}$	3/3	3/3	3/3	3/3	3/3	3/3
$10^{4.04}$	3/3	3/3	3/3	3/3	3/3	3/3

The LoD was confirmed by testing 20 individually extracted specimens at the estimated LoD and at least one 3-fold dilution above and below on both representative strains listed in Table 8-2. The lowest concentration that tested positive for ≥95% (19/20) of replicates was determined to be the confirmed LoD. All controls performed as expected. Results are summarized in Table 8-5.

Table 8-5: LoD Confirmation Study

Virus Strain	Assay	Log (EID_{50}/mL)	
		Invitrogen Platinum III SuperScript™	**Quanta qScript™**
A/Darwin/09/2021	H3_v2 (ZEN)	$10^{1.66}$	$10^{1.18}$
	H3_v2 (BHQ)	$10^{1.66}$	$10^{1.66}$
	H3_IVD	$10^{1.66}$	$10^{1.66}$
A/HongKong/4801/2014	H3_v2 (ZEN)	$10^{2.12}$	$10^{2.12}$
	H3_v2 (BHQ)	$10^{2.12}$	$10^{2.12}$
	H3_IVD	$10^{2.12}$	$10^{2.60}$

The confirmed LoD of the H3_v2 assay for both the ZEN and BHQ quenchers was found to be equivalent to the current H3_IVD assay. The LoD was confirmed to be $10^{2.12}$ or $1.23x10^{2.04}$ EID_{50}/mL.

Analytical Sensitivity- Inclusivity

The inclusivity of the modified H3 primers and probe was examined using influenza A(H3) viruses representing temporal, geographic, and genetic diversity within the subtype and prepared at a low titer (at or near the assay LoD) and a high titer. The proposed panel included influenza A strains targeted by both the H3 and the (H1)pdm09 primers and probes. The panel below includes only seasonal influenza A(H3) as these are the strains targeted with the H3_v2 primers and probe. Samples were tested in triplicate with the modified H3_v2 assay (both ZEN and BHQ) on RNA extracted by the Qiagen EZ1 Advanced XL DSP Virus Kit, on the ABI™ 7500 Fast Dx using two enzymes: the Invitrogen Superscript™ III Platinum and Quanta BioSciences qScript™. Inclusivity of influenza A(H3) strains was not impacted as all strains were detected with the modified H3_v2 assay (both ZEN and BHQ). Results are summarized in Tables 8-6 and 8-7.

Table 8-6: Inclusivity of the Modified Influenza A(H3) Primers and Probe with ZEN

Subtype/Clade	Virus Name	ID_{50}/mL	Invitrogen Platinum III SuperScript™	Quanta qScript™
H3N2	A/Massachusetts/10/2021	$10^{0.80}$	3/3	3/3
		$10^{1.80}$	3/3	3/3
	A/Cambodia/e0826360/2020	$10^{2.20}$	3/3	3/3
		$10^{3.20}$	3/3	3/3
	A/Hong Kong/2671/2019	$10^{2.50}$	3/3	3/3
		$10^{3.50}$	3/3	3/3
	A/Singapore/INFIMH-16-0019/2016	$10^{2.50}$	3/3	3/3
		$10^{3.50}$	3/3	3/3
	A/Texas/50/2012	$10^{2.50}$	2/3	3/3
		$10^{3.50}$	3/3	3/3
	A/Perth/16/2009	$10^{2.30}$	3/3	2/3
		$10^{3.30}$	3/3	3/3

Table 8-7: Inclusivity of the Modified Influenza A(H3) Primers and Probe with BHQ

Subtype/Clade	Virus Name	ID_{50}/mL	Invitrogen Platinum III SuperScript™	Quanta qScript™
H3N2	A/Massachusetts/10/2021	$10^{0.80}$	3/3	3/3
		$10^{1.8}$	3/3	3/3
	A/Cambodia/e0826360/2020	$10^{2.20}$	3/3	2/3
		$10^{3.20}$	3/3	3/3
	A/Hong Kong/2671/2019	$10^{2.50}$	3/3	3/3
		$10^{3.50}$	3/3	3/3
	A/Singapore/INFIMH-16-0019/2016	$10^{2.50}$	3/3	3/3
		$10^{3.50}$	3/3	3/3
	A/Texas/50/2012	$10^{2.50}$	3/3	3/3
		$10^{3.50}$	3/3	3/3
	A/Perth/16/2009	$10^{2.30}$	2/3	3/3
		$10^{3.30}$	3/3	3/3

