FDA U.S. FOOD & DRUG ADMINISTRATION

March 10, 2020

Centers for Disease Control and Prevention
Yon Yu
Regulatory Affairs and Clinical Guidelines Team Lead
1600 Clifton Rd; MS H24-11
Atlanta, Georgia 30329

Re: K200370
Trade/Device Name: CDC Human Influenza Virus Real-time RT-PCR Diagnostic Panel, Influenza A/B Typing Kit, CDC Human Influenza Virus Real-time RT-PCR Diagnostic Panel, Influenza A Subtyping Kit, CDC Human Influenza Virus Real-time RT-PCR, Influenza A/H5 Subtyping Kit
Regulation Number: 21 CFR 866.3980
Regulation Name: Respiratory Viral Panel Multiplex Nucleic Acid Assay
Regulatory Class: Class II
Product Codes: OZE, OOI, NSU, OEP, OQW, NXD
Dated: February 13, 2020
Received: February 14, 2020

Dear Yon Yu:

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 (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 located 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.

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 803) for devices or postmarketing safety reporting (21 CFR 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 4, Subpart A) for combination products; and, if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR 1000-1050.

Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR Part 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,

Steven Gitterman, M.D., Ph.D.
Deputy Director
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: 06/30/2020
See PRA Statement below.

510(k) Number *(if known)*
K200370

Device Name
CDC Human Influenza Real-Time RT-PCR Diagnostic Panel: Influenza A/B Typing Kit (VER 2), Influenza A Subtyping Kit (VER 3), and Influenza A/H5 Subtyping Kit (VER 4)

Indications for Use *(Describe)*

CDC Human Influenza Real-Time RT-PCR Diagnostic Panel: Influenza A/B Typing Kit (VER 2)

The Influenza A/B Typing 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 qualitative detection of influenza virus type A or B 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 epidemiological 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.

CDC Human Influenza Real-Time RT-PCR Diagnostic Panel: Influenza A Subtyping Kit (VER 3)

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 epidemiological 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.

CDC Human Influenza Real-Time RT-PCR Diagnostic Panel: Influenza A/H5 Subtyping Kit (VER 4)

The Influenza A/H5 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 the presumptive identification of virus in patients who may be infected with influenza A subtype A(H5) (Asian lineage) from viral RNA in human respiratory specimens and viral culture in conjunction with clinical and epidemiological risk factors;

- To provide epidemiological 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.

Testing with the influenza H5a and H5b primer and probe sets should not be performed unless the patient meets the most current U.S. Department of Health and Human Services (DHHS) clinical and epidemiological criteria for testing suspect A(H5) specimens. The definitive identification of influenza A(H5) (Asian lineage) either directly from patient specimens or from virus cultures requires additional laboratory testing, along with clinical and epidemiological assessment in consultation with national influenza surveillance experts.

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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8. 510(k) Summary

I. GENERAL INFORMATION

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Date Prepared: February 13, 2020

II. DEVICE INFORMATION

Proprietary Name: CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel, Influenza A/B Typing Kit (Ver2), Influenza A Subtyping Kit (Ver3), Influenza A/H5 Subtyping Kit (Ver4)

Common Name: Influenza A/B Typing Kit, Influenza A Subtyping Kit, Influenza A/H5 Subtyping Kit

Regulation Section: 866.3980-Respiratory viral panel multiplex nucleic acid assay

Subsequent Regulation Sections: 866.3332-Reagents for detection of specific novel influenza A viruses
862.2570-Instrumentation for clinical multiplex systems

Device Classification: Class II

Product Code: OZE

Subsequent Product Codes: NSU, NXD, OEP, OQW, OOI

Panel: Microbiology

III. PREDICATE DEVICE

CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel (K190302)

IV. DEVICE DESCRIPTION

The CDC Human Influenza Real-Time RT-PCR Diagnostic Panel is used in real-time RT-PCR (rRT-PCR) assays on an *in vitro* diagnostic real-time PCR system. The panel is configured in four separate kits. Each kit consists of oligonucleotide primers, fluorescently labeled hydrolysis probes, and controls which are used in rRT-PCR assays for the *in vitro* qualitative detection and characterization of influenza virus RNA in respiratory specimens from patients presenting with influenza-like illness (ILI). Oligonucleotide primers and probes for detection of influenza A, influenza B, and 2009 influenza A (swine origin) were selected from highly conserved regions of the matrix (M), non-structural (NS), and nucleoprotein (NP) genes, respectively. Oligonucleotide primers and probes for characterization and differentiation of influenza A(H3) and A(H1)pdm09 viruses and genetic lineages of influenza B were selected from highly conserved regions of their HA genes. Oligonucleotide primers and probes to detect the human RNase P gene (RP) in control samples and clinical specimens is also included in the panel.

V. INTENDED USE

CDC Human Influenza Real-Time RT-PCR Diagnostic Panel: Influenza A/B Typing Kit

The Influenza A/B Typing 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 qualitative detection of influenza virus type A or B 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.

CDC Human Influenza Real-Time RT-PCR Diagnostic Panel: Influenza A Subtyping Kit

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.

CDC Human Influenza Real-Time RT-PCR Diagnostic Panel: Influenza A/H5 Subtyping Kit

The Influenza A/H5 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 the presumptive identification of virus in patients who may be infected with influenza A subtype A(H5) (Asian lineage) from viral RNA in human respiratory specimens and viral culture in conjunction with clinical and epidemiological risk factors;

- 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.

Testing with the influenza H5a and H5b primer and probe sets should not be performed unless the patient meets the most current U.S. Department of Health and Human Services (DHHS) clinical and epidemiologic criteria for testing suspect A(H5) specimens. The definitive identification of influenza A(H5) (Asian lineage) either directly from patient specimens or from virus cultures requires additional laboratory testing, along with clinical and epidemiological assessment in consultation with national influenza surveillance experts.

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.

VI. TECHNOLOGICAL CHARACTERISTICS

The technological characteristics of the modified CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel remain the same as the predicate device. Modifications were made primarily to address recent evolutionary changes in circulating influenza A viruses that may impact the reactivity of the current Influenza A/B Typing Kit, Influenza A Subtyping Kit, and Influenza A/H5 Subtyping

Kit. No modifications were made to the assay designs of the Influenza B Lineage Genotyping Kit.

VII. SUBSTANTIAL EQUIVALENCE COMPARISON

The CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel (K190302), will serve as the predicate for the proposed change. See tables 8-1 to 8-3 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/B Typing Kit [K190302]**	**CDC Human Influenza Virus Real-Time RT- PCR Diagnostic Panel , Influenza A/B Typing Kit (Ver2)**
Intended Use	The Influenza A/B Typing 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 the CDC device in conjunction with clinical and epidemiological information: • For qualitative detection of influenza virus type A or B 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.	Same

	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), influenza B viruses	Same
Specimen Types	Nasopharyngeal swabs, nasal swabs, throat swabs, nasal aspirates, nasal washes and dual nasopharyngeal/throat swabs, bronchoalveolar lavages, bronchial aspirates, 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 • 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	Same
Enzyme Master Mix	Invitrogen SuperScript™ III Platinum® One-Step Quantitative RT-PCR Kit (with or without ROX) **OR** Quanta BioSciences qScript™ One-Step qRT-PCR • Kit, Low ROX	Same
Required Instrumentation	• Applied Biosystems™ 7500 Fast Dx Real-Time PCR Instrument with SDS software version 1.4 • Applied Biosystems™ QuantStudio™ Dx with version 1.0.3 software • QIAGEN Rotor-Gene® Q MDx with AssayManager® 1.0.4 and Epsilon version 1.0.1 software	Same

Table 8-2: Device Comparison

	Predicate Device	Proposed Device
Item	**CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel, Influenza A Subtyping Kit (Ver2) [K190302]**	**CDC Human Influenza Virus Real-Time RT- PCR Diagnostic Panel, Influenza A Subtyping Kit (Ver3)**
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 the CDC device 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	Same

	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.	
Organism Detected	Influenza A viruses (animal and human), Swine-origin influenza A viruses, Influenza A subtypes: seasonal A(H3), A(H1)pdm09	Same
Specimen Types	Nasopharyngeal swabs, nasal swabs, throat swabs, nasal aspirates, nasal washes and dual nasopharyngeal/throat swabs, bronchoalveolar lavages, bronchial aspirates, 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 • 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	Same
Enzyme Master Mix	Invitrogen SuperScript™ III Platinum® One-Step Quantitative RT-PCR Kit (with or without ROX) **OR** Quanta BioSciences qScript™ One-Step qRT-PCR Kit, Low ROX	Same
Required Instrumentation	• Applied Biosystems™ 7500 Fast Dx Real-Time PCR Instrument with SDS software version 1.4 • Applied Biosystems™ QuantStudio™ Dx with version 1.0.3 software • QIAGEN Rotor-Gene® Q MDx with AssayManager® 1.0.4 and Epsilon version 1.0.1 software	Same

Table 8-3: Device Comparison

	Predicate Device	**Proposed Device**
Item	**CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel, Influenza A/H5 Subtyping Kit (Ver3) [K190302]**	**CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel, Influenza A/H5 Subtyping Kit (Ver4)**
Intended Use	The Influenza A/H5 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 the CDC device in conjunction with clinical and epidemiological information: • For the presumptive identification of virus in patients who may be infected with influenza A subtype A(H5) (Asian lineage) from viral RNA in human respiratory specimens and viral culture in conjunction with clinical and epidemiological risk factors; • 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. Testing with the influenza H5a and H5b primer and probe sets should not be performed unless the patient meets the most current U.S. Department of Health and Human Services (DHHS) clinical and epidemiologic criteria for testing suspect A(H5) specimens. The definitive identification of influenza	Same