Analytical Specificity- Cross-Reactivity with other viruses in the panel

The cross-reactivity of the modified H3 primers and probe was examined using influenza viruses of different subtypes and lineages. Samples were tested in triplicate with RNA extracted from high titer preparations of each virus (10^5-10^9 ID_{50}/mL) using the ABI™ 7500 Fast Dx and the Invitrogen Superscript™ III Platinum enzyme. No cross-reactivity was seen with the H3_v2 assay with either ZEN or BHQ quenchers. Samples were also tested for Influenza A or Influenza B to ensure RNA extraction was successful. Results are summarized in Table 8-8.

Table 8-8: Cross-Reactivity of Modified Influenza A(H3) Primers and Probe

Influenza Virus Name	Subtype	ID_{50}/mL	H3_v2 (ZEN)	H3_v2 (BHQ)
A/H1N1pdm09	A/Brisbane/02/2018	$10^{7.90}$	Not Detected	Not Detected
	A/Michigan/45/2015	$10^{8.30}$	Not Detected	Not Detected
	A/California/08/2009	$10^{9.20}$	Not Detected	Not Detected
A/H1N2v_1A.1.1	A/Ohio/24/2017	$10^{6.30}$	Not Detected	Not Detected
A/H1N1v_1A.3	A/Ohio/09/2015	$10^{7.70}$	Not Detected	Not Detected
A/H1N2v_1B.2.1	A/Ohio/35/2017	$10^{6.90}$	Not Detected	Not Detected
A/H3N8	A/canine/Florida/43/2004	$10^{8.10}$	Not Detected	Not Detected
	A/equine/Ohio/01/2003	$10^{8.40}$	Not Detected	Not Detected
A/H5N8	A/Astrakhan/3212/2020 IDCDC-RG71A	$10^{8.68}$	Not Detected	Not Detected
	A/Gyrfalcon/Washington/41088-6/2014 CVV	$10^{8.90}$	Not Detected	Not Detected
A/H7N9	A/Taiwan/1/2017	$10^{5.54}$	Not Detected	Not Detected
A/H9N2	A/Bangladesh/0994/2011 CVV	$10^{9.50}$	Not Detected	Not Detected
B/Vic	B/Washington/02/2019	$10^{9.30}$	Not Detected	Not Detected
B/Yam	B/Phuket/3073/2013	$10^{9.90}$	Not Detected	Not Detected
Inf C	C/Minnesota/01/2016	NA	Not Detected	Not Detected

Analytical Specificity- Cross-Reactivity

The cross-reactivity of the influenza A(H3) primers and probe was evaluated with additional non-influenza respiratory pathogens to verify the modification does not impact the specificity of the assay design. The H3_v2 assay (ZEN and BHQ) was tested against RNA extracted from non-influenza human respiratory viruses, bacteria, and yeast representing common respiratory pathogens or flora commonly present in human respiratory specimens. RNA was extracted from high titer preparations (10^5-10^{10} ID_{50}/mL or equivalent CFU/mL) and tested using the ABI™ 7500 Fast Dx and the Invitrogen Superscript™ III Platinum enzyme. None of the organisms tested were detected with either the H3_v2 ZEN or BHQ assays. Results are summarized in Table 8-9.