	A(H5) (Asian lineage) either directly from patient specimens or from virus cultures requires additional laboratory testing, along with clinical and epidemiological assessment in consultation with national influenza surveillance experts. 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.	
Organism Detected	Influenza A viruses (animal and human), Influenza A subtype A(H5) (Asian lineage)	Same
Specimen Types	Human respiratory specimens and viral culture	Same
Technological Characteristics	Real-time RT-PCR based assay	Same
Nucleic Acid Extraction	• 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	Same
Enzyme Master Mix	Invitrogen SuperScript™ III Platinum® One-Step Quantitative RT-PCR Kit (with or without ROX) **OR** Quanta BioSciences qScript™ One-Step qRT-PCR Kit, Low ROX	Same
Required Instrumentation	• Applied Biosystems™ 7500 Fast Dx Real-Time PCR Instrument with SDS software version 1.4 • Applied Biosystems™ QuantStudio™ Dx with version 1.0.3 software • QIAGEN Rotor-Gene® Q MDx with AssayManager® 1.0.4 and Epsilon version 1.0.1 software	Same

VIII. ANALYTICAL PERFORMANCE EVALUATION

Analytical Sensitivity- Limit of Detection (LOD)

Analytical sensitivity and improved reactivity of the modified InfA, pdmInfA, and pdmH1 assays were determined in LOD studies. An LOD equivalency comparison between the modified and currently cleared InfA, pdmInfA and pdmH1 assays from the FDA-cleared CDC Human Influenza Real-Time RT-PCR Diagnostic Panel were examined. Serial dilutions of "benchmark" strains and current strains (see Table 8-4) of known titer [either Tissue Culture Infectious Dose 50% ($TCID_{50}$/mL) or Egg Infectious Dose 50% (EID_{50}/mL)] were prepared with a diluent consisting of a suspension of human A549 cells and viral transport medium (VTM). Triplicate samples of each serial dilution were tested using both the modified and cleared assays. The benchmark strains represented viruses previously characterized with the cleared InfA, pdmInfA, and pdmH1 assays. Current strains with reduced reactivity with the cleared InfA, pdmInfA, and pdmH1 assays were included to show the equivalent or improved reactivity of the modified InfA, pdmInfA, and pdmH1 assays. The acceptance criteria for LOD equivalency between the current FDA cleared assays and the modified assays was defined as demonstrating 100% positivity (3 out of 3 replicates) at either the same endpoint LOD concentration or within a 5-fold dilution of each other against the benchmark virus strain. Summary results for the modified InfA, pdmInfA, and pdmH1 assays with each virus strain are shown in Tables 8-5 through 8-14.

Table 8-4: Virus Selection for LOD Equivalency and Confirmation Studies

Virus	Type/subtype	Stock Titer (EID_{50}/mL or $TCID_{50}$/mL	Assay Tested
A/Michigan/45/2015*	A(H1N1)pdm09	$10^{8.3}$	InfA, pdmInfA, pdmH1
A/Illinois/20/2018	A(H1N1)pdm09	$10^{7.8}$	InfA, pdmInfA, pdmH1
A/Hong Kong/4801/2014*	A(H3N2)	$10^{7.9}$	InfA
A/Abu Dhabi/240/2018	A(H3N2)	$10^{9.1}$	InfA
A/duck/Vietnam/NCVD-1544/2012*	A(H5N1)	$10^{9.5}$	InfA
A/duck/Vietnam/NCVD-17A231/2016	A(H5N6)	$10^{9.3}$	InfA

*Indicates a benchmark strain previously characterized with the FDA-cleared InfA, pdmInfA, and pdmH1 assays.

Table 8-5: LOD Equivalency- InfA - A/Michigan/45/2015 (Presented as number of positive replicates out of three total replicates tested per condition)

Titer (EID_{50}/mL)	Invitrogen Superscript™		Quanta qScript™	
	InfA IVD	InfA Modified	InfA IVD	InfA Modified
$10^{4.3}$	3/3	3/3	3/3	3/3
$10^{3.6}$	3/3	3/3	3/3	3/3
$10^{2.9}$	3/3	3/3	3/3	3/3
$10^{2.2}$	3/3	3/3	3/3	3/3
$10^{1.5}$	1/3	2/3	3/3	3/3
$10^{0.8}$	0/3	0/3	0/3	1/3

Table 8-6: LOD Equivalency- InfA - A/Illinois/20/2018 (Presented as number of positive replicates out of three total replicates tested per condition)

Titer ($TCID_{50}$/mL)	Invitrogen Superscript™		Quanta qScript™	
	InfA IVD	InfA Modified	InfA IVD	InfA Modified
$10^{3.4}$	3/3	3/3	3/3	3/3
$10^{2.7}$	3/3	3/3	3/3	3/3
$10^{2.0}$	3/3	3/3	3/3	3/3
$10^{1.3}$	2/3	3/3	3/3	3/3
$10^{0.6}$	2/3	2/3	2/3	1/3
$10^{-0.1}$	0/3	0/3	0/3	0/3

Table 8-7: LOD Equivalency- InfA - A/Hong Kong/4801/2014 (Presented as number of positive replicates out of three total replicates tested per condition)

Titer (EID_{50}/mL)	Invitrogen Superscript™		Quanta qScript™	
	InfA IVD	InfA Modified	InfA IVD	InfA Modified
$10^{2.8}$	3/3	3/3	3/3	3/3
$10^{2.1}$	3/3	3/3	3/3	3/3
$10^{1.4}$	3/3	3/3	3/3	3/3
$10^{0.7}$	2/3	2/3	1/3	3/3
$10^{0.2}$	1/3	1/3	1/3	2/3
$10^{-0.7}$	0/3	0/3	0/3	0/3

Table 8-8: LOD Equivalency- InfA - A/Abu Dhabi/240/2018 (Presented as number of positive replicates out of three total replicates tested per condition)

Titer (EID_{50}/mL)	Invitrogen Superscript™		Quanta qScript™	
	InfA IVD	InfA Modified	InfA IVD	InfA Modified
$10^{3.4}$	3/3	3/3	3/3	3/3
$10^{2.7}$	3/3	3/3	3/3	3/3
$10^{2.0}$	2/3	3/3	2/3	3/3
$10^{1.3}$	2/3	2/3	0/3	1/3
$10^{0.6}$	0/3	1/3	0/3	0/3
$10^{-0.1}$	0/3	0/3	0/3	0/3

Table 8-9: LOD Equivalency- InfA - A/duck/Vietnam/NCVD-1544/2012 (Presented as number of positive replicates out of three total replicates tested per condition)

Titer (EID_{50}/mL)	Invitrogen Superscript™		Quanta qScript™	
	InfA IVD	InfA Modified	InfA IVD	InfA Modified
$10^{4.5}$	3/3	3/3	3/3	3/3
$10^{3.8}$	3/3	3/3	3/3	3/3
$10^{3.1}$	3/3	3/3	3/3	3/3
$10^{2.4}$	3/3	1/3	3/3	3/3
$10^{1.7}$	1/3	1/3	1/3	1/3
$10^{1.0}$	0/3	0/3	0/3	0/3

Table 8-10: LOD Equivalency- InfA - A/duck/Vietnam/NCVD-17A231/2016 (Presented as number of positive replicates out of three total replicates tested per condition)

Titer (EID_{50}/mL)	Invitrogen Superscript™		Quanta qScript™	
	InfA IVD	InfA Modified	InfA IVD	InfA Modified
$10^{4.3}$	3/3	3/3	3/3	3/3
$10^{3.6}$	3/3	3/3	3/3	3/3
$10^{2.9}$	3/3	3/3	3/3	3/3
$10^{2.2}$	3/3	0/3	3/3	2/3
$10^{1.5}$	1/3	0/3	0/3	0/3
$10^{0.8}$	0/3	0/3	0/3	1/3

Table 8-11: LOD Equivalency- pdmInfA - A/Michigan/45/2015 (Presented as number of positive replicates out of three total replicates tested per condition)

Titer (EID_{50}/mL)	Invitrogen Superscript™		Quanta qScript™	
	pdmInfA IVD	pdmInfA Modified	pdmInfA IVD	pdmInfA Modified
$10^{4.3}$	3/3	3/3	3/3	3/3
$10^{3.6}$	3/3	3/3	3/3	3/3
$10^{2.9}$	3/3	3/3	3/3	3/3
$10^{2.2}$	3/3	3/3	3/3	3/3
$10^{1.5}$	1/3	2/3	0/3	1/3
$10^{0.8}$	0/3	0/3	0/3	0/3

Table 8-12: LOD Equivalency- pdmInfA - A/Illinois/20/2018 (Presented as number of positive replicates out of three total replicates tested per condition)

Titer (EID_{50}/mL)	Invitrogen Superscript™		Quanta qScript™	
	pdmInfA IVD	pdmInfA Modified	pdmInfA IVD	pdmInfA Modified
$10^{3.4}$	3/3	3/3	3/3	3/3
$10^{2.7}$	3/3	3/3	3/3	3/3
$10^{2.0}$	3/3	3/3	3/3	3/3
$10^{1.3}$	3/3	0/3	3/3	1/3
$10^{0.6}$	1/3	0/3	0/3	0/3
$10^{-0.1}$	0/3	0/3	0/3	0/3

Table 8-13: LOD Equivalency- pdmH1 - A/Michigan/45/2015 (Presented as number of positive replicates out of three total replicates tested per condition)