Table 8-9: Modified Influenza A(H3) Primer and Probe Cross-Reactivity

Organism Tested				
Bacteria and Yeast	**Strain**	**CFU/mL**	**H3_v2 (ZEN)**	**H3_v2 (BHQ)**
Bordetella pertussis	Tohama I	$10^{10.0}$	Not Detected	Not Detected
Candida albicans	3147	$10^{8.49}$	Not Detected	Not Detected
Chlamydia pneumoniae	CM-1	40 IFU/mL	Not Detected	Not Detected
Corynebacterium diphtheriae	NCTC 13129	57.4 ng/µL	Not Detected	Not Detected
Escherichia coli	K12	$10^{9.60}$	Not Detected	Not Detected
Haemophilus influenzae	M15709	$10^{6.40}$	Not Detected	Not Detected
Lactobacillus plantarum	NA	$10^{8.80}$	Not Detected	Not Detected
Legionella pneumophila	Philadelphia-1	$10^{8.41}$	Not Detected	Not Detected
Moraxella catarrhalis	M15757	$10^{9.50}$	Not Detected	Not Detected
Mycobacterium tuberculosis	H37Ra	$10^{5.00}$	Not Detected	Not Detected
Mycoplasma pneumoniae	PI 1428	$10^{9.00}$	Not Detected	Not Detected
Neisseria elongata	NA	$10^{5.00}$	Not Detected	Not Detected
Neisseria meningitidis	M2578	$10^{7.90}$	Not Detected	Not Detected
Pseudomonas aeruginosa	NA	$10^{10.5}$	Not Detected	Not Detected
Staphylococcus epidermidis	NA	$10^{10.5}$	Not Detected	Not Detected
Staphylococcus aureus	NA	$10^{10.7}$	Not Detected	Not Detected
Streptococcus pneumoniae	249-06 (Thailand)	$10^{6.60}$	Not Detected	Not Detected
Streptococcus pyogenes	MGAS 8232	41 ng/µL	Not Detected	Not Detected
Streptococcus salivarius	DSM 13084	109 ng/µL	Not Detected	Not Detected
Organism Tested				
Virus Subtype	**Designation**	**$TCID_{50}$/mL**	**H3_v2 (ZEN)**	**H3_v2 (BHQ)**
Enterovirus	Echo 6	$10^{6.9}$	Not Detected	Not Detected
Human Adenovirus, type 1	Ad.71	$10^{9.2}$	Not Detected	Not Detected
Human Adenovirus, type 7a	S-1058	$10^{7.1}$	Not Detected	Not Detected
Human Coronavirus	299E	$2.8x10^{6}$	Not Detected	Not Detected
	OC43	$8.9x10^{6}$	Not Detected	Not Detected
	NL63	$1.6x10^{4}$	Not Detected	Not Detected
	HKU1	$5.5x10^{5}$ copies/µL	Not Detected	Not Detected
Human Rhinovirus A	1A	$10^{5.8}$	Not Detected	Not Detected
Human parainfluenza 1	NA	3.0 ng/µL	Not Detected	Not Detected
Human parainfluenza 2	Greer	$10^{3.1}$	Not Detected	Not Detected

Human parainfluenza 3	C-243	$10^{7.9}$	Not Detected	Not Detected
Respiratory syncytial virus	CH93-18b	$10^{6.8}$	Not Detected	Not Detected
Herpes Simplex virus	KOS	$5x10^{7.75}$	Not Detected	Not Detected
Varicella-zoster virus	AV92-3:H	$5x10^{3.75}$	Not Detected	Not Detected
Epstein Barr virus	B95-8	1.7 ng/µL	Not Detected	Not Detected
Measles	Edmonston	$5x10^{4.5}$	Not Detected	Not Detected
Mumps	Enders	$5x10^{6.5}$	Not Detected	Not Detected
Cytomegalovirus	AD-169	$5x10^{6.25}$	Not Detected	Not Detected
Influenza C	C/Minnesota/1/2016	NA	Not Detected	Not Detected
	C/Minnesota/4/2015	NA	Not Detected	Not Detected
	C/Minnesota/29/2015	NA	Not Detected	Not Detected
Mers-CoV	NA	$5.3x10^5$ copies/µL	Not Detected	Not Detected
SARS-CoV-2	NA	NA	Not Detected	Not Detected

In-silico Analysis:

Process 1 - Assessment of Primers:

Potential assay primer and probe sets from the CDC were assessed against an alignment of all available H3N2 Influenza sequence information deposited within the GISAID EpiFlu database. Each primer region in the alignment was compared against each given primer and probe sequence, with nucleotide mismatches calculated for each primer/probe position.