Titer (EID_{50}/mL)	Invitrogen Superscript™		Quanta qScript™	
	pdmH1 IVD	pdmH1 Modified	pdmH1 IVD	pdmH1 Modified
$10^{4.3}$	3/3	3/3	3/3	3/3
$10^{3.6}$	3/3	3/3	3/3	3/3
$10^{2.9}$	3/3	3/3	3/3	3/3
$10^{2.2}$	3/3	3/3	3/3	3/3
$10^{1.5}$	3/3	3/3	2/3	3/3
$10^{0.8}$	1/3	0/3	1/3	0/3

Table 8-14: LOD Equivalency- pdmH1 - A/Illinois/20/2018 (Presented as number of positive replicates out of three total replicates tested per condition)

Titer (EID_{50}/mL)	Invitrogen Superscript™		Quanta qScript™	
	pdmH1 IVD	pdmH1 Modified	pdmH1 IVD	pdmH1 Modified
$10^{3.4}$	3/3	3/3	3/3	3/3
$10^{2.7}$	3/3	3/3	3/3	3/3
$10^{2.0}$	1/3	3/3	3/3	3/3
$10^{1.3}$	0/3	1/3	0/3	3/3
$10^{0.6}$	0/3	0/3	0/3	0/3
$10^{-0.1}$	0/3	0/3	0/3	0/3

A confirmation of the LOD for the modified InfA, pdmInfA, and pdmH1 assays was determined by preparing and testing 20 individually extracted samples for the highest virus dilution where ≥95% of all replicates tested positive. An LOD was determined with both Invitrogen Superscript™ and Quanta qScript™ enzyme systems that are cleared for use with CDC Human Influenza Real-Time RT-PCR Diagnostic Panel. One of the currently cleared nucleic acid extraction methods was used to extract RNA and testing was performed on the Applied Biosystems 7500 Fast Dx. The results are summarized in Tables 8-15 and 8-16.

Table 8-15: LOD Confirmation Summary – modified InfA Assay

Influenza A Virus Subtype	Influenza Strain Designation	LOD (ID_{50}/mL)	
		Invitrogen SuperScript™	Quanta qScript™
A(H1N1)pdm09	A/Michigan/45/2015	$10^{2.2}$	$10^{2.2}$
	A/Illinois/20/2018	$10^{2.0}$	$10^{2.0}$
A(H3N2)	A/Hong Kong/4801/2014	$10^{1.4}$	$10^{1.4}$
	A/Abu Dhabi/240/2018	$10^{2.7}$	$10^{2.7}$
A(H5)	A/duck/Vietnam/NCVD-1544/2012	$10^{2.4}$	$10^{3.1}$
	A/duck/Vietnam/NCVD-17A231/2016	$10^{2.2}$	$10^{2.9}$

Table 8-16: LOD Confirmation Summary – modified pdmInfA and pdmH1 Assays[1]

Influenza A Virus Subtype	Influenza Strain Designation	LOD (ID_{50}/mL)	
		Invitrogen SuperScript™	Quanta qScript™
A(H1N1)pdm09	A/Michigan/45/2015	$10^{2.9}$	$10^{2.9}$
	A/Illinois/20/2018	$10^{2.0}$	$10^{2.0}$

[1]The LOD of the pdmInfA and pdmH1 assays is presented as the lowest virus concentration where InfA and both pdmInfA and pdmH1 primer and probe sets demonstrate uniform detection with ≥95% of all replicates testing positive.

Analytical Sensitivity – Inclusivity

The inclusivity of the modified pdmInfA and pdmH1 assays was examined using 10 influenza A(H1N1)pdm09 viruses representing temporal, geographic, and genetic diversity within the subtype and prepared at a low titer at or near the assay LOD. Samples were tested in triplicate. Results are summarized in Table 8-17. Similarly, the inclusivity of the modified InfA assay was examined with 24 influenza A viruses prepared at low titer at or near the LOD representing seasonal human viruses as well as influenza viruses of animal origin and of concern for their pandemic potential. Results are summarized in Table 8-18. Inclusivity studies were performed with both enzymes cleared with the CDC Human Influenza Real-Time RT-PCR Diagnostic Panel and one cleared extraction method. Testing was performed on the Applied Biosystems 7500 Fast Dx.

Table 8-17: Inclusivity of the modified pdmInfA and pdmH1 Assays (Presented as number of positive replicates out of three total replicates tested per condition)

Influenza Virus Strain Designation	EID_{50}/mL or $TCID_{50}$/mL	Invitrogen SuperScript™		Quanta qScript™	
		pdmInfA IVD	pdmInfA Modified	pdmH1 IVD	pdmH1 Modified
A/Florida/81/2018	$10^{3.1}$	3/3	3/3	3/3	3/3
A/Alaska/35/2018	$10^{3.5}$	3/3	3/3	3/3	3/3
A/Hawaii/17/2018	$10^{4.0}$	3/3	3/3	3/3	3/3
A/West Virginia/01/2016	$10^{1.4}$	3/3	3/3	3/3	3/3
A/Washington/24/2012	$10^{2.5}$	3/3	3/3	3/3	3/3
A/Florida/62/2014	$10^{3.3}$	3/3	3/3	3/3	3/3
A/Bangladesh/2021/2012	$10^{4.1}$	3/3	3/3	3/3	3/3
A/Utah/13/2016	$10^{2.5}$	3/3	3/3	3/3	3/3
A/Colorado/14/2012	$10^{1.1}$	3/3	3/3	3/3	3/3
A/North Carolina/4/2014	$10^{3.3}$	3/3	3/3	3/3	3/3

Table 8-18: Inclusivity of the modified InfA Assay (Presented as number of positive replicates out of three total replicates tested per condition)

Influenza Virus Designation	Subtype	EID_{50}/mL or $TCID_{50}$/mL	Invitrogen SuperScript ™	Quanta qScript™
A/Florida/81/2018	A(H1N1)pdm09	$10^{3.1}$	3/3	3/3
A/Alaska/35/2018	A(H1N1)pdm09	$10^{3.5}$	3/3	3/3
A/Hawaii/17/2018	A(H1N1)pdm09	$10^{4.0}$	3/3	3/3
A/Utah/13/2016	A(H1N1)pdm09	$10^{2.5}$	3/3	3/3
A/West Virginia/01/2016	A(H1N1)pdm09	$10^{1.4}$	3/3	3/3
A/Switzerland/8060/2017	A(H3N2)	$10^{2.2}$	3/3	3/3
A/Kansas/14/2017	A(H3N2)	$10^{2.9}$	3/3	3/3
A/Idaho/33/2016	A(H3N2)	$10^{2.9}$	3/3	3/3
A/Singapore/INFIMH-16-0019/2016	A(H3N2)	$10^{3.2}$	3/3	3/3
A/Texas/88/2016	A(H3N2)	$10^{2.5}$	3/3	3/3
A/Ohio/35/2017	A(H1N2)v	$10^{1.9}$	3/3	3/3
A/chicken/Pennsylvania/29810 1-4/2004	A(H2N2)	$10^{3.5}$	3/3	3/3
A/Ohio/13/2017	A(H3N2)v	$10^{1.9}$	3/3	3/3
A/equine/Ohio/01/2003	A(H3N8)	$10^{2.4}$	3/3	3/3
A/canine/Florida/43/2004	A(H3N8)	$10^{3.1}$	3/3	3/3
A/chicken/Alabama/1975	A(H4N8)	$10^{3.9}$	3/3	3/3
A/Northern pintail/Washington/40964/2014	A(H5N2)	$10^{3.4}$	3/3	3/3
A/gyrfalcon/Washington/41088 -6/2014	A(H5N8)	$10^{3.8}$	3/3	3/3
A/chicken/California/32213-1/2000	A(H6N2)	$10^{2.2}$	3/3	3/3
A/feline/New York/16-040082-1/2016	A(H7N2)	$10^{4.2}$	3/3	3/3
A/Taiwan/1/2017	A(H7N9)	$10^{2.5}$	3/3	3/3
A/Anhui/1/2013	A(H7N9)	$10^{4.9}$	3/3	3/3
A/duck/Vietnam/NCVD-227/2009	A(H9N2)	$10^{3.4}$	3/3	3/3
A/Bangladesh/0994/2011	A(H9N2)	$10^{3.5}$	3/3	3/3

Analytical Specificity – Cross-Reactivity

The cross-reactivity of the modified pdmInfA and pdmH1 assays was examined using influenza viruses of different types and subtypes or lineages. Samples were tested in triplicate with RNA extracted from high titer preparations of each virus ($\geq 10^6$ $TCID_{50}$/mL or EID_{50}/mL) using one of the cleared extraction methods. Testing was performed on the ABI 7500 Fast Dx using one of the enzyme systems cleared with the CDC Human Influenza Real-Time RT-PCR Diagnostic Panel. Results are summarized in Table 8-19. Cross-reactivity was seen with the modified pdmInfA assay with one non-targeted influenza virus at very high-titer.