Process 2 – Inclusivity/Exclusivity Analysis of Chosen Primers by BLAST:

Each primer sequence inclusivity/exclusivity was tested via NCBI BLAST+ against the nr/nt database. The database search parameters were as follows: 1) The nucleotide collection consists of GenBank+EMBL+DDBJ+PDB+RefSeq sequences, but excludes EST, STS, GSS, WGS, TSA, patent sequences as well as phase 0, 1, and 2 HTGS sequences and sequences longer than 100Mb; 2) The database is non-redundant. Identical sequences have been merged into one entry, while preserving the accession, GI, title and taxonomy information for each entry; 3) Database was updated on 12/22/2023; 4) The search parameters automatically adjust for short input sequences and the expect threshold is 1000; 5) The match and mismatch scores are 1 and -3, respectively; 6) The penalty to create and extend a gap in an alignment is 5 and 2 respectively. Results returned were >= 86% identity matches for all primers. These results were all confirmed to be H3 Influenza virus HA segments from the result description field or taxonomy ids.

IX. Clinical Performance Evaluation

The clinical performance of the modified influenza A(H3) primers and probe was evaluated using residual upper human respiratory clinical specimens collected from patients during previous influenza seasons in the United States that were previously tested with the CDC Human Influenza Real-Time RT-PCR Diagnostic Panel. The modified assay was tested against a set of positive and negative specimen panels. Given the decrease in sensitivity seen in strains

containing the 3' mutation with the cleared H3_IVD primers and probe, Next Generation Sequencing (NGS) was used as the comparator assay for performance evaluation to confirm the identity of positive specimens. The NGS methodology is outlined below.

Next Generation Sequencing (NGS) Methodology

The sequencing comparator method used here is the same high-throughput Illumina based sequencing pipeline we use for routine influenza surveillance. Extracted RNA is amplified using primers that target the universally conserved terminal ends of each influenza gene segment. Importantly, these primers are influenza type-specific and universally conserved for all viruses of that type. This amplification results in full length dsDNA amplicons that are sheared, indexed, and sequenced on the Illumina next-generation sequencing platform. A negative control from each extraction plate is included from the extraction step, negative and positive controls are added for the amplification step, and a final negative control is added for the library preparation step. Raw sequencing reads are assembled into consensus sequences using IRMA (Iterative Refinement Meta Assembler). IRMA uses segment and subtype specific reference sequences to build an initial assembly. That assembly is then used as a reference to repeat the assembly. This process happens iteratively until no additional reads are captured by the assembly. Reads that do not match the reference sequence, such as those that may be contaminating the sample, are excluded from the assembly.

BioInformatics Analysis Pipeline

IRMA is a unique genome assembly algorithm that first chooses a reference sequence per influenza genome segment by sorting each sequence read into bins based on the top BLAT match to those seed references. Reads in each segment's bin are then individual mapped to the reference with SAM and a consensus is generated. These generated consensus sequences are then added as additional seed references into another round of read-capture by BLAT and consensus mapping with the SAM algorithm. This process is repeated up to 5 times, stopping when the new consensus is no longer more refined than the previous round. This generated BLAT+SAM consensus is then further used as the reference sequence for sorted-read mapping with the SSW algorithm. This second assembly process also iterates up to 5 times. The analysis pipeline requires greater than 25X coverage at 100% of the bases in the reading-frame for a read to be included in the analysis. Median coverage for the HA gene for the 60 H3 positive clinical samples was ~3746.

For further details see: Shepard SS, Meno S, Bahl J, Wilson MM, Barnes J, Neuhaus E. Viral deep sequencing needs an adaptive approach: IRMA, the iterative refinement meta-assembler. *BMC Genomics.* 2016;17(1):708.

Retrospective Study

The positive specimen panel consisted of a total of 60 influenza A(H3) specimens. These positive specimens consisted of 30 specimens confirmed to be influenza A(H3) clade 3C2a1b.2a (i.e., contain the 3' mutation) with NGS and 30 specimens archived at CDC that were confirmed to not contain the 3' mutation with NGS. The negative specimen panel consisted of a total of 60 negative specimens. Negative specimens were taken from symptomatic patients known to be positive for influenza H1N1.

For all clinical studies, RNA was extracted using the Qiagen EZ1 Advanced XL DSP Virus Kit, and tested on the ABI™ 7500 Fast Dx using two enzymes: the Invitrogen Superscript™ III Platinum and Quanta BioSciences qScript™. See Table 8-10 for an outline of the retrospective panel composition.