Table 8-19: Modified pdmInfA and pdmH1 Assay Cross-Reactivity

Influenza Virus Designation	Subtype	EID_{50}/mL or $TCID_{50}$/mL	Invitrogen SuperScript™	
			pdmInfA	pdmH1
A/Perth/16/2009	A(H3N2)	$10^{8.3}$	-	-
A/Victoria/361/2011	A(H3N2)	$10^{9.2}$	-	-
A/Iowa/1/2006	A(H1N1)v	$10^{8.2}$	+	+
A/Texas/14/2008	A(H1N1)v	$10^{8.3}$	+	+
A/Ohio/09/2015	A(H1N1)v	$10^{7.7}$	+	+
A/Minnesota/19/2011	A(H1N2)v	$10^{7.1}$	+	-
A/Ohio/35/2017	A(H1N2)v	$10^{6.9}$	+	-
A/fowl/New Jersey/38092/2014	A(H2N2)	$10^{9.2}$	-	-
A/Ohio/13/2017	A(H3N2)v	$10^{6.6}$	+	-
A/equine/Ohio/01/2003	A(H3N8)	$10^{8.4}$	-	-
A/Northern pintail/Washington/40964/2014	A(H5N2)	$10^{9.4}$	-	-
A/gyrfalcon/Washington/41088 -6/2014	A(H5N8)	$10^{9.8}$	+	-
A/Anhui/01/2013	A(H7N9)	$10^{10.9}$	-	-
A/Bangladesh/0994/2011	A(H9N2)	$10^{10.5}$	-	-

The cross-reactivity of the modified InfA assay was examined using influenza viruses of different types or lineages. Samples were tested in triplicate with RNA extracted from high titer preparations of each virus (≥ 10^6 $TCID_{50}$/mL or EID_{50}/mL). Testing was performed using one of the enzyme systems cleared with the CDC Human Influenza Real-Time RT-PCR Diagnostic Panel, one cleared extraction method, and the Applied Biosystems 7500 Fast Dx. The results are summarized in Table 8-20.

Table 8-20: Modified InfA Assay Cross-Reactivity

Influenza Virus Designation	Type and subtype or lineage	EID_{50}/mL	Invitrogen SuperScript™	
			InfB	InfA
B/Maryland/15/2016	B/Victoria	$10^{8.5}$	+	-
B/Colorado/06/2017	B/Victoria	$10^{9.4}$	+	-
B/Texas/81/2016	B/Yamagata	$10^{8.3}$	+	-
B/Phuket/3073/2013	B/Yamagata	$10^{8.9}$	+	-
C/Minnesota/1/2016	Influenza C	nd[1]	-	-

[1]Infectious dose titer not determined for influenza C.

Analytical Specificity – Exclusivity

The exclusivity of the pdmInfA assay was evaluated with additional non-influenza respiratory pathogens to verify that the incorporation of non-specific AT-rich overhangs do not impact the specificity of the assay design. The pdmInfA assay was tested for cross-reactivity with non-influenza human respiratory viruses, bacteria, and yeast. Nucleic acids were extracted from high titer preparations (typically ≥ 10^6 $TCID_{50}$/mL or EID_{50}/mL, ≥ 10^6 CFU/mL) of 35 organisms (16 viruses, 18 bacteria, and 1 yeast) representing common respiratory pathogens or flora commonly present in human respiratory specimens. Testing was performed using one of the enzyme systems cleared with

the CDC Human Influenza Real-Time RT-PCR Diagnostic Panel, one cleared extraction method, and the Applied Biosystems 7500 Fast Dx. The results are summarized in Table 8-21.

Table 8-21: Modified pdmInfA Assay exclusivity with respiratory viruses, bacteria, and yeast

Organism Tested			Invitrogen SuperScript™
Bacteria and Yeast	Strain	cfu / mL	
Bordetella pertussis	Tahoma	$10^{10.0}$	-
Candida albicans	3147	$10^{8.5}$	-
Chlamydia pneumoniae[1]	CM-1	40 IFU/mL	-
Corynebacterium diphtheriae[2]	NCTC 13129	57.4 ng/ µL	-
Escherichia coli	K12	$10^{9.6}$	-
Haemophilus influenzae	M15709	$10^{6.4}$	-
Lactobacillus plantarum	NA[3]	$10^{8.8}$	-
Legionella pneumophila	Philadelphia-1	$10^{8.4}$	-
Moraxella catarrhalis	M15757	$10^{9.5}$	-
Mycobacterium tuberculosis	H37Ra	$10^{10.5}$	-
Mycoplasma pneumoniae	PI 1428	$10^{9.0}$	-
Neisseria elongata	NA[3]	$10^{5.0}$	-
Neisseria meningitidis	M2578	$10^{7.9}$	-
Pseudomonas aeruginosa	NA[3]	$10^{10.5}$	-
Staphylococcus epidermidis	NA[3]	$10^{10.5}$	-
Staphylococcus aureus	NA[3]	$10^{10.7}$	-
Streptococcus pneumoniae	249-06 (Thailand)	$10^{6.6}$	-
Streptococcus pyogenes	7790-06	$10^{7.5}$	-
Streptococcus salivarius[2]	DSM 13084	109 ng/ µL	-
Viruses	Strain	$TCID_{50}$/mL	Invitrogen SuperScript™
Enterovirus	Echo 6	$10^{6.9}$	-
Human Adenovirus, type 1	Ad.71	$10^{9.2}$	-
Human Adenovirus, type 7a	S-1058	$10^{7.1}$	-
Human Coronavirus virus[2]	OC43	50.4 ng /µL	-
Human Coronavirus virus[2]	299E	31.6 ng /µL	-
Human Rhinovirus A	1A	$10^{5.8}$	-
Human Parainfluenza 1 virus[2]	NA[3]	3.0 ng/ µL	-
Human Parainfluenza 2 virus	Greer	$10^{3.1}$	-
Human Parainfluenza 3 virus	C-243	$10^{7.9}$	-
Respiratory Syncytial virus	CH93-18b	$10^{6.8}$	-
Herpes Simplex Virus	KOS	$10^{8.4}$	-
Varicella-zoster Virus	AV92-3	$10^{4.4}$	-
Epstein Barr Virus[2]	B95-8	1.7 ng/µL	-
Measles Virus	Edmonston	$10^{5.2}$	-
Mumps Virus	Enders	$10^{7.2}$	-
Cytomegalovirus	AD-169	$10^{6.9}$	-

[1] Organism quantified by Infectious Forming Units (IFU)
[2] Organism quantified by spectrophotometry (ng/µL)
[3] NA = not applicable

IX. CLINICAL PERFORMANCE EVALUATION

Retrospective Study

The clinical performance of the modified InfA, pdmInfA, and pdmH1 assays was evaluated using residual human respiratory clinical specimens collected from patients during previous influenza seasons in the United States in 2011-12 and 2013-14 and tested with the CDC Human Influenza Real-Time RT-PCR Diagnostic Panel. The modified InfA assay was tested with a total of 62 positive and 50 negative specimens identified with the cleared CDC Human Influenza Real-Time RT-PCR Diagnostic Panel. The positive specimens consisted of 35 influenza A(H1N1)pdm09 and 27 influenza A(H3N2). The modified pdmInfA and pdmH1 assays were tested with a total of 35 positive specimens for influenza A(H1N1)pdm09 and 50 negative specimens. Testing was performed using both enzymes cleared for use with the CDC Human Influenza Real-Time RT-PCR Diagnostic Panel, one of the cleared extraction methods, and the Applied Biosystems 7500 Fast Dx. Results are summarized in Tables 8-22 through 8-25.

Table 8-22: Modified InfA Assay-Retrospective Positive Clinical Study Results

	Invitrogen SuperScript™		Quanta qScript™	
Specimen Type	# of Positives[1]	% Positive Agreement (95% CI)	# of Positives[1]	% Positive Agreement (95% CI)
NPS, NS	51/51	100.0 (93.0-100.0)	51/51	100.0 (93.0-100.0)
NPS/TS	2/2	100.0 (34.2-100.0)	2/2	100.0 (34.2-100.0)
TS	4/4	100.0 (51.0-100.0)	4/4	100.0 (51.0-100.0)
NW	3/3	100.0 (43.9-100.0)	3/3	100.0 (43.9-100.0)
Sputum	1/1	100.0 (20.7-100.0)	1/1	100.0 (20.7-100.0)
BW	1/1	100.0 (20.7-100.0)	1/1	100.0 (20.7-100.0)

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

Table 8-23: Modified InfA Assay-Retrospective Negative Clinical Study Results

	Invitrogen SuperScript™		Quanta qScript™	
Specimen Type	# of Negatives[1]	% Negative Agreement (95% CI)	# of Negatives[1]	% Negative Agreement (95% CI)
NPS	54/54	100.0 (93.4-100.0)	54/54	100.0 (93.4-100.0)

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

Table 8-24: Modified pdmInfA and pdmH1 Assays-Retrospective Positive Clinical Study Results

	Invitrogen SuperScript™		Quanta qScript™	
Specimen Type	# of Positives[1]	% Positive Agreement (95% CI)	# of Positives[1]	% Positive Agreement (95% CI)
BW	1/1	100.0 (20.7-100.0)	1/1	100.0 (20.7-100.0)
NPS, NS	28/28	100.0 (87.9-100.0)	28/28	100.0 (87.9-100.0)
NW	3/3	100.0 (43.9-100.0)	3/3	100.0 (43.9-100.0)
TS	3/3	100.00 (43.9-100.0)	3/3	100.00 (43.9-100.0)

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

Table 8-25: Modified pdmInfA and pdmH1 Assays-Retrospective Negative Clinical Study Results

	Invitrogen SuperScript™		Quanta qScript™	
Specimen Type	# of Negatives[1]	% Negative Agreement (95% CI)	# of Negatives[1]	% Negative Agreement (95% CI)
NPS	54/54	100.0 (93.4-100.0)	54/54	100.0 (93.4-100.0)

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

X. CONCLUSION

The modification of the CDC Human Influenza Virus rRT-PCR Diagnostic Panel, Influenza A/B Typing Kit, Influenza A Subtyping Kit, and Influenza A/H5 Subtyping Kit to ensure comprehensive detection of influenza A viruses does not substantially change the device. Analytical and clinical data demonstrate that the performance of the device to detect influenza A viruses is accomplished with high positive and negative percent 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.