Table 8-10: Number of Specimens for PCR Testing Performed

	Invitrogen Superscript™ III			**Quanta qScript™**		
	NGS- Positive		**NGS-Negative**	**NGS- Positive**		**NGS-Negative**
	3' mutation	**no 3' mutation**		**3' mutation**	**no 3' mutation**	
H3_v2 (ZEN)	30	30	60	30	30	60
H3_V2 (BHQ)						
H3_IVD						

Performance was evaluated in two ways. In the first evaluation, the positive percent agreement (PPA) was determined for both the modified H3_v2 primers and probe as well as the current H3_IVD primers and probe when compared to the NGS data. The PPA in this evaluation was determined for the total number of 60 positive specimens. Negative percent agreement (NPA) was also calculated for both primer and probe sets across all 60 negative specimens. Confidence intervals were calculated using the Wilson method. PPA of the H3_v2 assay was determined to be 100% (93.4-100) when tested using the Invitrogen Superscript™ III and 96.67% (89-99) when using the Quanta qScript™. NPA was determined to be 100% (93.4-100) when tested using both master mixes. For both PPA and NPA the modified H3_v2 assay performed equivalent or better than the current H3_IVD assay. Results are outlined in Tables 8-11 and 8-12.

Table 8-11: Modified Influenza A(H3) Primers and Probe Retrospective Positive Clinical Study Results- Total Positive Specimens

Primer/Probe Set	**Invitrogen Superscript™ III**		**Quanta qScript™**	
	# of Positives[1]	**% Positive Agreement (95% CI)**	**# of Positives[1]**	**% Positive Agreement (95% CI)**
H3_v2 (ZEN)	60/60	100 (93.4-100)	58/60	96.67 (89-99)
H3_v2 (BHQ)	60/60	100 (93.4-100)	58/60	96.67 (89-99)
H3_IVD	60/60	100 (93.4-100)	57/60	95 (86-98)

[1]Proportion of positive samples correctly identified versus the comparator

Table 8-12: Modified Influenza A(H3) Primers and Probe Retrospective Negative Clinical Study Results

Primer/Probe Set	Invitrogen Superscript™ III		Quanta qScript™	
	# of Negatives[1]	% Negative Agreement (95% CI)	# of Negatives[1]	% Negative Agreement (95% CI)
H3_v2 (ZEN)	60/60	100 (93.4-100)	60/60	100 (93.4-100)
H3_v2 (BHQ)	60/60	100 (93.4-100)	60/60	100 (93.4-100)
H3_IVD	60/60	100 (93.4-100)	60/60	100 (93.4-100)

[1]Proportion of negative samples correctly identified versus the comparator

The second evaluation of performance was a comparison of the average Ct values for the modified H3_v2 primers and probe to the current H3_IVD primers and probe. Only positive specimens that generated a positive result with both sets of primers and probes were included in this evaluation. There was no significant shift in Ct values seen with the modified H3_v2 assay. Summary results are in Table 8-13.

Table 8-13: Comparison of Average Ct Values

Invitrogen Superscript™ III			Quanta qScript™		
H3_v2 (ZEN)	H3_v2 (BHQ)	H3_IVD	H3_v2 (ZEN)	H3_v2 (BHQ)	H3_IVD
25.08	25.87	25.43	25.38	25.66	25.59

To highlight the improvement in sensitivity with this known mutation in circulating influenza A(H3) viruses, one positive specimen (see Specimen ID 3003572756 in Attachment F), has a double mutation present in the target region of this assay. The clinical analysis demonstrates the shift in Ct value from an average of 31.1 for the current H3_IVD assay to 22.94 and 22.77 with the modified H3_v2 (ZEN) assay and H3_v2 (BHQ) assay, respectively.

X. Conclusion

The modification of the CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel, Influenza A Subtyping Kit to ensure comprehensive detection of influenza A(H3) viruses does not substantially change the device. Analytical and clinical data demonstrate that the performance of the device to detect influenza A(H3) viruses is accomplished with high positive and negative agreement in a manner substantially equivalent to the predicate. The change raises no new issues of safety and effectiveness and the indications for use remain the same.